EUROPEAN HEMATOLOGY ASSOCIATION

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ABSTRACT BOOK

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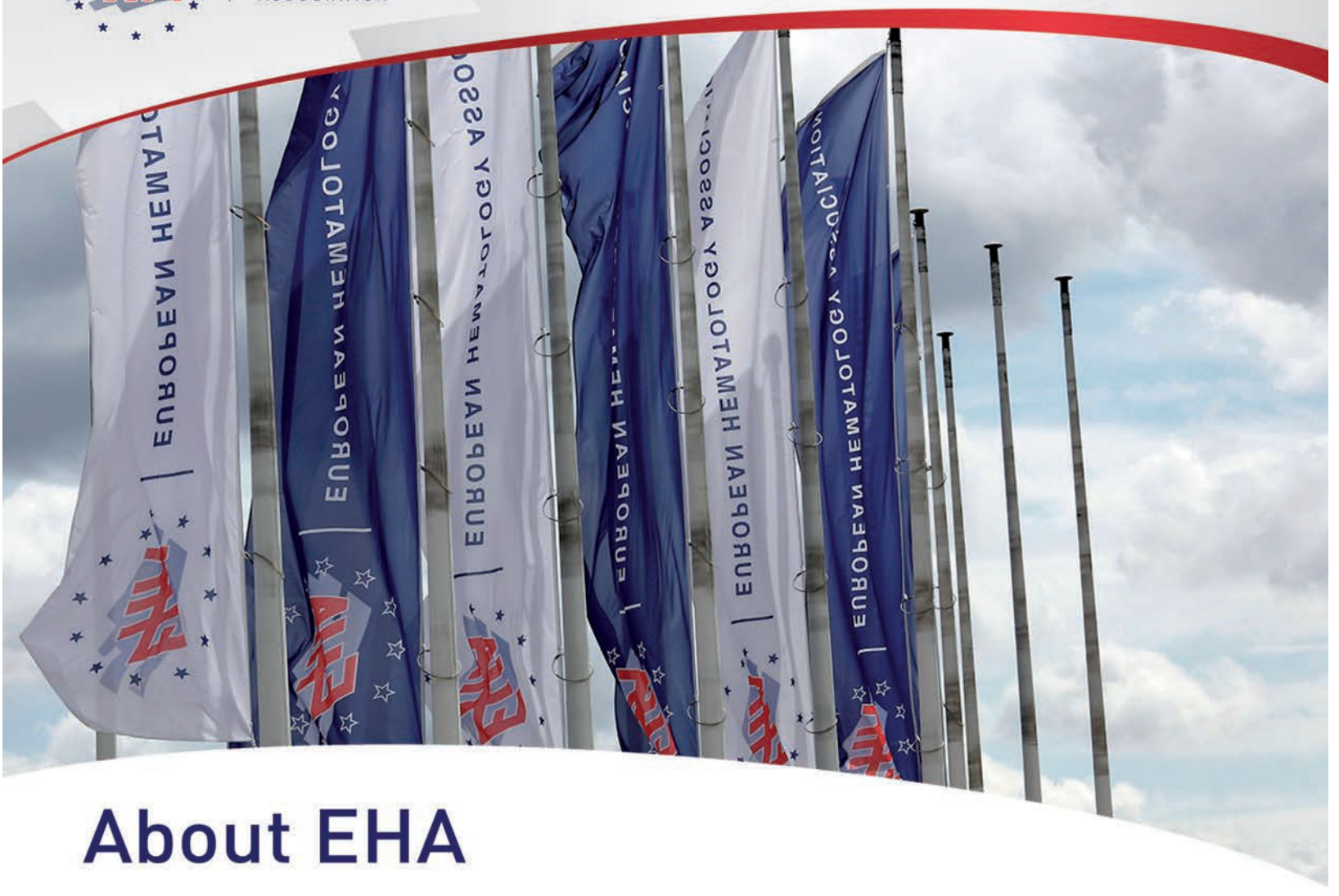

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The European Hematology Association (EHA) is a non-profit scientific association that represents European medical professionals with an active interest in hematology.

The Annual Congress, organized in a major European City, offers the opportunity to learn about new data from basic, translational and clinical research and gives access to knowledge that directly impacts the clinical practice. Not only the size of the congress increased over the years but also the first steps towards creating an education and career development program were taken.

Educational needs are the focus of our continuing medical education program. Not only through live events, but also through the EHA Learning Center, a recently launched online platform. EHA supports high quality science: we encourage research by creating a network and sharing knowledge.

EHA offers education and training and supports the careers of hematologists in Europe and travelling to Europe through its fellowships and grants program. Different fellowships are available for basic, translational and clinical researchers both in their early or advanced career.

As the largest organization of hematologists in Europe, EHA has taken it upon itself to serve and further their political interests. We advocate for you on the EU level for more research funding, improved research environment and better access to hematology care.

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Word of Welcome

On behalf of the EHA Board and the Scientific Program Committee of the 21st Congress of EHA we are pleased to introduce this year's Abstract Program.

The Scientific Program Committee has compiled an exciting up-to-date program of Simultaneous Oral and Poster Sessions from over 2400 abstracts submitted. Selected posters will be presented during the traditional Poster Walks allowing more time for discussion of results and conclusions. To better promote basic research in hematology, we introduced a new special presentation type: the poster pitch! During selected oral sessions, 5-8 presenters will have the opportunity to pitch their abstract/poster to the attendees of the session.

There are also E-posters available on the E-poster screens, for which a specific time is allocated during the Poster Browsing Time at the end of each Walk. All presented posters and E-posters can be viewed on the E-poster screens from Friday morning to Saturday evening. Posters will also be available on the EHA Learning Center, for which you have complimentary access after the congress: learningcenter.ehaweb.org.

The six Best Abstracts will be presented during the Presidential Symposium on Friday afternoon. One of them has been selected from the record number of "late breaking abstracts" with "hot" data. Only the most exciting results have been selected and will be presented in the Late Breaking Oral Session on Sunday morning. There are also late breaking posters that are included in a poster walk of the relevant topic.

On behalf of the EHA Board, the committees and all the people involved in this years' EHA congress, we thank you for coming to Copenhagen and wish you an exciting meeting.

Andreas Engert
Chair Scientific Program Committee 21st Congress

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Table of Contents

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Table of Contents

Simultaneous sessions II

Poster session II

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Table of Contents

Simultaneous sessions III

E-posters

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Table of Contents

Publication Only

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The origin of a name that reflects Europe's cultural roots.

Ancient Greek

αἷμα [haima] = blood
αἵματος [haimatos] = of blood
λόγος [logos]= reasoning

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

haematologica (adjective, plural and neuter, used as a noun) = hematological subjects

Modern English

The oldest hematology journal, publishing the newest research results.
2014 JCR impact factor = 5.814

Haematologica, as the journal of the European Hematology Association (EHA), aims not only to serve the scientific community, but also to promote European cultural identify.

SIMULTANEOUS SESSIONS I

New agents for myeloma treatment

S100

CARFILZOMIB WEEKLY PLUS MELPHALAN AND PREDNISONE IN NEWLY DIAGNOSED ELDERLY MULTIPLE MYELOMA (IFM 2012-03)

X Leleu[1,*], G Fouquet[2], L Karlin[3], B Kolb[4], M Tiab[5], C Araujo[6], N Meuleman[7], T de Revel[8], P Bourquard[9], P Lenain[10], M Roussel[11], A Jaccard[12], MO Petillon[2], K Belhadj-Merzoug[13], G Lepeu[14], ML Chretien[15], J Fontan[16], P Rodon[17], A Schmitt[18], F Offner[19], L Voillat[20], S Cerejat[21], F Kuhnowsko[22], S Rigaudeau[23], O Decaux[24], C Humbrecht-Kraut[25], J Frayfer[26], O Fitoussi[27], D Roos Weil[28], JC Eisenmann[29], V Dorvaux[30], EG Voog[31], C Hulin[32], M Attal[33], P Moreau[34], T Facon[2]

[1]Oncologie Hématologique Et Thérapie Cellulaire, Chu Poitiers, Poitiers, [2]Chu Lille, Lille, [3]Chu Lyon Sud, Lyon, [4]Chu Reims, Reims, [5]Ch La Roche Sur Yon, La Roche Sur Yon, [6]Ch Bayonne, Bayonne, [7]institut Bordet, Bruxelles, [8]Ch Clamart, Clamart, [9]Chu Nimes, Nimes, [10]Centre Henri Becquerel, Rouen, [11]Cancer Research Center, Toulouse, [12]Chu Limoges, Limoges, [13]Chu Henri Mondor, Creteil, [14]Ch Avignon, Avignon, [15]Chu Dijon, Dijon, [16]Chu Besançon, Besancon, [17]Ch Périgueux, Perigueux, [18]Institut Bergonié, Bordeaux, France, [19]Universitair Ziekenhuis Gent, Gent, Belgium, [20]Ch Chalon Sur Saone, Chalon Sur Saone, [21]Ch Corbeil Essonnes, Corbeil Essonnes, [22]Institut Curie, Paris, [23]Chu Versailles, Versailles, [24]Chu De Rennes, Rennes, [25]Ch Colmar, Colmar, [26]Ch Meaux, Meaux, [27]Polyclinique Bordeaux Nord, Bordeaux, [28]Groupe Hospitalier Pitié Salpétrière, Paris, [29]Ch Mulhouse, Mulhouse, [30]Ch Metz-Thionville, Metz-Thionville, [31]Centre Jean Bernard, Le Mans, [32]Chru Nancy, Nancy, [33]chu Toulouse, Toulouse, [34]Chu Nantes, Nantes, France

Background: Melphalan plus prednisone and bortezomib combination is the most frequent standard of care used upfront for newly diagnosed elderly myeloma (eNDMM). Despite significant improvements with bortezomib, such as subcutaneous administration and weekly schedule, safety profile issues remain with MPV, that only can be resolved with lowering the doses, albeit of the potential loss of efficacy. Carfilzomib, a novel generation proteasome inhibitor, has different safety profile with absence of neuropathy. Carmysap, a phase I/II trial of twice weekly Carfilzomib plus MP in eNDMM, demonstrated carfilzomib MTD at 36mg/m^2. The safety profile appeared otherwise good for this frail population.
Aims: We hypothesized that Carfilzomib can be used on a weekly schedule allowing to increase the dose of Carfilzomib given its positive safety profile.
Methods: IFM2012-03 (carmysap weekly) is a phase 1/2 multicenter open label single arm study to determine MTD during the phase 1 part and VGPR+CR rate (IMWG criteria) during the phase 2 part of KMP (Carfilzomib Weekly Plus Melphalan and Prednisone) regimen. The inclusion/exclusion criteria of interest were eNDMM (65 and older), with CRAB and measurable disease, with absolute neutrophils ≥1G/L, untransfused platelet count ≥75G/L, hemoglobine ≥8.5g/dL and clairance creatinine ≥30ml/min.
Induction comprised nine 5 weeks cycles. Carfilzomib is given 36, 45, 56 and 70 mg/m^2 on days 1, 8, 15, 22 IV route in combination to oral Melphalan 0.25mg/kg/j and oral prednisone 60mg/m2, both on days 1 to 4.
Maintenance: Carfilzomib. 36 mg/m^2 weekly, every two weeks IV route for 1 year. Melphalan and Prednisone is not pursued at maintenance. Analysis is done on an Intent to Treat. Recruitment was 6 patients per cohort, 3 DLTs meant MTD at the lower N-1 dose. The following are defined as DLTs: Any hematologic toxicity of grade 4 intensity or preventing administration of 2 or more of the 4 carfilzomib doses of the first treatment cycle; Grade ≥3 febrile neutropenia; Grade ≥3 gastrointestinal toxicities; Any other grade ≥3 nonhematologic toxicity considered related to CMP by the principal investigator; Grade ≥3 peripheral neuropathy persisting for more than 3 weeks after discontinuation of study drugs. We report herein the phase 1 part of the study with the 2 cohorts at 70mg/m^2.
Results: 32 NDMM recruited, 30 treated in the study, 6 per cohort at 36 mg/m² carfilzomib +MP, then 45, 56, and finally at 70mg/m². At the end of the first 70 cohort, the DSMB decided to add a second 70 cohort. The median age was 76 with 15 patients older than 75, sex ratio M/F 1.2, R-ISS 2 and 3 in 80%. There was one DLT at 36 mg/m² carfilzomib (grade 4 lymphopenia), one at 45 (lysis syndrome complicated with grade 4 renal insufficiency, two at 56 (cardiac insufficiency grade 3 and febrile neutropenia grade 3) and 2 at 70 (vomiting grade 3 and liver cholestase enzyme grade 3). As a whole for the study, the ORR is 87.5%, with 33% at least in CR. At data cut-off, with a median follow-up at 12 months, one patient had progressed and one patients had died of cardiac dysfunction considered related to Carfilzomib at the dose of 56mg/m^2. Along with the previous 2 patients, 2 other patients have stopped treatment for lysis syndrome (at 45) and pulmonary hypertension later in the disease course on cycle 5 at 56. Overall, there are 22 SAE reported for a total of greater than 200 cycles administered of KMP. An extra 3 patients have had Carfilzomib dose reduction, 2 patients at 36 from 45 and one at 45 from 56, for neutropenia grade 4, thrombocytopenia grade 4, and dyspnea grade 3, respectively.
Summary/Conclusions: IFM2012-03, the study of KMP, Carfilzomib (Kyprolis) plus Melphalan and Prednisone in elderly NDMM has not reached MTD up to 70mg/m^2 of carfilzomib. The RP2D could then be at 70 for carfilzomib. KMP appears feasible and manageable, the primary cause of AEs coming from dose adaptation of Melphalan in very elderly NDMM. Updated data will be presented at ASCO.

S101

IMPROVED EFFICACY AFTER INCORPORATING AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) INTO KRD TREATMENT WITH CARFILZOMIB (CFZ), LENALIDOMIDE (LEN), AND DEXAMETHASONE (DEX) IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: In a phase 1/2 trial (N=53), extended treatment with KRd without (w/o) ASCT was highly active in newly diagnosed myeloma (NDMM) with stringent complete response (sCR) 55% and 3-year progression-free survival (PFS) 79%.
Aims: In a subsequent phase 2 trial, we are evaluating whether extended KRd can be further improved by incorporating ASCT (KRd+ASCT). We report results from both trials after completion of enrollment into KRd+ASCT and after completion of KRd w/o ASCT (median follow-up [f/u] 4 years) as a historical control.
Methods: Both studies enrolled patients (pts) with NDMM based on similar eligibility criteria except KRd+ASCT excluded transplant ineligible pts. The treatment schemas were generally similar between studies. In KRd+ASCT, pts received: four 28-day cycles of induction with CFZ IV 36 mg/m^2 on Days (D) 1-2, 8-9, 15-16 (CFZ 20 mg/m^2 for D1-2, C1 only), LEN PO D121 at 25 mg, DEX PO 40 mg/wk; followed by stem cell collection (SCC), melphalan 200 mg/m^2 and ASCT; then KRd consolidation (C5-8) using the same doses and schedule except LEN 15 mg in C5 with the option to escalate to prior dose, and DEX reduced to 20 mg/wk; then KRd maintenance (C9-18) using the same doses as in C8 except CFZ on Days 1-2, 15-16 only. In KRd w/o ASCT, transplant-eligible pts underwent SCC after C4 then resumed KRd, and KRd maintenance was longer (C9-24). Both studies recommended single-agent LEN off study. The primary endpoint in KRd+ASCT is sCR at the end of C8. We hypothesized that an improvement of sCR from 30% at the end of C8 (historical KRd w/o ASCT) to >50% (KRd+ASCT) represents added benefit of ASCT with 5% type I error (2 sided) and supports further evaluation. Minimal residual disease (MRD) is evaluated by 10-color multiparameter flow cytometry (MFC, threshold 10^{-4}-10^{-5}) and by next-generation sequencing (NGS, LymphoSIGHT™, threshold at 10^{-6} for MRD negativity).
Results: The current KRd+ASCT study enrolled 76 pts with 72 evaluable. Baseline characteristics were comparable between the KRd+ASCT and KRd w/o ASCT study populations, including median age (59 and 59y) and high-risk IMWG cytogenetics (36% and 33%). In the ongoing KRd+ASCT, 69 pts proceeded to ASCT at data cut-off (Jan 1, 2016), 50 completed KRd consolidation, and 26 KRd maintenance, with remaining pts on treatment, except 1 patient who progressed prior to transplant. At the end of C8, sCR was 72% for KRd+ASCT (n=50) *vs* 30% for KRd w/o ASCT (n=44) and 88% (n=26) *vs* 51% (n=41) at the end of C18. At median f/u of 17.8 months, 2-year PFS was 99% for KRd+ASCT *vs* 92% for KRd w/o ASCT at median f/u of 47.5 months. In KRd+ASCT, MRD by MFC was negative in 94% of pts tested (n=31) at the end of C8 and 95% of pts tested (n=19) at the end of C18. In KRd w/o ASCT, 4-year PFS was 69% overall, 78% in MRD-negative pts *vs* 60% in positive/unknown pts by MFC, and 100% in pts with MRD negative status by NGS. Updated MRD analyses and outcomes based on MRD status by both MFC and NGS will be

presented at the meeting. The types and rates of adverse events (AEs) pre- and post-ASCT were comparable to AEs in KRd w/o ASCT.
Summary/Conclusions: Recognizing limitations of cross-study comparisons, KRd+ASCT shows superior outcomes *vs* historical KRd w/o ASCT, supporting further evaluation in the randomized setting. Both KRd studies compare favorably to other NDMM studies.

S102

WEEKLY CARFILZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (WKCYD) FOLLOWED BY MAINTENANCE WITH WEEKLY CARFILZOMIB (WK) IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM)

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Background: Carfilzomib is a novel second-generation proteasome inhibitor approved as a single agent and in combination with lenalidomide and dexamethasone for the treatment of relapsed MM. The approved schedule of carfilzomib is twice-weekly, on days 1, 2, 8, 9, 15, and 16 of 28-day cycles. In a recent phase I/II study in relapsed/refractory patients (pts) a more convenient schedule of weekly Carfilzomib in combination with dexamethasone showed to be effective (77% overall response rate; ORR) and safe (ASH 2015). The ongoing phase III ARROW study compares weekly *vs* twice weekly Carfilzomib. In the newly diagnosed setting, no data about weekly Carfilzomib are available. We designed a phase 1/2 study of wKCyd for NDMM pts.
Aims: The primary objective was to determine the maximum tolerated dose (MTD) of weekly Carfilzomib. The secondary objectives were to determine the safety and the efficacy of wKCyd as induction and of wK as maintenance, including response rate, progression-free survival and overall survival.
Methods: NDMM pts not eligible for autologous stem cell transplantation due to age or co-morbidities were enrolled. Carfilzomib was administered intravenously on days 1,8,15; cyclophosphamide orally on days 1, 8, 15 and dexamethasone orally once weekly. Dose escalation used a standard 3+3 schema with dose-limiting toxicities (DLTs) assessed during cycle 1. Three dose levels were studied with Carfilzomib escalated from 45 to 70 mg/m^2 with the standard doses of cyclophosphamide 300 mg/m2 and dexamethasone 40 mg. After completion of 9 28-day cycles, patients receive 28-day maintenance cycles with Carfilzomib (days 1, 8, 15) at the MTD defined by the phase I study until disease progression or intolerance. Adverse events were graded by NCI-CTCAE v4. Response was assessed according to the modified International Uniform Response Criteria.

Table 1. Adverse events of any grade and grade ≥3 occurring in ≥5% of patients during induction and maintenance.

Adverse events	Induction (N=54)		Maintenance (N=29)	
	Any grade, n (%)	Grade ≥3, n (%)	Any grade, n (%)	Grade ≥3, n (%)
Anaemia	19 (35)	3 (6)	1 (3)	0
Thrombocytopenia	16 (30)	3 (6)	4 (14)	0
Neutropenia	13 (24)	10 (18)	3 (10)	0
Nausea/Vomiting	8 (15)	1 (2)	2 (7)	0
Hypertension	7 (13)	0	4 (14)	2 (7)
Fever	7 (13)	1 (2)	3 (10)	0
Bronchitis	6 (11)	2 (4)	0	0
Fatigue	4 (7)	0	0	0
Acute kidney injury	4 (7)	1 (2)	0	0
Pulmonary edema	4 (7)	4 (7)	0	0
Cardiac event	3 (6)	2 (4)	1 (3)	1 (3)
Diarrhea	3 (6)	0	0	0
Sepsis	2 (4)	2 (4)	0	0
Constipation	2 (4)	0	0	0

Results: Results of dose escalation phase 1 study have been previously reported (Palumbo A et al, Blood 2014), the MTD of weekly Carfilzomib was established as 70 mg/m^2. Results are presented for all patients treated with KCyd at the Carfilzomib MTD in both the phase 1 (n=3) and phase 2 (n=51) portions of the study. Among these 54 pts, median age was 72 years (range 60-85), 33% had ISS stage III, 49% had unfavorable FISH profile [t(4;14) or t (14;16) or del17p, del1 or amp1]. Median Carfilzomib treatment duration was 9.1 months (range 0.13-25.4). During induction, the ORR was 88%, the very good partial response (VGPR) rate was 71%, the complete response (CR) rate was 12%. Twenty-nine pts could be evaluated for maintenance. After a median duration of maintenance of 14.5 months, 7 pts (35%) improved response. During maintenance, the ORR was 95%, the VGPR rate was 80%, the CR rate was 40%, including a stringent CR rate of 20%. The 1-year progression-free survival was 79.4%. Ten pts (18%) discontinued treatment due to an adverse event. The most common adverse events of any grade and of grade ≥3 during induction and during maintenance are shown in the Table. Six pts died on study, cause of death included disease progression, pulmonary edema, pulmonary embolism, second primary malignancy, sudden death and pneumonia (1 each).
Summary/Conclusions: This is the first prospective study evaluating once weekly Carfilzomib in NDMM. wKCyd as induction and wK as maintenance appeared to be safe and effective. Responses became deeper with subsequent cycles and improved during maintenance. The response rate observed with weekly Carfilzomib compares favorably with similar studies with standard twice weekly Carfilzomib infusion.

S103

A META-ANALYSIS OF OVERALL SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH LENALIDOMIDE MAINTENANCE AFTER HIGH-DOSE MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANT

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Background: Several studies demonstrate that lenalidomide (LEN) maintenance post autologous stem cell transplant (ASCT) reduces the risk of disease progression or death in patients with multiple myeloma (MM) by approximately 50% (Attal, *NEJM,* 2012; McCarthy, *NEJM,* 2012; Palumbo, *NEJM,* 2014). However, these studies were not powered for overall survival (OS).
Aims: To conduct a meta-analysis assessing the effect of LEN maintenance post ASCT on OS.
Methods: A prospectively planned meta-analysis assessed OS with LEN *vs* placebo/no maintenance (control) after ASCT. A search identified 17 randomized controlled trials (RCTs) using LEN post ASCT. Three RCTs (IFM 2005-02, CALGB 100104 [Alliance], GIMEMA RV-209) met prespecified inclusion criteria (had patient-level data, had a control arm, and achieved database lock for primary efficacy analysis of patients with newly diagnosed MM receiving LEN post ASCT). A March 2015 cutoff of the 3 RCTs enabled sufficient OS events to test treatment effect (hazard ratio [HR]=0.78).

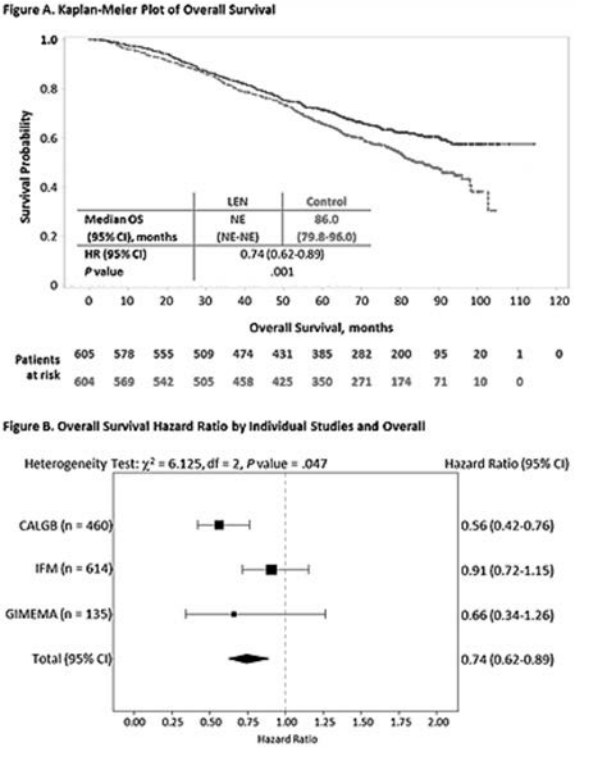

Figure A and Figure B.

Results: From 2005 to 2009, 1209 patients were randomized in the 3 RCTs to receive LEN (n=605) 10 mg/day on days 1-21 (GIMEMA) or 1-28 (IFM and CALGB) of 28-day cycles or control (n=604). With a median follow-up of 6.6

years, 491 patients (41%) had died. Baseline characteristics were generally balanced in the pooled data. After induction and single (82%) or tandem (18%) ASCT, 55% of patients achieved a complete response (CR) or very good partial response (VGPR). Median OS for the LEN maintenance group was not reached compared with 86 months for the control group (HR=0.74; 95% CI, 0.62-0.89; log-rank *P*=.001; **Figure A**), and 5-, 6-, and 7-year OS were longer in the LEN maintenance *vs* the control group (71% *vs* 66%, 65% *vs* 58%, and 62% *vs* 50%, respectively). Fisher's combination test confirmed the significant OS benefit in the meta-analysis (*P*=.001). Patients who achieved ≤PR post ASCT benefited from LEN maintenance (HR=0.86; 95% CI, 0.65-1.15) as did patients with CR/VGPR (HR=0.70; 95% CI, 0.54-0.90). OS benefit was generally consistent across subgroups. Based on a heterogeneity test (Pignon, *Lancet Oncol,* 2001), the OS study results from the efficacy meta-analysis were considered significantly heterogeneous (*P*=.047; **Figure B**) across studies for both the intention-to-treat post-ASCT population and for all patients randomized. Potential factors contributing to the heterogeneity between the clinical trials were explored and identified, including baseline/disease characteristics (such as International Staging System stage, cytogenetics), study conduct, and second-line therapy. Second primary malignancy data will be presented.

Summary/Conclusions: This large meta-analysis demonstrates that LEN maintenance significantly prolonged OS *vs* control (placebo/no maintenance) post ASCT, including in patients who achieved CR, demonstrating benefit in patients in all response categories.

S104

UPFRONT OR RESCUE TRANSPLANT IN YOUNG PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: A POOLED ANALYSIS OF 529 PATIENTS

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Background: In patients with newly diagnosed myeloma, upfront Melphalan 200 mg/m^2 followed by ASCT (MEL200-ASCT) prolongs progression-free survival-1 (PFS1) in comparison with chemotherapy plus lenalidomide (CC+R) but ASCT as salvage therapy at first relapse may still effectively rescue patients who did not receive upfront ASCT.

Aims: The primary aim was to evaluate the long-term benefit of upfront ASCT *vs* CC+R, including the impact of MEL200-ASCT *vs* CC+R in specific subgroups of patients with different prognostic features. The secondary aim was to evaluate the efficacy of salvage ASCT in patients receiving upfront CC+R.

Methods: We performed an individual patient data meta-analysis of patients enrolled in 2 phase III trials (RV-MM-209 and EMN-441) that randomized patients to MEL200-ASCT *vs* CC+R. Primary endpoints were PFS1, PFS2, overall survival (OS). Subgroup analyses according to baseline features, protocol and post MEL200-ASCT/CC+R maintenance were performed. We calculated the odds ratio (ORs) with 95% confidence intervals (CIs), as measure of association between the upfront and rescue therapy (MEL200-ASCT *vs* CC+R followed by rescue ASCT) with the 4-year risk of second progression/death (PFS2) and death (OS).

Results: In the pooled analysis, 268 patients were randomized to MEL200-ASCT and 261 to CC+R. Median follow-up was 46 months. MEL200-ASCT significantly improved PFS1 in comparison with CC+R (median 42 *vs* 24 months, HR 0.53; P<0.001), with a significant advantage in all the subgroups analyzed. In patients with R-ISS Stage I the 4-year PFS1 was 53% with MEL200-ASCT *vs* 36% with CC+R; in patients with R-ISS Stage II/III the 4-year PFS1 was 41% with MEL200-ASCT *vs* 23% with CC+R. 134 MEL200-ASCT patients and 176 CC+R patients experienced first progression. 125 patients in the MEL200-ASCT group and 174 in the CC+R group received second-line therapy. MEL200-ASCT significantly prolonged PFS2 in comparison with CC+R (4-year PFS2: 71% *vs* 54%, HR 0.53, p<0.001). The long-term advantage of MEL200-ASCT was evident in good and bad prognosis patients: in patients with R-ISS Stage I, the 4-year PFS2 was 83% with MEL200-ASCT *vs* 71% with CC+R; in patients with R-ISS Stage II/III the 4-year PFS2 was 67% with MEL200-ASCT and 48% with CC+R. The PFS1 and PFS2 advantage translated into a significant OS benefit for patients randomized to MEL200-ASCT in comparison with CC+R (4-year OS: 84% *vs* 70%, HR 0.51, P<0.001). Subgroup analyses of OS were limited by a lower number of events but the advantage with MEL200-ASCT was still evident in most of the subgroups analyzed. At data cut off, in the MEL200-ASCT group, 9% of patients received again ASCT, 68% bortezomib, 16% immunomodulatory agents (IMIDs), 7% other therapies. In the CC+R group, ASCT was recommended but not mandatory at relapse, and the choice of therapy was based on patient's will and physician discretion according to patient eligibility to ASCT: 53% of patients received ASCT, 38% bortezomib regimens, 6% IMIDs, and 3% other therapies. Upfront ASCT regardless of salvage therapy significantly reduced the risk of second progression/death (OR 0.31; P<0.001) and death (OR 0.41; P=0.003) at 4 years in comparison with CC+R followed by salvage ASCT.

Summary/Conclusions: MEL200-ASCT significantly improved PFS1, PFS2 and OS in comparison with CC+R in good and bad prognosis patients. Upfront ASCT significantly reduced the risk of death in comparison with CC+R and salvage ASCT. These data confirm the role of upfront ASCT as the standard approach for all young myeloma patients.

First-line treatment of Hodgkin Lymphoma

S105

BASELINE TOTAL METABOLIC VOLUME (TMTV) PREDICTS THE OUTCOME OF PATIENTS WITH ADVANCED HODGKIN LYMPHOMA (HL) ENROLLED IN THE AHL2011 LYSA TRIAL

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Background: The TMTV assessed on the baseline FDG-PET is a novel approach of tumor burden measurement quantifying the most active part of the tumor. It has been reported to influence HL outcome in a retrospective series (Kanoun et al, EJNM 2014; 41: 1735).
Aims: We designed a study evaluating the TMTV prognosis value in patients (pts) prospectively enrolled in a phase III randomized trial testing a treatment strategy driven by PET, compared to a standard treatment not monitored by PET.
Methods: Eligible pts for the present study had to be enrolled in the AHL2011 trial (NCT01358747) and to have a baseline PET (PET0) available for central review and TMTV calculation. Pts were 16-60 y, with a previously untreated advanced HL (Ann Arbor stage III, IV or high risk IIB) and were randomly assigned to a treatment strategy driven by PET after 2 escalated BEACOPP (BEA) cycles (PET2), delivering 4 cycles of ABVD for PET2 negative (PET2-) pts and 4 cycles of BEA for PET2 positive (PET2+) pts or a standard treatment not monitored by PET and delivering 6 cycles of BEA. PET2 were centrally reviewed and interpreted according to Deauville criteria. TMTV was computed on PET0 by summing the metabolic volumes of the individual lesions using the 41% SUVmax thresholding method already described in lymphoma (Meignan et al, EJNM 2014; 41: 113).
Results: 392 pts with a median age of 30 years were included: 64% were male, 89% had stage III/IV, and 59% an IPS≥3. Median TMTV was 200 ml (23-2149). Using a X-tile method a 350ml cut off value was firstly identified in a training set of patients (n=262; 67%) randomly obtained from the whole population, and found to predict PFS in both the training and validation sets of pts (n=130; 33%). With a median follow up of 16 months, 2y-PFS was 81% *vs* 93% in pts with high and low TMTV respectively in the whole population (p=0.0015; HR=3). PET2 positivity was also related to a lower 2y-PFS compared to PET2- pts (76% *vs* 92%; p<0.0001). Then 3 groups could be identified: pts with either [high TMTV and PET2+ (n=23; 6%)], or [high TMTV and PET2-, or low TMTV and PET2+ (n=103; 27%)], or [low TMTV and PET2- (n=261; 67%)] had a 61%, 88%, 94% 2y-PFS respectively (p<0.0001).
Summary/Conclusions: The TMTV predicts the outcome of young advanced HL pts independently of the early metabolic response to treatment. The combination of TMTV and PET2 allows identifying 3 subsets of HL pts with significantly different outcome that may help clinician to better tailor therapy.

S106

LONG-TERM FOLLOW-UP OF CONTEMPORARY TREATMENT IN EARLY-STAGE HODGKIN LYMPHOMA (HL): UPDATED ANALYSES OF THE GERMAN HODGKIN STUDY GROUP HD7, HD8, HD10 AND HD11 TRIALS

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Background: Combined modality treatment (CMT) is currently considered as standard of care in patients with early-stage HL. During the years, a gradual toxicity reduction through balancing extent and intensity of radiotherapy (RT) and chemotherapy was achieved.
Aims: We evaluated long-term follow-up (FU) of pivotal trials, to ensure the applied therapies are safe and beneficial for our patients.
Methods: We analyzed updated FU data of 4299 patients who were treated for primary early-stage HL in previously published GHSG trials between 1993 and 2003. Patients with favorable disease were randomized in HD 7 to either CMT with 2xABVD or to 40Gy extended-field (EF)-RT only, and in HD10 to either 4x or 2xABVD and 20 or 30Gy involved-field (IF)-RT, respectively. Patients with unfavorable HL in HD8 had received either 30Gy IF- or extended-field (EF)-RT after 2xCOPP/ABVD and in HD11 either 20 or 30Gy IF-RT after 4xABVD or 4x$BEACOPP_{bas}$. Progression-free (PFS) and overall survival (OS) were analyzed according to the Kaplan-Meier method. Cumulative incidences of secondary neoplasias (SN) were calculated and compared between groups using Pepe&Mori's test. Type of SN, salvage therapies and causes of death were analyzed descriptively.
Results: The median FU was 120 and 98 months for patients in HD7 (n=627) and HD10 (n=1190) and 153 and 106 months for patients in HD8 (n=1064) and HD11 (n=1395), respectively. New FU data beyond the last evaluation was available for only <50% of patients and last information was obtained from population registries in 18-30%. In HD7, CMT was superior to EF-RT with 15-year PFS estimates of 72.8% *vs* 52.2% and a hazard ratio (HR) of 0.45 (0.33-0.61). No significant differences were observed regarding OS (HR: 0.81 (0.56-1.18)) or the cumulative incidence of SN. In HD10, non-inferiority of 2xABVD+20Gy IF-RT to more intensive treatment was confirmed with HRs of 1.0 (0.6-1.5) and 0.9 (0.5-1.6) and 10-year estimates of 87.2% and 94.1% for PFS and OS, respectively. No significant differences in SN were observed. In HD8, non-inferiority of IF- compared to EF-RT was confirmed with HRs of 0.98 (0.76-1.25) and 0.88 (0.66-1.16) for PFS and OS, respectively. We observed a non-significant trend towards more SN (15-year cumulative incidence 17.1% and 14.2%, respectively, p=0.3) and deaths from SN after EF-RT. In HD11, no difference in PFS was found with intensified chemotherapy compared to standard ABVD with either 30Gy IF-RT (HR: 1.1 (0.7-1.5)) or 20Gy IF-RT (HR: 0.8 (0.6-1.1). In contrast, there was a significant difference in 10-year PFS rates estimated at 77.6% *versus* 83.3% to the detriment of ABVD-treated patients who had received 20Gy instead of 30Gy IF-RT with a HR of 1.5 (1.0-2.1). After $BEACOPP_{bas}$, 20Gy IF-RT was non-inferior to 30Gy IF-RT with a HR of 1.0 (0.7-1.5) for PFS. No differences in terms of OS or SN could be observed.
Summary/Conclusions: Long-term FU data of four large randomized GHSG phase III trials confirm the current risk-adapted therapeutic strategies in early-stage HL. Outcome in patients with early-stage favorable HL is optimal with CMT consisting of 2xABVD+20Gy IF-RT with 10-year PFS and OS estimates of 87.2% and 94.1%, respectively. 10-year PFS and OS estimates in early-stage unfavorable HL treated with 4xABVD+30Gy IF-RT leave room for improvement with 83.3% and 90.0%, respectively. Moderate intensification of chemotherapy does not improve efficacy outcome but might facilitate the reduction of IF-RT-dose. Continued FU is necessary to assess long-term effects of currently applied risk-adapted therapies.

S107

ADJUSTMENT OF THERAPY FOR HODGKIN LYMPHOMA BASED ON INTERIM PET IS BENEFICIAL AND RADIOTHERAPY MAY BE SUBSTITUTED WITH CHEMOTHERAPY IN PATIENTS WITH NEGATIVE INTERIM STUDY: FINAL RESULTS OF H2 TRIAL

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Background: The main goal of therapy for Hodgkin lymphoma (HL) is to maximize response while minimizing long-term treatment-related toxicity.
Aims: The study aimed to explore the effect of therapy adjustment based on early interim PET/CT (PET-2) on the HL patient outcome.
Methods: This prospective multicenter study recruited patients (pts) between 9/2006-8/2013. Pts with classic HL aged 18-60 years, stages I-IV were eligible. Early HL (ED) was categorized into favorable (EF) and unfavorable (EU) disease. After 2 ABVD cycles, pts with EF and negative PET-2 underwent involved site radiation therapy (ISRT) and EU pts received 2 more ABVD cycles (total 4) followed by ISRT In pts with negative PET-2, ISRT could be substituted by two further ABVD courses. Pts with positive PET-2 received a total 4 or 6 ABVD courses for EF and EU, respectively, followed by obligatory ISRT. Pts with advanced HL (AD) (B symptoms or stages III/IV) were assigned to therapy based on IPS: those with IPS 0-2 initially received ABVDx2 while pts with IPS ≥3 received escalated BEACOPP (EB) x2. If PET-2 was negative, therapy was completed using ABVDx4. If PET-2 was positive, therapy was escalated to EB with ISRT given to bulky mediastinal masses. A dynamic visual score (DS) comparing PET-2 to baseline PET was used for decision-making regarding therapeutic changes (Dann et al, Haematologica, 2010).Briefly, for pts with an initial single site of uptake, PET-2 was considered positive if the intensity of residual uptake was ≥normal mediastinal or liver blood pool (the hottest of the two). For pts with multiple initial sites of HL, disappearance of uptake in all sites or a residual single site uptake with markedly lower intensity on PET-2

was considered a negative result. The Deauville score (performed post-hoc) less or equal to 3 was defined as negative.
Results: HL progression was documented in 50 out of 355 pts, of whom 42 relapsed and 8 had primary progressive disease. PET-2 was negative in 86% and positive in 7% of pts, according to both DS and Deauville score (93% concordance by both systems; kappa 0.627). At a median follow-up of 47 months (4-114), among 170 ED pts a highly significant difference in the 5-year progression-free survival (PFS) was demonstrated between those with negative and positive PET-2 [0.91 and 0.68, respectively; hazard ratio (HR) 3.66; 95% CI 1.4-9.7; p=0.009] by DS. The differences were even more marked after reassessment using the Deauville criteria (0.91 compared to 0.47,HR 7.6; 95% CI 2.7-21.4 P<0.001). The 5-year PFS for pts with a negative PET-2 who did not receive ISRT was 0.94 compared to 0.89 for the group receiving ISRT. In the AD group (n=185), 18% (n=33) of pts progressed and 15% (n=27) had a positive PET-2. The 5-year PFS for the whole group, PET-2 negative and positive subgroups was 0.80, 0.82 and 0.68, respectively (p=0.07). The 5-year OS for ED and AD groups was 0.99 and 0.97, respectively.
Summary/Conclusions: Seventy six percent of relapses still occur in pts with a negative PET-2. A positive PET-2 portends an adverse prognosis at all stages of HL. ED pts with positive PET-2 have a poor prognosis if their therapy is continued with ABVD followed by ISRT. ISRT does not appear to improve the outcome for ED pts with negative PET-2. The prognosis of AD patients with positive PET-2 could be improved with administration of 4 additional EB cycles. If PET-2 is negative, EB can be safely changed to ABVD in AD pts.

S108

PET-CT ADAPTED THERAPY AFTER 3 CYCLES OF ABVD TO ALL STAGES OF HODGKIN LYMPHOMA. GATLA TRIAL HL-05

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Background: Positron emission tomography using fluoro-2-deoxy-d-glucose (PET-CT) is an important tool for treatment response assessment in Hodgkin Lymphoma (HL) treated with ABVD.
Aims: Adapt therapy to all stage of Hodgkin Lymphoma to the result of PET-CT after 3 ABVD (PET-CT+3). Reduce therapy in pts. who achieve early CR with negative PET-CT. Intensify treatment, in pts. with positive PET-CT after 3 ABVD. Achieve CR, event free survival (EFS) and overall survival (OS), as good as in our historical control (LH -96), when we used 3-6 ABVD adapted to stage plus involved field radio therapy (IFRT) in all pts
Methods: Four hundred and one newly diagnosed pts. with HL Stages I-IV have been included (LH-05). All pts. received 3 ABVD and were evaluated with a PET-CT (PET-CT +3). Pts. with a negative PET-CT+3 were considered in CR and received no further therapy. Pts in partial response (PR) completed 6 ABVD and IFRT on PET-CT positive areas. Pts with less than PR received salvaje chemotherapy. Three hundred and seventy seven pts have been evaluated. With a median age of 35 yrs.and 38% age older than 59 years. Two hundred and twenty eight 860%9 presented with localized stage (I-IIA) and 144 (40%) presented with advanced stage (IIB-III-IV).

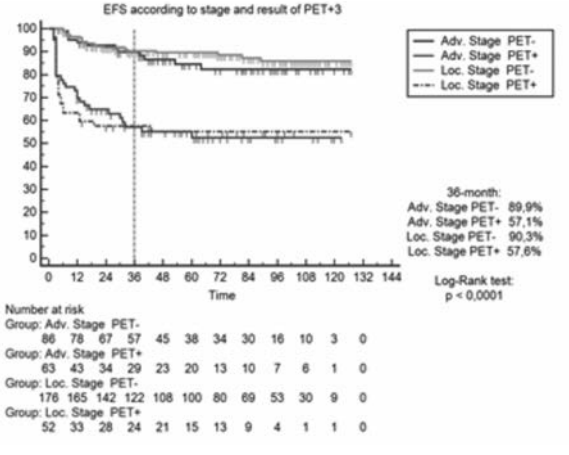

Figure 1.

Results: Of all pts. 260 (69%) achieved CR with negative PET-CT+3, 117 (31%) were PET-CT+3 positive, 101 pts. were in PR with chemo-sensitive disease and completed a total of 6 ABVD+IFRT in PET-CT positive areas. Eighty-two (81%) achieved CR. With a median follow up of 68 months the EFS and OS ***at 3 years*** is 80% and 96% respectively. Pts with negative PET-CT +3 had an EFS of 90% both for localized and advances stage, compared to 58% for pts. with positive PET-CT+3 and loc. stage and 5%for with a positive PET-TC+3 and adv. stage. We perform a multivariate analysis for EFS which included age, stage, IPS, bulky disease, extranodal areas and the result of the PET+3. This last parameter together with age were the only ones with statistical significance (p=0.001 and 0.046 respectively), finding stage at diagnosis not significant. When comparing the results LH-05 with our previous clinical trial (LH-96) there is no difference in EFS and OS at 36 months (83% *vs* 85% and 97 *vs* 96%) but in LH-05 only 30% received more than 3 cycles of ABVD and IFRT compared to 61% and 100% in LH-96. This reduces the exposure to chemo and radiotherapy.
Summary/Conclusions: With PET-CT adapted therapy for all stages of HL after 3 ABVD, 260 pts. received only 3 cycles of ABVD as initial therapy with an EFS and OS of 91% and 98% at 36 months. In the Cox regression model, PET-CT at completion of treatment was the most significant factor associated to EFS. Treatment with 3 cycles of ABVD can be adequate for pts. with negative PET-CT+3 regardless their stage at diagnosis

S109

OSTEONECROSIS AS A TREATMENT COMPLICATION IN HODGKIN LYMPHOMA PATIENTS: AN ANALYSIS OF THE GERMAN HODGKIN STUDY GROUP (GHSG)

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Background: Hodgkin Lymphoma (HL) is one of the best curable hematological malignancies. Therefore, a major focus for future improvements in the treatment of this disease will be a reduction of short- and long-term side effects. Osteonecrosis (ON) has been reported as an infrequent but sometimes debilitating late effect of HL therapy in a few case report series.
Aims: The aim of the present study is to provide a detailed analysis of ON as a late effect after multimodality treatment for HL.
Methods: A total of 12,083 patients treated within the GHSG trials HD10-15 and HD18 between 05/98 and 07/14 were evaluated. During the trial duration and prolonged follow-up, complications and late effects of trial participants are registered. Using a trial-database-aided search for ON and related symptoms as well as manual chart review, 66 incident cases of osteonecrosis were identified. Detailed information on patient characteristics, localizations, interventions and outcomes were collected. Risk factors for ON after HL therapy were analyzed by multivariate logistic regression.
Results: The cumulative incidence of ON was 0.16% [CI: 0.08-0.30] in early (favorable or unfavorable) stage HL (n=10) and 0.93% [CI: 0.71-1.21] in advanced stage HL (n=54). The majority of ON cases were male (75%). A total of 66 patients had a total of 140 ON. Most patients had more than one area affected (71%) and the most commonly affected location was the femoral head (73%). Most patients with available information needed surgical intervention (54%) and had continuing symptoms despite treatment (66%), including inability to walk and severe pain. Joint-saving interventions were attempted and successful in 6 of 8 patients. The first ON event occurred within the first 3 years after HL diagnosis in 83% of cases. Peak incidence was observed in the second year after diagnosis with 41% of cases occurring between 12 and 24 months after therapy. In a multivariate logistic regression including all patients, male gender (OR 2.1; CI: 1.2-3.7) and advanced stage (OR 3.9; CI: 2.3-6.9) were identified as risk factors for ON. Because ON was a very rare complication in early stage HL, the following results focused on survivors of advanced stage HL. The median cumulative prednisone dose in ON cases after advanced stage HL was 8,400mg (range: 3,920-10,800) *versus* 7,350mg (range: 0-16,800) in not affected patients. In a multivariate logistic regression model including only patients with advanced stage HL, young age (OR 0.7 for each additional 10 years of age; CI: 0.5-0.9) and a higher cumulative dose of prednisone during therapy (OR 1.3 for each additional 1gr; CI: 1.1-1.5) were identified as additional risk factors. Nodal pain after alcohol ingestion (p=0.78), radiotherapy (p=0.29), a large mediastinal tumor (p=0.13), international prognostic score (IPS) (p=0.09) and body-mass-index (BMI) (p=0.99) were evaluated as potential risk factors but did not significantly influence the risk for ON.
Summary/Conclusions: We provide the largest and most comprehensive analysis of ON after HL therapy performed so far. ON after HL therapy, despite being an infrequent event, often leads to significant disease burden in affected patients and sometimes has dramatic effects on mobility and quality of life. The described risk factors and peak incidence timeframe could be helpful in order to identify patients at high risk for ON. Early evaluation of these often young patients is recommended in case of symptoms suggestive of ON. This might help to identify affected patients early and attempt bone and joint saving interventions whenever possible. High prednisone doses were identified as a risk

factor for ON and future modifications of HL therapy leading to a decrease in the cumulative corticosteroid dose might be able to decrease the incidence of ON after HL therapy in the future.

Myeloproliferative neoplasms - Clinical 1

S110

OUTCOMES OF 121 PREGNANCIES IN PATIENTS WITH POLYCYTHEMIA VERA (PV)

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Background: Very limited data are available regarding pregnancy (preg) outcomes in patients with PV, less than 40 pregs being reported in the literature. Within the European LeukemiaNet we collected 121 pregs in 48 PV patients from 7 centers in France, Italy, Serbia, Romania, Switzerland and Germany.
Aims: Outcomes of pregancies in patients with polycythemia vera (PV).
Methods: Pregs were categorized in two groups. Group 1 consisted of pregs before the diagnosis of PV (n=39). Group 2 (n=82) included all pregs after the diagnosis of PV. Most patients in group 2 received low dose aspirin during preg and low molecular weight heparin after delivery until the 6th week postpartum. The target hematocrit during preg was 40% and therapeutic phlebotomy was performed, if needed. Iron supplementation was not advised during preg.
Results: Median age at diagnosis of PV was 29 yrs (range 18-40), median age at delivery 32 yrs (21-43). 13 out of 48 patients (27%) had high-risk PV due to severe thromboembolic complications either at the time of diagnosis or during follow up. These included 5 Budd Chiari syndromes, 4 portal vein thromboses, 2 *superior sagittal sinus thromboses*, 3 deep vein thromboses, and one pulmonary embolism. In group 2, a planned medical interruption was performed in 6/82 pregs due to patients´ choice. The outcome of another 6 pregs cannot be reported yet (still ongoing in 3 and missing in another 3). Thus, a total of 70 pregs are evaluable at time of abstract submission in group 2. In group 1 live birth was recorded in 19/39 pregs (49%), while 20 pregs were unsuccessful due to spontaneous abortion (n=9; 23%), stillbirth (n=8; 21%) or late fetal loss (n=3; 8%). In contrast, outcome was significantly better in group 2 with 54 live births out of 70 pregs (77%) [chi square, p=0,002]. In group 2 we observed 12 (17%) spontaneous abortions, one ectopic preg (1%) and 3 (4%) stillbirths. Interferon alpha was used in 12 high risk cases with 10 live births (83%) and 2 spontaneous abortions. Concerning maternal complications, the rate of severe thromboembolic events was similar between the two groups (group 1: one pulmonary embolism with cardiogenic shock (2,6%), group 2: two Budd Chiari syndromes in 3rd trimester (2,8%). There were no deaths. Of note, the bleeding rate was significantly higher in group 2 (8 minor and 3 major bleedings) which was probably due to the treatment with aspirin and low molecular weight heparin in this group while only one minor bleeding was observed in group 1 [chi square, p=0,036]. Most PV patients' hematocrit values spontaneously decreased during preg, thus phlebotomies were mainly performed during the first trimester. 6 twin pregs were managed in group 2, five of them resulted in live births, and one patient had a spontaneous abortion in the 22th week.
Summary/Conclusions: This is the largest series of PV pregs reported to date collected across the European LeukemiaNet network. The success rate of pregs was significantly better (49% *versus* 77%, respectively) for patients in whom the diagnosis of PV was known and appropriate management performed according to current guidelines. This high success rate of almost 80% is encouraging in this higher risk PV population. However, despite the use of aspirin and molecular weight heparin major thrombotic events still occur and an increased bleeding rate is observed.

S111

INCREASED RISK OF SECOND MALIGNANCIES IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS DIAGNOSED IN SWEDEN 1973-2009. A POPULATION-BASED COHORT STUDY OF 9,379 PATIENTS

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Background: The risk of developing acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) in patients with myeloproliferative neoplasms (MPNs) is well established. Less is known, however, about the risk of non-hematologic malignancies in patients with MPNs.

Aims: To assess the risk of developing a wide range of second malignancies in a large population-based study of MPN patients compared to matched controls.
Methods: All patients diagnosed with MPNs and reported to the Swedish Cancer Registry or the Inpatient Registry between 1973 and 2009 were included. Four controls matched by age, sex, and region of residence were randomly selected from the Register of Total Population. End of follow-up was December 31st 2010. Patients and controls were excluded if they had any malignancy prior to the MPN diagnosis or corresponding time for controls. We identified incident cases of cancer during follow-up by cross-linking to the Swedish Cancer Registry. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated using a Cox regression model. A landmark analysis was performed in order to reduce detection bias, where all patients with a new malignant diagnosis within one and two years respectively from the MPN diagnosis were excluded.
Results: In total, 9,379 MPN patients and 35,682 controls were identified. The MPN cohort included 4,502 males (48%) and 4,877 females (52%), and the median age at MPN diagnosis was 67.5 years. We found an overall significantly increased risk of non-hematologic cancer with an HR of 1.60 (95% CI 1.50-1.71). The relative risk of developing a second malignancy was similar across all MPN subtypes, men and women, and calendar periods of MPN diagnosis. The non-hematologic malignancies with the greatest observed risk increase included non-melanoma skin cancer (HR 2.92, 2.51-3.39), malignant melanoma (HR 1.85, 1.36-2.53), kidney cancer (HR 2.85, 2.01-4.03), brain cancer (HR 2.60, 1.76-3.86), and endocrine cancers including thyroid cancer HR 2.41, 1.59-3.64) (Table). MPN patients were also at a significantly increased risk of developing second malignancies of the lung, head and neck, pancreas, esophagus, and stomach compared to matched controls. The risk of developing a non-hematologic malignancy tended to increase with follow-up time after MPN diagnosis. The risk of developing a second hematologic malignancy was significantly increased, the HRs for lymphoproliferative malignancies (including acute lymphoblastic leukemia) were 2.62 (2.06-3.34) and for AML 47.04 (33.17-66.69). The relative risk of a second non-hematological and hematological malignancies in MPN patients remained similarly significantly elevated in the landmark analysis starting one and two years after the MPN diagnosis.

Table 1. Risk of different types of malignancies in patients with myeloproliferative neoplasms compared to matched controls. HR=hazard ratio, CI=confidence interval, ALL=acute lymphoblastic leukemia.

Type of malignancy	HR	95% CI
All non-hematologic cancer	1.60	1.50 - 1.71
Lung cancer	1.73	1.37 - 2.19
Non-melanoma skin cancer	2.92	2.51 - 3.39
Melanoma	1.85	1.36 - 2.53
Head and neck cancer	1.83	1.23 - 2.73
Breast cancer	1.35	1.09 - 1.67
Pancreas cancer	1.59	1.07 - 2.35
Esophagus and stomach cancer	1.71	1.26 - 2.32
Colon, rectum and anal cancer	1.13	0.94 – 1.36
Kidney cancer	2.85	2.01 - 4.03
Prostate cancer	1.26	1.08 - 1.48
Brain cancer	2.60	1.76 - 3.86
Endocrine cancer	2.41	1.59 - 3.64
Lymphoproliferative malignancies (including ALL)	2.62	2.06 - 3.34
Acute myeloid leukemia	47.04	33.17 - 66.69

Summary/Conclusions: This population-based study is, to our knowledge, the largest to date to support an increased risk of non-hematologic and hematologic malignancies in MPN patients. The reason behind the increased risk are not fully understood, but several possible factors exist; a genetic propensity to develop malignancies, acquired somatic mutations, an altered immune function and mutagenic cytoreductive treatments. Clinicians should be aware of the increased cancer risk and direct adequate attention to new symptoms in MPN patients.

S112

RUXOLITINIB PROVES SUPERIOR TO BEST AVAILABLE THERAPY IN PATIENTS WITH POLYCYTHEMIA VERA (PV) AND A NONPALPABLE SPLEEN: RESULTS FROM THE PHASE IIIB RESPONSE-2 STUDY

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Background: PV is a myeloproliferative neoplasm characterized by erythrocytosis, burdensome symptoms, and increased risk of thrombosis. A key treatment goal is to maintain hematocrit (HCT) control. In the phase 3 RESPONSE study, the JAK1/JAK2 inhibitor ruxolitinib was superior to best available therapy (BAT) in maintaining HCT control without phlebotomy (PBT), normalizing blood cell count, reducing spleen volume, and improving symptoms in HU-resistant/intolerant PV patients (pts) with splenomegaly.
Aims: RESPONSE-2 is an open-label phase 3b study comparing RUX with BAT in HU-resistant/intolerant PV pts without palpable splenomegaly.
Methods: HU-resistant/intolerant pts without palpable splenomegaly who required PBT for HCT control were randomized 1:1 to RUX 10 mg bid or BAT. The primary endpoint was the proportion of pts who achieved HCT control at wk 28 (defined as the absence of PBT eligibility [HCT >45% and ≥3 percentage points from baseline, or HCT >48%] from wk 8 to 28, with ≤1 PBT eligibility from wk 0 to 8). The key secondary endpoint was the proportion of pts who achieved complete hematologic remission (CHR) at wk 28. Other endpoints included patient-reported outcomes and safety. The MPN-SAF TSS was used to assess 10 PV-related symptoms, each on a scale of 0 (absent) to 10 (worst imaginable). BAT pts could cross over to RUX from wk 28. Primary analysis occurred when all pts reached wk 28 or discontinued.

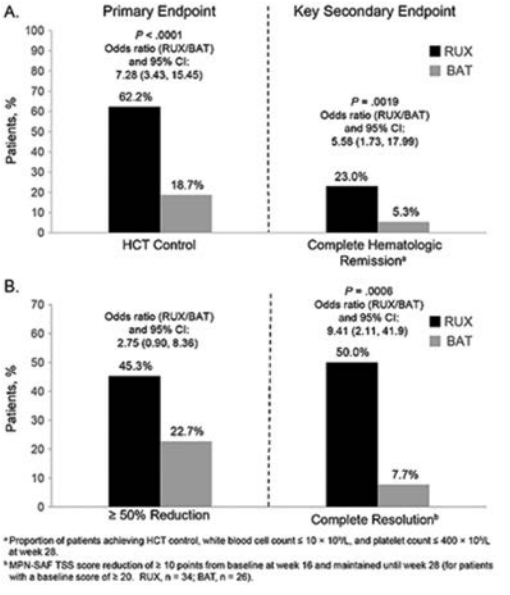

Figure. Percentage of Patients Achieving (A) the Primary and Key Secondary Endpoints and (B) Improvement in MPN-SAF TSS.

Results: 74 and 75 pts were randomized to RUX and BAT, respectively. The median time since PV diagnosis was 6.5 and 6.7 y; 28% and 24% of pts had a history of thromboembolic events; and 78% and 76% had ≥2 PBTs within 24 wk of screening, respectively. Overall, 29.5% of pts received >1 prior line of PV-directed therapy. By the cutoff date (29 Sep 2015), 2 (3%) RUX and 56 (75%) BAT pts had discontinued randomized treatment, of which 1.4% and 8.0% were due to adverse events (AEs), respectively; 51 (68%) pts crossed over to RUX. The median duration of exposure to RUX and BAT was 42 and 28 wk, respectively; median dose intensity for RUX was 20 mg/day. HCT control (primary endpoint) was achieved in 62% of RUX *vs* 19% of BAT pts (*P*<.0001); CHR (key secondary endpoint) was achieved in 23% and 5% of RUX and BAT pts, respectively (*P*=.0019; Fig A). At wk 28, 45% of RUX pts, *vs* 23% of BAT pts, had ≥50% improvement in MPN-SAF TSS; 50% *vs* 7.7% achieved complete symptom resolution (Fig B). RUX pts had improvements in individual symptoms; most symptoms worsened with BAT. Pruritus improved in RUX pts, but did not change in most BAT pts; 60% of RUX *vs* 5.3% of BAT pts had a much or very much improved condition in PGIC. Anemia or thrombocytopenia occurred in 16.2% and 2.7% of pts in the RUX arm *vs* 2.7% and 8.0% in the BAT arm. Corresponding grade 3/4 events were reported in 0% of RUX pts *vs* 1.3% and 4.0% of BAT pts, respectively. The most common nonhematologic AEs (>10% of pts) in either the RUX or BAT arm were headache (12.2%; 10.7%), constipation (10.8%; 5.3%), hypertension (10.8%; 4.0%), pruritus (10.8%; 20.0%), and weight increase (10.8%; 1.3%). All were grade 1/2, except hypertension (6.8%; 4.0%) and pruritus (0%; 2.7%). No deaths were reported in the RUX arm; 2 (2.7%) occurred in the BAT arm.
Summary/Conclusions: In this study, RUX was well tolerated and superior to BAT in controlling HCT without PBT, normalizing blood counts, and improving PV-related symptoms in pts with PV resistant to or intolerant of HU, thus extending the results from RESPONSE to pts without palpable splenomegaly.

S113

PHENOTYPIC DIFFERENCES AND TREATMENT RESPONSES IN MOLECULAR SUBGROUPS OF ESSENTIAL THROMBOCYTHEMIA FROM ANALYSIS OF THE PT1 COHORT

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Background: Somatically acquired mutations in JAK2, MPL or CALR are found in 85% of patients with essential thrombocythemia (ET) and 15% of patients are 'triple-negative' (TN). However, no prospective data exist on the disease characteristics or response to treatment of the different molecular subgroups of ET.
Aims: We aimed to characterise, in the prospective setting, whether baseline clinical characteristics, bone marrow histology, adverse outcomes, and response to treatment are influenced by mutation status in ET.
Methods: Patients aged 18 years or older with newly diagnosed or previously diagnosed ET were enrolled into low, intermediate or high-risk PT-1 studies depending on their risk of vascular complications. Median prospective follow-up duration was 36 months (range, 2-87 months). JAK2, MPL and CALR screening utilised PCR based methods. Clinical and laboratory features were compared between the four molecular subgroups. Time-to-event data was analyzed using Kaplan-Meier curves, log-rank analyses and Cox proportional hazards models. Response to treatment and drug dosage were analyzed using non-parametric regression and linear mixed effects modeling respectively.
Results: JAK2, MPL and CALR mutated ET represented 53%, 4% and 26% of patients and were mutually exclusive in most cases. 1% of patients harbored >1 mutation in these genes and analysis of such patients using individual hematopoietic colonies showed that JAK2, MPL or CALR mutations can be successively acquired within the same tumour subclone. Within the PT-1 cohort, CALR- and MPL-mutated subgroups affected males and females equally, in contrast to the female predominance observed in JAK2-mutated and TN subgroups wherein only 37% and 31% of patients respectively were male (p=0.0008, Chi-squared test). TN patients were significantly younger than any of the other molecular subgroups with a median age of 44 years (p<0.0001 for all comparisons, ANOVA). CALR-mutated patients were also significantly younger than JAK2- (p=0.003, ANOVA) and MPL-mutated (p=0.005, ANOVA) patients, with a median age of 54 years. *CALR*-mutated trephines had more prominent megakaryocyte atypia (increased cluster frequency, cluster size, tight clusters, paratrabecular megakaryocytes and fibrosis), and TN trephines had relatively milder megakaryocyte abnormalities. CALR-mutated ET patients presented with lower hemoglobin levels and total white cell counts, and higher platelet counts. Whilst the majority of venous thrombotic events occurred in the JAK2-mutated subgroup (21 of 27 events), CALR-mutated ET patients suffered increased rates of myelofibrotic transformation (hazard ratio 3.15 compared to the *JAK2*-mutated subgroup, p=0.03, Cox proportional hazards model correcting for age, gender and treatment received). No interaction was noted between treatment with either hydroxyurea or anagrelide and the incidence of these adverse outcomes. Patients with CALRins5 mutations presented with higher platelet counts than patients with CALRdel52 mutations but no other differences in disease features or outcome. Platelet control was equivalent between all molecular subgroups following treatment with either hydroxyurea plus aspirin or anagrelide plus aspirin in high-risk patients. However, CALR-mutated and MPL-mutated patients developed lower hemoglobin levels during anagrelide therapy. This observation was not due to differences in anagrelide dosage between molecular subgroups.
Summary/Conclusions: These results demonstrate that molecular testing identifies distinct biological subgroups in ET with different clinical outcomes and treatment responses.

S114

A GREATER UNDERLYING MUTATIONAL COMPLEXITY MAY CONTRIBUTE TO THE DIFFERENTIAL PROGNOSTIC IMPACT OF PHENOTYPIC-DRIVER MUTATION IN PRIMARY MYELOFIBROSIS

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Background: In primary myelofibrosis (PMF), *JAK2/CALR/MPL* mutational status is prognostically informative, with "triple-negative" (TN) patients (pts) displaying the worst survival (Rumi E, Blood 2014). Also, survival was significantly shorter in *CALR* type 2/like (Ty2) *vs* type 1/like (Ty1) mutated pts (Tefferi A, Blood 2014; Guglielmelli P, BCJ. 2015). A high-molecular risk category (HMR) identifies pts with reduced survival if harboring any one of *ASXL1, EZH2, IDH1/2* and *SRSF2* mutated genes (Vannucchi A, Leukemia 2013; Guglielmelli P, Leukemia 2014).
Aims: To analyze the molecular landscape of PMF pts categorized according to their driver mutation status and correlate with clinical endpoints and outcome.
Methods: All PMF pts (WHO-2008) provided informed consent. Established methods were used for JAK2, MPL and CALR mutations. NGS analysis with Ion Torrent platform was used to genotype 18 genes (referred as subclonal, SC): all coding sequence of c-KIT, TET2, RUNX1, NRAS, KRAS, DNAMT3A, IKZF1, EZH2, TP53; hot spot of IDH1/2 and SRSF2 and selected exons of CBL, IDH2, ASXL1, SF3B1, NFE2, SH2B3, U2AF1. The nonparametric Wilcoxon rank-sum test, Kaplan-Meier estimate of survival and log-rank test were used as appropriate.
Results: We analyzed 126 pts: 43 were *JAK2*+ (34.1%), 42 TN (33.3%) and 41 *CALR* mut (32.6%; 26 (63.4%) Ty1 and 15 (36.6%) Ty2. Overall, 17 pts (13.5%) progressed to AML. Death occurred in 69 pts (43.4%) after a median follow up of 3.8y. There was no difference between Ty1 and Ty2 for common hematologic and clinical variables; conversely, both Ty1 and Ty2 differed from *JAK2*+ counterpart for younger age, lower leukocyte and higher platelet counts; males were more represented among Ty1 pts that other genotypes. More deaths occurred among Ty2 (45.8%), *JAK2*+ (37.0%) and TN (72.7%) pts compared to Ty1 (20.0%) (P<.0001). Median survival of *CALR* Ty1 was 26.4y *vs* 8.59y for *CALR* Ty2, 10.3y for *JAK2*+ and 2.1 for TN (P<.0001). The corresponding hazard ratio, taking *CALR* Ty1 pts as reference, was 3.0 (95%CI, 1.2-7.6), 2.4 (95%CI, 1.0-5.7) and 11.1 (95%CI, 5.0-25.01) for *CALR* Ty2, *JAK2*+ and TN pts, respectively. HMR status was associated with shortened survival ((HR 3.3, 95%CI 1.9-5.8; P<.001) as it was the number of HMR mutations (HR 5.4, 95%CI 2.8-10.6; P<.001). Considering the individual mutations, only *EZH2, ASXL1* and *SRSF2* predicted for shorter survival (2.7y, 1.93y and 1.03y respectively; P=.001; P=.01 and P<.0001). Pts harboring any one of SC mutations were similarly distributed among groups: Ty1 61%, Ty2 67%, JAK2+ 77%, TN 74%; exceptions were *SRSF2* mutations (n=26) that were 0% Ty1, 6.7% Ty2, 14% JAK2+, 45% TN (P<.0001), and *SF3B1* that was mutated in 3 Ty2 (20%) *vs* 1 pts in both Ty1 (4%) and JAK2+ (2%) and no TN pt (P=.009). Conversely, the proportion of pts with ≥2 SC mutations was significantly higher in TN (52%) *vs* the others (Ty1 15%, Ty2 20%, JAK2+ 42%)(P=.02). The proportion of HMR was significantly higher in TN (67%) and JAK2+ (54%) pts compared with *CALR* subtypes (35% in Ty1, 27% in Ty2) (P=.01), as it was the percentage of pts having ≥2 HMR mutations (P=.006).
Summary/Conclusions: Overall, our findings support previous reports that the prognostic advantage of *CALR* mutation in PMF regards only Ty1 mutation; however, we did not find evidence that such difference might be ascribed to a greater molecular complexity of Ty2. Conversely, the dismal outcome of TN pts might be explained, at least in part, by occurrence of greater number of prognostically negative HMR mutations, particularly of *SRSF2* mutations.

CLL natural history and progression

S115

INTEGRATIVE ANALYSIS OF GENE MUTATIONS AND CHROMOSOMAL ABNORMALITIES BY NEXT-GENERATION SEQUENCING AND FISH IN HEMATOPOIETIC PROGENITORS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is a highly genetically heterogeneous disease. Although CLL has been traditionally considered as a mature B cell leukemia, few independent FISH and Next-Generation Sequencing (NGS) studies have shown that the genetic alterations may appear in CD34+ early hematopoietic progenitors. However, the presence of both chromosomal aberrations and gene mutations in CD34+ cells from the same patients has not been explored.
Aims: To analyze both chromosomal abnormalities and gene mutations in CD34+ hematopoietic progenitors from CLL patients to elucidate whether these genetic events appear previously during the B-cell differentiation process.
Methods: NGS and FISH studies were carried out in isolated CD19+ mature B cells from 57 CLL bone marrow samples to assess the presence of gene mutations and/or cytogenetic aberrations, respectively. Amplicon-based deep NGS (454 Life Sciences, Roche) was performed to study the mutational status of *TP53*, *NOTCH1*, *SF3B1*, *FBXW7*, *MYD88* and *XPO1*. The presence of genetic lesions on 11q, 12, 13q, 14q and 17p was studied by FISH. All cases with any mutations in CD19+ cells were also sequenced in their corresponding CD34+ early progenitors (coverage=1159X). Moreover, FISH was also performed in the CD34+ fraction of a subset of CLL patients with cytogenetic changes (n=9).
Results: NGS studies revealed a total of 28 mutations in 24 CLL patients (24/57, 42.1%). *NOTCH1* was the most frequently mutated gene (22.8%), followed by *XPO1* (8.8%), *SF3B1* (7%), *FBXW7* (5.3%), *TP53* (3.5%) and *MYD88* (1.8%). CLLs with mutations in any of these genes, except to *MYD88*, were associated with an unmutated *IGHV* status (*P*=0.001). Most of the patients showed the same mutations in their corresponding CD34+ cells (21/24, 87.5%). Comparing the mutations in both cell populations, two different patterns could be identified. In one group, the mutational burden remained similar between both cell fractions. Mutations in *NOTCH1* (11/13) and *XPO1* (5/5) were predominant in this group. By contrast, the other group showed a high decrease or even absence of these mutations in the CD34+ cells than the corresponding B lymphocytes being mutations in *TP53* (2/2), *FBXW7* (2/3) and *SF3B1* (3/4) the most recurrent in this group. FISH analysis revealed that 5 out of 9 CLL cases with cytogenetic alterations in B mature lymphocytes presented also the same aberrations on the CD34+ cells. Of note, *IGH* alterations did not appear in any of the CD34+ cases (0/2), whereas 11q- (2/2) and 13q- (3/5) were present in the CD34+ early progenitors. Gathering both techniques, 2 out of 9 CLLs had both chromosomal aberrations and mutations in their mature lymphocytes. Noteworthy, one of these cases showed an *IGH* alteration and *NOTCH1* mutation (altered cells=80% and 51%, respectively) on the CD19+ population. However, only *NOTCH1* mutation (31%) was found on the CD34+ cells. The other patient presented 13q- (93%), 11q- (64%) and *XPO1* mutation (39%) on the CD19+ B cells. The same alterations were also present in hematopoietic progenitors (72%, 49% and 31%, respectively).
Summary/Conclusions: Our data confirmed that chromosomal abnormalities and gene mutations are present not only in mature B lymphocytes, but also in the CD34+ early progenitors of CLL patients. Moreover, our findings shed light about the hierarchy existing in the appearance of these events: 11q-, 13q-, *NOTCH1* and *XPO1* mutations could be early hits on CLL pathogenesis, whereas *IGH* alterations, *TP53*, *SF3B1* and *FBXW7* mutations seemed to show in a later maturational level. **Funding:** PI15/01471; Junta de Castilla y León (MHS)

S116

DISTINCT HOMOTYPIC B-CELL RECEPTOR INTERACTIONS SHAPE THE OUTCOME OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Signaling initiated by antigen binding to the B-cell receptor immunoglobulin (BcR IG) is paramount for CLL development and evolution. Indeed, BcR signaling pathways are constitutively active in all CLL cases and recently, a cell-autonomous model of CLL cell activation has been proposed, whereby all CLL-derived BcR IGs could promote Ca^{2+} influx and NF-κB target gene transcription through the binding of a common internal epitope on the BcR IG.
Aims: We investigated the relevant molecular interactions underlying cell-autonomous signaling in CLL cases with distinct and opposite clinical outcome in order to understand the molecular basis of the clinical disease heterogeneity. We focused on CLL stereotyped subset #4 (IGHV4-34/IGKV2-30), a particularly indolent clinical subgroup with a characteristic anergic functional phenotype of the malignant cells, and stereotyped subset #2 (IGHV3-21/IGLV3-21), noted for a dismal prognosis, largely independent of p53 dysfunction.
Methods: We produced recombinant IG molecules from CLL leukemic cells. The BcR IGs were analyzed for self-association using analytical ultracentrifugation, and their structure was determined by x-ray crystallography. Recombinant BcRs were transfected in the RAG2/λ5/SLP65 triple knockout murine pre B-cell line (TKO cells) engineered to include a tamoxifen-inducible ERT2-SLP65 fusion protein. Addition of 4-hydroxy tamoxifen in the absence of exogenous antigen induces an increase in Ca^{2+} influx in case of autonomous signaling of the tested BcR.
Results: The BcR IGs of both subsets interact in the crystals in a geometry highly indicative of specific homotypic recognition, albeit with distinct features. In subset #4, the VH CDR3 binds to a conformational epitope spanning the Heavy chain (VH and CH1 domains), while in subset #2 the VL CDR1 and CDR2 loops contact a surface composed of residues from the Light chain (VL FR1 and the linker between the VL and CL domains). Using analytical ultracentrifugation, we confirmed that the contacts observed in the experimental crystal structures for both subsets were also occurring in solution. Interestingly, the association in solution of the receptors was markedly different for the two subsets with persistent binding in subset #4 cases with indolent disease, *versus* weaker interactions in subset #2 aggressive cases. For both subsets, using the TKO tamoxifen-inducible system and Ca^{2+} influx as a readout, we found that the observed interactions activate cell-autonomous intracellular signaling in the absence of exogenous antigen. Conversely, cells expressing epitope or paratope mutants of either receptor did not demonstrate significant variations in Ca^{2+} influx, confirming the relevance of the homotypic interactions revealed by the crystal structures.
Summary/Conclusions: We provide the first description to high resolution of a homotypic association process in BcRs that resembles antibody-antigen recognition and leads to intracellular signaling in CLL cells. BcR IGs from CLL cases with different prognosis bind homotypically via their combining sites to specific, diverse epitopes to initiate intracellular signalling. Our results suggest that the avidity of BcR self-recognition may directly underlie the clinical course of CLL, since tight, persistent binding was noted in cases with indolent disease whereas weaker interactions characterized the aggressive progressive cases.

S117

ESTABLISHMENT OF A PRE-CLINICAL *IN VIVO* PLATFORM SPANNING LOW-RISK TO HIGH-RISK CLL

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Background: CLL is the most common leukemia in the Western world. During CLL development, incipient CLL cells undergo a multistep mutational process, during which they acquire a set of genetic and/or epigenetic lesions, which ultimately result in the leukemic state. CLL-associated mutations can be classified into so-called driver mutations, which are essential for malignant growth and passenger mutations, which are functionally less significant. Recent work has revealed the identity of early, so-called *trunk lesions* and later-occurring *subclonal mutations* that are associated with disease progression and (chemo-)therapy resistance. Prominent examples for subclonal additional genetic events are *ATM* and *TP53* mutations, which are both associated with resistance against genotoxic therapies.
Aims: We aimed at overcoming one of the biggest hurdles in preclinical CLL research-the lack of mouse models that faithfully mimic the genetic events leading to CLL development.

Methods: We combined the well established Eμ-*TCL1* CLL mouse model with a floxed *Tp53*- or *Atm*-allele. We made use of the *Cd19*-Cre mouse to specifically delete *Tp53* or *Atm* in the B cell compartment. The resulting Eμ-*TCL1*;*Cd19*$^{Cre/wt}$;*Tp53*$^{fl/fl}$ (TCP) and Eμ-*TCL1*;*Cd19*$^{Cre/wt}$;*Atm*$^{fl/fl}$ (TCA) mice were carefully characterized and subsequently used for treatment strategy testing.
Results: The increase of leukemic burden in the blood was significantly faster in TCP and TCA mice, when compared to the Eμ-*TCL1*;*Cd19*$^{Cre/wt}$ (TC) control. Thrombocytopenia and splenomegaly developed faster in the *Atm*- and *Tp53*-deficient models. In agreement with this faster disease progression, TCP and TCA mice succumbed faster to the disease than TC control animals (31.4 weeks, 36.9 weeks and 49.9 weeks, respectively). Despite faster diesase progression in TCP and TCA mice, splenomegaly at time of death was comparable in all three cohorts. Histologically, the three lines were indistinguishable. Richter's transformation, as defined by loss of Cd5 expression, a strong increase in cell size and a high proliferative index, was occassionally observed in TCP and TC mice, but not in the TCA cohort. Interestingly, TCA mice showed a significantly better response to cyclophosphamide when compared to TCP animals, but TC mice did not. Looking more closely at disease kinetics, we observed a significant increase in disease aggressiveness in the TC group up to the level of TCP mice, suggesting the selection of an aggressive clone under insufficient therapy. Lastly, we used the TCA model to investigate whether pharmacological inhibition of PARP, a treatment strategy proven to be effective in homologous recombination-deficient tumors of other entities, might be beneficial in *ATM*-deficient CLL as well. Indeed, we could show a strong and significant survival benefit by daily application of Parp inhibitor in our mice, an effect not observed in the *Atm*-proficient controls.
Summary/Conclusions: We have, based on the Eμ-*TCL1* allele, generated mouse models mimicking the genetic alterations found in high-risk CLL patients, namely the loss of *TP53* and *ATM*. These novel mouse models might be used as valuable tools for the development of new treatment strategies for these high risk groups. We have done so by assessing the effectiveness of Parp inhibition specifically in the *Atm*-deficient setting in this mouse model of CLL.

S118

AKT ACTIVATION IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS PROMOTES TRANSFORMATION TOWARDS AGGRESSIVE RICHTER'S SYNDROME LYMPHOMA

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Background: Richter's syndrome (RS) is an aggressive transformation of Chronic Lymphocytic Leukemia (CLL) refractory to current therapies with dismal prognosis. RS arises from CLL cells independent of common DLBCL-mutations. Frequently mutations in p53, CDKN2 or cMyc genes are involved, but a significant proportion displays no specifically acquired driver mutation.
Aims: Here we aim to elucidate the role of AKT in transformation towards Richter´s syndrome in human patients and functionally address constitutively AKT-activation *in vivo*. Ultimately, we aim to develop mouse models that can be used to develop novel treatment options towards Richter's syndrome.
Methods: FFPE sections of Richter syndrome patients were used for pAKT staining. We have developed a mouse model allowing for Cre-activatable expression of a myristoylated AKT constitutive active allele from the *Rosa26* locus (*AKT-C*). We crossed that mice with Cd19^{Cre}- and *Cg1*^{Cre}-mediated B cell-specific *AKT-C* expression in the *Eμ:Tcl1* CLL mouse model.
Results: In biopsies revealed from patients with RS one third of cases showed enhanced AKT activation. Strikingly, Richter's transformation was recapitulated when AKT was genetically over-activated in Eμ-Tcl-1 mice, where AKT-C expressing cells developed a high-grade lymphoma phenotype leading to significantly decreased survival. AKT-C expression in TCL1 mouse model CLL cells furthermore induced morphology changes displaying features of aggressive lymphoma such as large transformed B-cell phenotype and frequent mitotic figures, enhanced proliferation indicated by KI67. The phenotype of transformed aggressive lymphoma could be revealed independently in both Cd19^{Cre}- and *Cg1*^{Cre}-mediated activation of AKT. Thus, *Eμ:Tcl1* transformed CLL cells act in concert with constitutively active AKT to develop RS. Noteworthy, *Cd19*^{Cre};*AKT-C* double transgenic mice fail to develop leukemia and lymphoma, indicating that CLL development in *Eμ:Tcl1* mice is a precondition to transformation. As for the downstream mechanisms of AKT-mediated transformation we identified GSK-3b inhibition and subsequent cMyc and Mcl-1 stabilization. This might confer contribute to resistance of RS cells against DNA-damaging and PI3K-inhibiting compounds in this novel model of Richter´s syndrome.
Summary/Conclusions: Collectively, we have generated the first murine Richter's Syndrome model providing novel mechanistic insights into the molecular understanding of Richter's transformation as an AKT-driven disease. The new model is amenable to model therapeutic strategies and to address the efficacy of synergistic treatment combinations in transformed lymphoma.

AML Biology - Novel mechanisms of leukemogenesis

S119

FREQUENT RECURRING MUTATIONS DISRUPT THE ANTI-PROLIFERATIVE FUNCTION OF ZBTB7A IN ACUTE MYELOID LEUKEMIA WITH T(8;21) TRANSLOCATION

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Background: The t(8;21)(q22;q22) translocation results in the *RUNX1/RUNX1T1* rearrangement and is one of the most frequent chromosomal aberrations in AML. However, *in vivo* models indicate the requirement of additional lesions, such as of *KIT* or *FLT3* mutations, for leukemogenesis as the RUNX1/RUNX1T1 fusion gene alone is not sufficient to induce leukemia.
Aims: We set out to identify cooperating mutations in AML patients with t(8;21) translocation.
Methods: Exome and targeted sequencing, DNA pull-down, transcription assay, immunofluorescence, retroviral transduction, gene expression profiling.

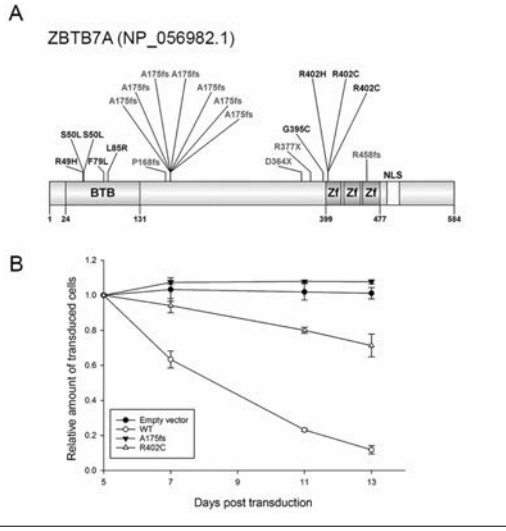

Figure A and Figure B.

Results: To identify additional cooperating mutations, we performed exome sequencing of matched diagnostic and remission samples from two AML patients with t(8;21) translocation. *ZBTB7A* was the only mutated gene identified in both patients. ZBTB7A is a member of the POZ/BTB and Krüppel (POK) transcription factor family. Previous studies suggested that ZBTB7A may act both as proto-oncogene and as tumor suppressor. Using targeted amplicon sequencing of *ZBTB7A* and 45 leukemia relevant genes, we screened 56 diagnostic AML t(8;21) samples. *ZBTB7A* mutations were identified in 13 of 56 patients (23%). Two recurring mutational hotspots (R402 and A175fs) in exon 2 were identified (Figure A). Variant allele frequency (VAF) ranged from 5.4-76.2% and 4 of 13 patients (31%) harbored two mutations of *ZBTB7A*. On the protein level, we confirmed the expression of a truncated ZBTB7A mutant (R377X) by Western blot for one patient with available material. In our cohort, *ZBTB7A* and *ASXL2* mutations occurred at similar frequencies and 5 of 13 patients carried mutations in both genes, however, there was no significant association of mutated *ZBTB7A* and mutations in *ASXL2* (Fisher's exact test, p=0.12) or any other recurrently mutated gene. On a functional level, the ZBTB7A mutants R402H, R402C, A175fs or R377X failed to repress a luciferase reporter containing ZBTB7A-binding elements derived from the ARF-promoter. Structural modeling revealed that Arginine 402 binds into the major groove of the DNA double helix and likely contributes to the affinity or sequence specificity of the DNA interaction of the zinc finger domain of ZBTB7A. Through DNA pull-down assays we confirmed impaired DNA binding of A175fs and R402H. The truncating ZBTB7A mutants A175fs and R377X showed altered cytoplasmic protein localization. In the t(8;21) translocation positive AML cell line Kasumi-1 retroviral expression of wild-type ZBTB7A inhibited cell growth, whereas this anti-proliferative effect was not observed upon expression of the A175fs ZBTB7A mutant. The R402C mutant expressing Kasumi1-cells showed a trend towards reduced cell growth, suggesting residual activity (Figure B). Based on this observation, we expressed *ZBTB7A* wild-type or its mutants together with the *RUNX1/RUNX1T1* fusion in lineage negative murine bone marrow cells and performed colony forming cell (CFC) assays. *ZBTB7A* expression led to a significant decrease in the number of colonies in primary CFC (87±12.6 *versus* 45±5.8, p<0.0001), while this effect was lost for both mutants A175fs and R402C. These findings support an oncogenic synergism between *RUNX1/RUNX1T1* and *ZBTB7A* mutations. We correlated *ZBTB7A* expression with clinical outcome in a large cohort of AML patients (GSE37642). Remarkably, in over 200 cytogenetically normal AML patients treated on clinical trial (NCT00266136), high expression of *ZBTB7A* was associated with a favorable outcome (p=0.0004), suggesting a relevance in AML beyond the t(8;21) subgroup.
Summary/Conclusions: In summary, we have identified *ZBTB7A* as one of the most frequently mutated genes in t(8;21) positive AML. Considering that *ZBTB7A* mutations result in loss of function, we suggest that *ZBTB7A* acts as a tumor suppressor in t(8;21) positive AML.

S120

HNRNP K: AN ONCOGENE AND TUMOR SUPPRESSOR, TWO DISCRETE PATHS TO AML THROUGH ON GENE

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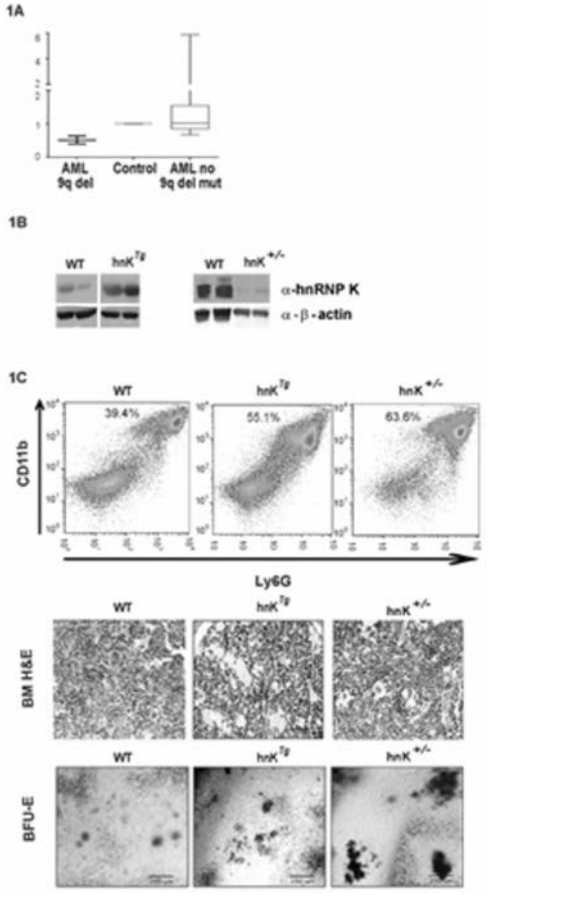
Background: We have recently demonstrated that *HNRNPK* is a critical haploinsufficient tumor suppressor residing at the 9q21.32 locus that is deleted in a subset of patients with AML (Gallardo et al, *Cancer Cell*, 2016). In this study, we generated a haploinsufficient *Hnrnpk* (*Hnrnpk*$^{+/-}$) mouse model that exhibited reduced survival and developed significant hematologic and myeloid malignancies. However, in addition to *HNRNPK* haploinsufficiency, we found that a large subset of AML patients without a 9q21.32 deletion, actually overexpress HNRNP K, resulting in poor prognoses. These data suggest that hnRNP K may have both tumor suppressive or oncogenic functions, depending on its expression levels.
Aims: This work will investigate the role of hnRNP K in AML, and to understand how *HNRNP K* behaves as an oncogene or as a tumor suppressor.
Methods: We analyzed copy number variations and aberrant expression of *HNRNPK* by FISH, qRT-PCR, and RPPA analyses in AML patients with and without 9q21.32 deletions. To directly examine the contribution of aberrant hnRNP K expression to malignant phenotypes, we generated two distinct cohorts of genetically engineered mice. One cohort was haploinsufficient for *Hnrnpk*, while the other specifically overexpressed hnRNP K in hematopoietic progenitors (*Hnrnpk*^{Tg}). Differences in survival, genomic stability, proliferation and differentiation potential, tumor formation, and molecular analyses were performed on hematological tissues using qRT-PCR, immunohistochemistry, colony formation assays, western blot analyses, and transplantation assays. Molecules of interest were further analyzed for interaction with hnRNP K via ChIP and RIP assays.
Results: Initial evaluation of *HNRNPK* expression levels in AML patients revealed that patients with the 9q21.32 deletion exhibited reduced hnRNP K levels, while many patients without this deletion had significant *HNRNPK* overexpression (Fig. 1A). This result was echoed in FISH analyses, as patients with the 9q21.32 deletion showed *HNRNPK* loss, while nearly 37% of AML (n=43) patients without the 9q deletion showed gene amplifications. In order to investigate the role of *HNRNP K* in AML, we generated both *hnRNP K*$^{+/-}$ and *hnRNP K*^{tg} mice. While these two mouse models have significant differences in hnRNP K expression compared to wild type (reduced *versus* overexpression), they surprisingly, had extremely similar phenotypes (Fig. 1B). Mice from each cohort suffered reduced survival, development of myeloid malignancies with high penetrance, genomic instability, enhancement in proliferation and differentiation potential in HSPCs, and the ability to generate myeloid hyperplasias following transplantation (Fig. 1C). Even though these opposite hnRNP K expression patterns result in extremely similar phenotypes, the molecular consequence of aberrant hnRNP K expression is facilitated through discrete molecular mechanisms. While hnRNP K haploinsufficiency directly decreased expression of anti-proliferation and differentiation genes likes *p21*, *C/EBPa*, and *C/EBPβ*, its over-

expression resulted in the direct activation of pro-growth genes like c-Myc. Given that hnRNP K expression is known to be tightly in all eukarotyic organisms, these data suggest that any alteration in hnRNP K expression may result in drastic cellular consequences.



Figures.

Summary/Conclusions: These data provide evidence that hnRNP K has the unique capacity to behave as an oncogene when overexpressed, or as a tumor suppressor when is its expression is reduced. In either case, these imbalances in hnRNP K expression are capable of directly contributing to the pathogenesis of AML.

S121

INTEGRATED ANALYSIS OF THE HUMAN HEMATOPOIETIC NON-CODING RNA LANDSCAPE REVEALS LNC-RNA STEM CELL SIGNATURE IN AML

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Background: Long non-coding RNAs (lncRNAs) and miRNAs have emerged as crucial regulators of gene expression, epigenetics and cell fate decisions.
Aims: We sought to establish a comprehensive resource for the exploration of non-coding RNAs across the human hematopoietic hierarchy, to understand their role in the pathogenesis of acute myeloid leukemia (AML).
Methods: Here we present an integrated quantitative and functional analysis of the miRNA-, lncRNA- and mRNA-transcriptome of purified human hematopoietic stem cells (HSCs) and their differentiated progenies, including granulocytes, monocytes, T-cells, NK-cells, B-cells, megakaryocytes and erythroid precursors, which we correlated with the ncRNA expression profile of 46 pediatric AML samples to establish a core lncRNA stem cell signature in AML.
Results: For each blood cell population, RNA from 5 healthy donors was hybridized onto three microarray platforms (Arraystar lncRNA V2.0, NCode™-miRNA/-ncRNA), yielding a coverage of more than 40,000 lncRNAs, 25,000 mRNAs and 900 miRNAs on 146 arrays. Compared to mRNAs, the mean expression level of lncRNAs was nearly 2-fold lower ($p<2.2x10^{-16}$), highlighting the challenge for RNA-Seq to provide adequate coverage of these rare transcripts. T-distributed stochastic neighbor embedding (t-SNE) on lncRNA and miRNA genes robustly structured the dataset into groups of samples that matched the input populations, demonstrating their unique ncRNA expression profiles. Self-organizing maps revealed clusters of lncRNAs and mRNAs that were coordinately expressed in HSCs and during lineage commitment. To demonstrate their functionality, we knocked down LINC00173 from the granulocytic core signature using two independent shRNA constructs, which resulted in diminished granulocytic *in vitro* differentiation (2-fold reduction in percentage of $CD66b^+/CD13^+$ granulocytes, $p\leq0.05$), myeloid colony-formation (1.5-2-fold, $p\leq0.05$) and nuclear lobulation (MGG-staining). Accordingly, CRISPR-mediated transcriptional repression of nuclear localized *LINC00173* (RNA-FISH validated by qRT-RNA of fractionated RNA) using dCas9-KRAB and three sgRNAs per locus reduced proliferation of myeloid NB4 cells (2-3-fold, $p\leq0.01$). Having established a global human hematopoietic lncRNA expression resource, we extended our findings to malignant hematopoiesis. Linear (PCA) and nonlinear (t-SNE) dimensionality reduction of 46 pediatric AML samples including Down syndrome AMKL, core-binding factor AMLs (inv[16] or t[8;21]) and MLL-rearranged leukemias mapped most samples to a space between HSCs and differentiated cells together with the myeloid progenitors. A subset of AML-samples mapped closely to healthy HSCs, including most of the DS-AMKLs and MLL-AMLs. We identified a stem-cell associated lncRNA signature that was absent in healthy differentiated progenies, but upregulated in AML samples. A mesoscale CRISPRi screening in AML cell lines suggested the importance of the lncRNA stem cell core signature for the maintenance of leukemic growth.
Summary/Conclusions: The definition of a core lncRNA stem cell signature in normal HSCs and AML blasts will guide our way towards an improved understanding of self-renewal and the underlying transcriptional program, which is hijacked during malignant transformation.

S122

THE IMPACT OF CELLULAR AGE ON LEUKEMIC TRANSFORMATION

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Background: Treatment of pediatric acute myeloid leukemia (AML) is largely extrapolated from adult trials despite age related disease heterogeneity. Indeed, during healthy ageing there are age related hemopoietic stem and progenitor (HSPC) cell proliferative and differentiative differences. We hypothesise that cellular age influences leukemic transformation, having implications for disease phenotype and response to therapy.
Aims: To determine if HSPC age affects oncogene-mediated leukemic transformation.
Methods: Murine HSPCs including $lin^-sca1^+cKit^+$ (LSK), common myeloid progenitor (CMP) and granulocyte-macrophage progenitor (GMP) cells from fetal liver (FL), 3 week(w), 10w and >60w adult bone marrow were isolated and transduced with the fusion oncogenes Nup98HoxA9 (NH9), AML1ETO (AE) and the mutant FLT3-ITD. Leukemic transformation was assessed *in vitro* by serial colony forming cell (CFC) assays, by growth in liquid culture and in stromal OP9 co-culture. *In vivo* leukemogenesis was assessed by transplantation of pre-leukemic LSKs transduced with NH9 after 2 rounds of colony formation (CFC2), into sublethally irradiated C57BL/6 mice. Gene expression was assessed by QPCR using fluidigm technology.
Results: NH9 transformed LSKs from all 4 ages *in vitro*. NH9 did not result in FL CMP and GMP transformation, while post-fetal (3w, 10w and >60w) CMPs and GMPs all transformed. Consistent with this, AE and FLT3-ITD transformed FL LSKs but not FL GMPs *in vitro*. This suggests that fetal transformation relies on specific features of the LSK that are absent from committed myeloid progenitors, independent from the oncogenic insult. To further assess age related transformation differences, NH9 transformed FL, 3w, 10w and >60w LSKs were assessed for AML *in vivo*. Older (10 and >60w) transformed cells led to a shorter latency with more penetrance than young (FL and 3w) transformed LSKs. Further, acute lymphoblastic leukemia (ALL) was observed in animals transplanted with FL and 3w transformed LSKs, but not 10w or >60w LSKs, suggesting young LSKs retain a lymphoid bias. As all NH9 transformed LSKs *in vitro* acquired self-renewal properties, the transformation differences observed *in vivo* may be cell autonomous or non-cell autonomous via the bone marrow microenvironment. Gene expression analysis of microenvironmental receptors and targets show the BMP pathway is upregulated in 10w and >60w LSK transformed cells suggesting the BMP pathway may have a role in age related transformation potential.
Summary/Conclusions: While LSKs from all 4 ages acquire self-renewal *in vitro*, progression to leukemia *in vivo* is slower and less penetrant in young compared to older transformed cells. This is in accordance with the lower incidence of AML in childhood compared to older adults. Activation of the BMP pathway has previously been associated with AML transformation, and our data suggest that the BMP pathway may play a role in the progression to AML specifically in older cells. The observation of ALL specifically in younger transformed LSKs suggests that young HSPCs retain lymphoid programs after myeloid oncogene expression, consistent with the higher incidence of ALL in childhood. Our findings support that age defined therapies are appropriate as HSPC age related biological differences are retained in leukemia and may impact not only disease phenotype but response to therapy.

Stem cell transplantation - Experimental

S123

NBEA: A NOVEL REGULATOR OF HEMATOPOIETIC STEM CELL *IN VIVO* REPOPULATION

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Background: Hematopoietic Stem Cells (HSCs) differentiate to generate all blood cells in a hierarchical manner. This has been exploited in HSC transplantation (HSCT) to treat hematologic diseases. Better understanding the molecular mechanisms implicated in HSCT should improve current transplant protocols. In our recently published work (Holmfeldt *et al.* J.Exp.Med. 2016), we have identified 17 new regulators of HSCT. Neurobeachin (*Nbea*) scored as a positive regulator of HSCT. NBEA protein is localized near the Golgi apparatus and regulates vesicular and protein trafficking. Patients with a disrupted NBEA allele present platelets with an abnormal morphology.
Aims: To understand the role of *Nbea* in HSCT and normal hematopoiesis.
Methods: *Nbea-/-* mice. Bone marrow (BM) transplant: Competitive congenic transplant into lethally irradiated recipients: Test cells (CD45.2+), competitor cells (CD45.1+), recipient mice (CD45.1+/CD45.2+). Flow Cytometry Analysis of Peripheral Blood (PB) and BM. RNA-microarray.
Results: Both adult BM Lineage-Sca-1+c-Kit+ (LSK) and E14.5 Fetal Liver (FL) LSK cells lentivirally transduced with shRNAs targeting *Nbea* (>70% transcript knockdown), and competitively transplanted within 40 hours post-infection into lethally irradiated recipient mice, suffer from a strong defect in short- and long-term repopulating activity, as measured by PB chimerism of recipient mice (>80% reduced). Thus, *Nbea* is required in LSK cells at different developmental stages for efficient HSCT. As transplanted *Nbea*-deficient cells are detected in the BM of recipients at 12 days post-transplantation, thus, a defect in homing or in LSK cell BM retention in recipients can be excluded. 4 months post-transplant, analysis of the major BM hematopoietic progenitor compartments (HPCs) of recipients showed a 50% decreased in the chimerism of *Nbea*-deficient cells in the HSC compartment. In contrast, *Nbea*-deficient cells were nearly absent from downstream progenitor compartments, suggesting a differentiation block. *Nbea-/-* mice die perinatally due to lack of synaptic transmissions There was no difference in the frequency of HPCs in the E14.5 FL of *Nbea-/-* and *Nbea+/+* littermates. *Nbea-/-* E14.5 FL-LSK cells showed no defect in engraftment ability compared to *Nbea+/+* controls, suggesting that embryonic plasticity has allowed for compensation. When CD45.2+ LSK cells derived from primary recipients of *Nbea-/-*E14.5 FL-LSK (CD45.2+) cells were isolated and transplanted into secondary recipients, a 25% reduction in repopulating activity was observed, supporting a role for *Nbea* in HSCT and HSC self-renewal. To understand how *Nbea* regulates HSC biology, we looked for differentially expressed genes using arrays on total RNA isolated from E14.5 FL-CD150+CD48- LSK cells sorted from both *Nbea+/+* and *Nbea-/-* embryos. Gene sets implicated in Snare interactions, vesicular transport, and Adherens junctions interactions were upregulated in *Nbea-/-* cells, supporting a role for *Nbea* in these processes.
Summary/Conclusions: *Nbea* is a *bona fide* regulator of HSC *in vivo* repopulation. Although Nbea is dispensable for native hematopoiesis, differences present in genes with a role in both vesicular trafficking and Adherens Junctions Interactions highlight the role of *Nbea* in these processes and how important they are for proper HSC interactions with the BM niche during HSCT.

S124

ACUTE MYELOID LEUKEMIA (AML) PATIENTS CURED AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION GENERATE TUMOR-SPECIFIC CYTOTOXIC ANTIBODIES THAT KILL AML BLASTS

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) can cure acute myeloid leukemia (AML) when the donor immune system generates a potent graft *versus* leukemia (GvL) response. While the role of T cells and NK cells in GvL immune responses has been established, the contribution of B cells to GvL responses is less clear. Using SEREX and other techniques, the presence of antibodies directed against established tumor antigens following allogeneic HSCT has been demonstrated, but because these antibodies were not obtained in monoclonal format, the function of these antibodies could not be analyzed.
Aims: To investigate the role of antibodies produced by donor-derived B cells in GvL responses.
Methods: We selected five patients with high-risk AML who remained disease-free for more than 5 years after allogeneic HSCT and thus have mounted a potent GvL response. From the peripheral blood of these patients we isolated memory B cells that we transduced with Bcl-6 and Bcl-xL, to establish antibody-producing clonal B cell lines. Blood was obtained 2 years after allogeneic HSCT. B cell lines were screened for the production of antibodies that specifically bound to surface antigens on AML cell lines and AML blasts isolated from patients in our clinic. Target identification was performed by immunoprecipitation and mass-spectometry.
Results: We identified patient derived clonal B cell lines producing antibodies that recognized antigens expressed on the cell surface of AML cells, but not on normal hematopoietic and non-hematopoietic cells. Antibodies were donor-derived, and a number of these antibodies recognized the U5 snRNP200 complex. The U5 snRNP200 complex is a component of the spliceosome that in normal cells is located in the nucleus but that is exposed on the cell membrane of AML cells. U5 snRNP200 complex-specific antibodies were specific for allogeneic HSCT recipients with AML, as they were found in in 4 out of 5 AML patients screened, but were not found in multiple myeloma patients who received an allogeneic HSCT or in healthy individuals. Strikingly, U5 snRNP200 complex-specific antibodies induced death of AML cells *in vitro*, and, in a human AML mouse model, *in vivo*. Cell death was induced in the absence of cytotoxic leukocytes or of complement, through a non-apoptotic process that depended on destabilization of the cytoskeleton as cell death could be blocked by incubation of the target cells with cytochalasin D, an actin polymerization inhibitor. Cytotoxicity of the U5 snRNP200 antibodies was present at 4°C and 37°C, suggesting that cell death was induced by a passive process. Indeed, interaction of the antibodies with their target cells did not induce a calcium flux. Cytotoxicity of the antibodies depended on the Fc region of the antibody, since recombinant U5 snRNP200 complex-specific antibodies with a defective Fc region were not cytotoxic.
Summary/Conclusions: Allogeneic HSCT recipients with robust donor anti-AML immunity generate antibodies against a component of the spliceosome, the U5 snRNP200 complex, that is expressed on the membrane of AML blasts. U5 snRNP200 antibodies are cytotoxic *in vivo* and *in vitro*, demonstrating the potency of the humoral immune system in tumor immunology.

S125

INCREASED REACTIVE OXYGEN SPECIES AND EXHAUSTION OF QUIESCENT CD34-POSITIVE BONE MARROW CELLS MAY CONTRIBUTE TO POOR GRAFT FUNCTION AFTER ALLOTRANSPLANT

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Background: Poor graft function is an important, often fatal complication following allogeneic hematopoietic stem cell transplantation. However, the underlying mechanism is unclear. In murine study, effective cross-talk between hematopoietic stem cells (HSCs) and the bone marrow microenvironment is important for normal hematopoiesis. Normal HSCs reside in a hypoxic bone marrow microenvironment protecting them from oxidative stress which would otherwise inhibit self-renewal resulting in bone marrow failure. We recently reported an impaired bone marrow endosteal and vascular microenvironment in subjects with poor graft function posttransplant. However, whether an increased level of reactive oxygen species (ROS) causes poor graft function following allotransplant remains to be elucidated.
Aims: The aim of our study is to determine whether quantitative and/or functional abnormalities of donor $CD34^+$ bone marrow cells result in poor graft function post-allotransplant.
Methods: In the current prospective case-pair study,apoptosis, cell-cycle state and colony forming capacity of $CD34^+$ bone marrow cells were quantified in subjects with poor or good graft function post-allotransplant. Moreover, expression of intracellular proteins including ROS, γ-H2AX, p53, phospho-p53, p21, phospho-p38, caspase-3 and caspase-9 were analyzed by flow cytometry. To study the effect of oxidative stress on post-allotransplant hematopoiesis, $CD34^+$ cells from subjects with good graft function were treated of H_2O_2 with and without N-acetyl-L-cysteine. Subsequently, the hematopoietic reconstituting activity of the donor $CD34^+$ bone marrow was evaluated using a NOD-$Prkdc^{scid}IL2rg^{null}$ xenograft assay by intra-bone marrow injection.
Results: Increased levels of ROS were identified in $CD34^+$ bone marrow cells in transplant recipients with poor graft function. This increase in ROS levels was associated with an elevated frequency of DNA double-strand breaks, apoptosis, exhaustion of quiescent $CD34^+$ cells and defective colony-forming unit plating efficiency, particularly in the $CD34^+CD38^-$ fraction. Up-regulated intracellular p53, phospho-p53, p21, caspase-3 and caspase-9 levels (but not phospho-p38) were detected in $CD34^+$ cells, particularly in the $CD34^+CD38^-$ fraction. To further study the potential role of ROS levels in post-transplant hematopoiesis, $CD34^+$ bone marrow cells from subjects with good graft function and normals were treated with H_2O_2. Treatment with H_2O_2 increased ROS levels, resulting in defective $CD34^+$ cells, an effect partially reversed by N-acetyl-L-cysteine. Moreover, $CD34^+$ bone marrow cells from the donors to subjects with poor or good graft function exhibited comparable hematopoietic reconstitution capacities in the xeno-transplanted NOD-$Prkdc^{scid}IL2rg^{null}$ mice.

Summary/Conclusions: Even if the transplanted donor's bone marrow CD34$^+$ cells are functionally normal pre-transplant, ROS-induced apoptosis may contribute to the exhaustion of CD34$^+$ bone marrow cells in subjects with poor graft function following allotransplant. This effect could be partially reversed by N-acetyl-L-cystine. Thus, our data suggest a potential approach for treating poor graft function post-allotransplant.

S126

MDSC (MYELOID-DERIVED SUPPRESSOR CELLS) DIFFERENTIATE UNDER GVHD (GRAFT-VERSUS-HOST DISEASE) CONDITIONS AND PREVENT GVHD BY INDUCING TYPE 2 T CELL RESPONSES

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Background: Graft-*versus*-host disease (GVHD) still represents the major complication after allogeneic bone marrow transplantation (BMT) since allogeneic transplant-derived T cells attack and destroy recipient tissues. Therefore, a prerequisite for GVHD prevention is the interference with T cell activation, proliferation and functions. Myeloid-derived suppressor cells (MDSCs) are a population of immature myeloid cells, which inhibit T cell functions by versatile mechanisms.
Aims: Therefore, we tested whether co-transplantation of *in vitro*-generated MDSCs prevents GVHD and elucidated the influence of GVHD conditions on the differentiation of MDSCs.
Methods: MDSCs were generated *in vitro* by culturing BM cells in the presence of GM-CSF and G-CSF. After 4 days more than 90% exhibited a Gr-1$^+$CD11b$^+$ MDSC phenotype. To test whether and how MDSCs prevent allogeneic T cell functions, we co-transplanted MDSCs into lethally irradiated recipient mice together with allogeneic BM and spleen cells or we cultivated MDSCs in medium containing serum derived from GVHD developing mice.
Results: *In vitro*-generated MDSCs efficiently prevented T cell proliferation *in vitro*. If co-transplanted with allogeneic BM and spleen cells, MDSCs inhibited clinical GVHD and GVHD-associated death and attenuated histological GVHD. Interestingly, MDSCs did not predominantly reduce the numbers of allogeneic T cells but induced type 2 allogeneic T cells characterized by the expression of Th2 specific cytokines and transcription factors. *In vitro*-generated MDSCs represent a mixed population of CD11b$^+$CD11c$^-$ and CD11b$^+$CD11c$^+$ cells. To further investigate the Th2-inducing capacity of MDSCs, we cultured MDSCs in medium supplemented with GVHD serum, which induced an expansion of CD11b$^+$CD11c$^+$ cells. CD11b$^+$CD11c$^+$ cells expressed MHC class II^{high}, Gr-1^{med}, CD301b^{pos} while CD11b$^+$CD11c$^-$ cells were MHC class II^{low}, Gr-1^{high} and CD301b^{neg}. CD301 expression is associated with a Th2 inducing phenotype in dendritic cells just as the presence of transcription factors IRF4 and Klf4, whose expression was also higher in CD11b$^+$CD11c$^+$ cells. Similarly, MDSCs co-injected with the allogeneic transplant exhibited an expansion of CD11b$^+$CD11c$^+$ cells in spleen and GVHD target organs preferentially in mice developing GVHD. Comparable to the *in vitro* conditions, these CD11b$^+$CD11c$^+$ cells acquired a phenotype similar to antigen-presenting cells characterized by CD80, CD86, CD40, and MHC class II expression. Isolation of the different MDSC subpopulations *in vitro* and subsequent incubation with allogeneic spleen cells showed that CD11b$^+$CD1c$^+$ MDSCs induce the secretion of Th2 cytokine IL-5, while CD11b$^+$CD11c$^-$ cells provoked only a weak Th2 response.
Summary/Conclusions: In summary, we could show that transplantation of MDSCs prevents GVHD after allogeneic BMT by inducing type 2 T cells. Th2 inducing capacity, however, was preferentially mediated by a subpopulation of MDSCs. Further studies will elucidate the molecular mechanisms of Th2 induction and will clarify whether MDSC differentiation and functions are dependent on the type of inflammatory environment.

S127

INDUCTION OF NAÏVE T CELLS TRANSITION INTO AN EFFECTIVE CD4+CD25+FOXP3+CD127- REGULATORY T CELLS, *IN VITRO*, AS A POTENTIAL ORDNANCE IN THE PREVENTION OF GRAFT-VERSUS-HOST DISEASE

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Background: Allogeneic haematopoietic stem cell transplantation (HSCT) has been used to treat some of haematological malignancies and inherited or acquired non-malignant diseases. Unfortunately, graft-*versus*-host disease (GVDH) occurred approximately 15% in transplant recipients and decreases the success of allogeneic HSCT. At present, no effective treatment can completely prevent the GVHD from allogeneic HSCT patients. CD4+CD25+FoxP3+ regulatory T cells (Tregs) have been shown to be important in maintaining immune homeostasis and preventing autoimmunity. However, 5% to 10% Tregs could be measured in human CD4+ T cells and few Tregs would convert to conventional activated T cells because of losing FoxP3 expression. It had been reported to correlate with the occurrence and severity of GVHD in some study.
Aims: In order to study the potential use of Treg cells for GVHD prevention, we attempt to evaluate the better method to increase the number of induced Treg cells (iTregs) in donor's PB and stabilize the FoxP3 in iTreg cells. To isolate the effective CD4+CD25+Foxp3+CD127-iTreg cells for clinical application and to establish a quick method to identify the functional iTreg cells is the study goal. Therefore, naïve T cells isolation for regulatory T cell induction is an important issue.
Methods: Mouse splenocytes were prepared from mouse spleen. Human PBSC were prepared from peripheral blood (PB) of healthy donors by Ficoll-Hypaque density gradient centrifugation. All T cells were isolated by negative selection; then CD4+naïve T cells were harvested. After that, the CD4+naïve T cells were activated by anti-CD3/CD28 beads in the presence of IL-2, TGF-β and retinoic acid (RA) containing RPMI1640 medium. The cells cultured with 3-day-nutrient-deprived medium (only 5% FBS), then refreshed the cells into the full nutrient supplement (10% FBS) for another 5 days. The harvested cells were analyzed by flow cytometry method with fluorescence-conjugated CD-antibodies, including CD4, CD25, CD127 and FoxP3. After trypanblue staining, the number of iTreg cell was counted by hemacytometer. The iTreg cells also harvested and the expression of functional marker genes in iTreg cells were analyzed via qPCR.
Results: In nutrient-deprived (5% FBS for 3 days in advance) culture system, we found the TGF-β triggered the mouse iTreg cells formation in a dose-dependent manner and increased iTreg cells formation efficiency under retinoic acid condition. Our data showed that we could induce the CD4+CD25+ Foxp3+CD127-iTreg cells more than 90%. The supplement of retinoic acid (0.1 and 0.5 nM) stabilized the *FoxP3*+ gene expression in iTregs during this incubation period; and the stability of FoxP3 expression and iTreg cell number could be maintained at least 12 days *in vitro*. The stability of iTreg cells is an important criterion for clinical use. Furthermore, we have analyzed the *FoxP3* gene and the bio-functional marker genes expression in iTreg cells to confirm the functional cells (Fig.1A). Based on these results, we consider the human T cells should be used. Therefore, we have investigated the human regulatory T cell induction. Human CD4+ naïve T cells were isolated from PB and activated via antibodies. The CD4+CD25+Foxp3+CD127-iTreg cells were induced to around 60~80% under IL-2 and TGF-β1 containing media, even without retinoic acid supplement (Fig.1B). It indicated we could harvest more iTreg cells under such condition. As we know, nTreg could suppress the induction of iTreg *in vivo*; further, we should remove the nTreg for improving the iTreg formation under cytokines supplement condition.

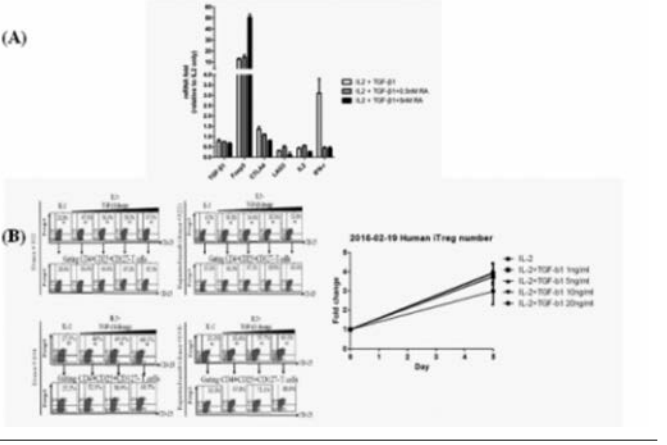

Figure 1. Functional iTreg cells induction. (A) The relative changes of gene expression in functional i Treg cells. (B) The human CD4+naïve T cells were isolated from human peripheral blood, and then activated with anti-CD3/CD28 antibodies. After TGF-β1 induction, the number of CD4+CD25+FoxP3+CD127- iTreg cells increased significantly in dose-dependent manner, even without retinoic acid supplement.

Summary/Conclusions: Our study showed that the combination of IL-2, TGF-β1 and RA in 3-day-nutrient-deprived medium could convert CD4+naïve T cells to CD4+CD25+FoxP3+CD127- iTreg cells and stabilize FoxP3 expression in the iTreg cells markedly. Further, we use the marker genes to clarify the biological function of iTregs *in vitro*. It may be to identify the functional iTreg cells quickly, after iTreg cells induction. Based on this method, we could harvest more and effective iTreg cells ready for use. It should be helpful for clinical application. GVHD mouse model will be established by using allogeneic HSCT to verify iTreg's function *in vivo*.

Myelodysplastic syndromes - Clinical

S128

ARCADE (20090160): A PHASE 3 RANDOMIZED PLACEBO-CONTROLLED DOUBLE-BLIND TRIAL OF DARBEPOETIN ALFA IN THE TREATMENT OF ANEMIA IN PATIENTS WITH LOW OR INTERMEDIATE-1 RISK MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Although erythropoiesis-stimulating agents (ESAs) are recommended in clinical guidelines to treat anemia in patients with lower-risk MDS, ESAs are not widely approved for this indication.
Aims: To evaluate the efficacy and safety of darbepoetin alfa (DAR) in IPSS low / intermediate-1 (int-1) risk MDS patients with anemia, in a phase 3, randomized, placebo(PBO)-controlled trial (EudraCT#2009-016522-14, NCT# 01362140).
Methods: Patients were enrolled from Dec 21, 2011 to Aug 27, 2014 in 9 European countries. Eligible patients had low/int-1 MDS, anemia [hemoglobin (Hgb) ≤10 g/dL], low transfusion burden (<4 transfusion units in each of 2 consecutive 8-week periods prior to randomization), no previous treatment with ESAs or biologic response modifiers, and endogenous erythropoietin (EPO) levels ≤500 mU/mL. Patients were randomized 2:1 to receive 24 weeks of subcutaneous DAR 500 µg or PBO every 3 weeks (Q3W), stratified by IPSS status (low or int-1). The dose was reduced if Hgb was >12.0 g/dL or if Hgb increased by >1.5 g/dL in 3 weeks without transfusion. Investigational product (IP) was discontinued and the patient entered follow-up if >3 dose reductions were needed. Key efficacy endpoints included (1) transfusion incidence from weeks 5-24 and (2) erythroid response (HI E) per IWG 2006 criteria, ie, ≥1.5 g/dL increase from baseline in Hgb with a mean rise of ≥1.5 g/dL for 8 weeks without transfusions. Results from the 24-week double-blind period are reported here; patients could then receive open-label DAR 500 µg Q3W for 48 weeks and were followed up for survival and progression to AML status for up to 3 years (ongoing).
Results: A total of 147 patients were randomized; 50.7% of patients were IPSS low risk and 49.3% were int 1 risk, median Hgb levels were 9.3 (Q1:8.8, Q3:9.7) g/dL, median EPO levels were 69 (Q1:36, Q3:158) mU/mL, rates of good / intermediate / poor IPSS karyotype were 91% / 9% / 0%, respectively, and% WHO classifications were RA:15%, RARS:14%, RCMD:44%, del5q:9%, RAEB-1:16%, and MDS U/unknown:2%. There were 146 (97 DAR, 49 PBO) patients in the primary analysis set. Baseline demographic and disease characteristics were generally similar between the two arms. Transfusion incidence from weeks 5-24 was significantly reduced with DAR *vs* PBO (DAR:36.1% *vs* PBO:59.2%, p=0.008). The proportion achieving HI-E was significantly increased with DAR *vs* PBO; DAR:14.7% (11 of 75 evaluable) *vs* PBO:0% (0 of 35 evaluable), p=0.016. All patients with HI-E (n=11) had a baseline serum EPO level <100 mU/mL. Adverse events (AEs) occurring ≥5% more frequently in the DAR arm than the PBO arm were fatigue, pyrexia, headache, and myalgia. Safety results from this trial were consistent with the previous DAR phase 2 MDS trial (Gabrilove BJH 2008, 142:379-393).

Table 1.

	Placebo (N=48), n (%)	Darbepoetin alfa (N=98), n (%)
AEs leading to IP discontinuation	2 (4.2)	3 (3.1)
Grade ≥3 / grade ≥4	13 (27.1) / 6 (12.5)	15 (15.3) / 5 (5.1)
Fatal AEs (none treatment-related)	2 (4.2)	1 (1.0)
Serious AEs	8 (16.7)	11 (11.2)
Treatment-related serious AEs	0 (0)	1 (1.0)
Venous thromboembolic events	0 (0)	1 (1.0)
Progression to AML	1 (2.2)	2 (2.1)

Summary/Conclusions: In this phase 3, randomized, double-blind, PBO-controlled trial in low/int-1 MDS patients with anemia, 24 weeks of DAR Q3W significantly reduced transfusions and increased rates of erythroid response with no new safety signals.

S129

CC-486 (ORAL AZACITIDINE) IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) WITH OR WITHOUT PRETREATMENT THROMBOCYTOPENIA

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Background: The estimated prevalence of thrombocytopenia in MDS is 40-65% (Kantarjian, *Cancer*, 2007). Severe thrombocytopenia (platelet count <30x10^9/L) is one of the worst prognostic factors for patients (pts) with MDS (Garcia-Manero, *Am J Hematol*, 2015). It has been suggested that pts with IPSS Low or Intermediate (Int)-1 risk MDS with severe thrombocytopenia should be considered higher-risk and receive disease-modifying therapy (DMT) at diagnosis (Gonzalez-Porras, *Cancer*, 2011). Like other DMTs, azacitidine (AZA) is associated with transient exacerbation of cytopenias during early treatment (Tx), which improve as Tx continues (Santini, *Eur J Haematol*, 2010). CC-486, the oral formulation of AZA, is in clinical development for Tx of hematologic malignancies, including MDS.
Aims: Evaluate the safety and efficacy of CC-486 monotherapy in pts with MDS with or without pretreatment thrombocytopenia.
Methods: Pts with MDS from three phase 1/2 CC-486 studies, two of which included dose-finding periods, were included in these analyses. CC-486 dosing regimens were: 120-600mg x7 days (d) (following a single SC AZA 75mg/m^2 x7d/28d cycle), or (with no initial SC AZA cycle) 300mg QD or 200mg BID x14d or 21d. All dosing regimens were administered in repeated 28d cycles. Thrombocytopenia was defined as platelet count ≤75x10^9/L. Overall Response included complete remission (CR), partial remission (PR), hematologic improvement (HI), and transfusion independence (TI) (Cheson, *Blood*, 2006).
Results: In all, 137 MDS pts participated in the three studies, including 72 pts with platelet counts ≤75x10^9/L (LowPlt group). Median age of all pts was 72 years (range 31-91). Median platelet count at baseline in the LowPlt group was 32.5x10^9/L (range 2-75) and in pts with platelet counts >75x10^9/L (HiPlt group) was 162x10^9/L (78-593). At entry, pts in the LowPlt cohort were less likely to have WHO-defined refractory anemia (15% *vs* 42% of pts in the HiPlt group) and more likely to have higher-risk (IPSS Int-2 or high) MDS (31% *vs* 12%, respectively). Rate of CR+PR was 22% in the LowPlt cohort and 7% in the HiPlt cohort, and Overall Response was the same in both groups (42%, **Table**). In the LowPlt group, 2 pts (14%) attained platelet TI and 17 pts (24%) attained HI-P. The most frequent Grade 3-4 treatment-emergent adverse events were hematological; anemia, neutropenia, febrile neutropenia, and thrombocytopenia occurred in 17%, 13%, 18%, and 21%, respectively, of pts in the LowPlt group, and in 26%, 26%, 14%, and 14% of pts in the HiPlt group. Grade 3-4 bleeding events were infrequent (n=9, 6.6%) and did not occur more often in the LowPlt group (GI [n=2], vaginal or cerebral hemorrhage [n=1 each]) than in the HiPlt group (GI [n=4] or ear hemorrhage [n=1]). Six deaths occurred during CC-486 treatment, 4 in the LowPlt group and 2 in the HiPlt group, including a 93-yr-old HiPlt pt who died of GI hemorrhage related to a duodenal ulcer.

Table 1.

Hematologic Response	LowPlt ≤75x10^9/L (n=72) n/N (%)	HiPlt >75x10^9/L (n=64)* n/N (%)	All patients (n=136)* n/N (%)
Overall Response Rate (CR + PR + Any HI + Any TI)	30/72 (42)	27/64 (42)	57/136 (42)
CR†	8/36 (22)	2/29 (7)	10/65 (15)
PR†	0/24	0/28	0/52
Any HI	24/72 (33)	21/62 (34)	45/134 (34)
HI-E	12/65 (18)	16/55 (29)	28/120 (23)
HI-P	17/72 (24)	6/13 (46)	23/85 (27)
HI-N	4/37 (11)	4/21 (19)	8/58 (14)
Any TI	9/32 (28)	14/33 (42)	23/65 (35)
RBC TI	7/27 (26)	14/33 (42)	21/60 (35)
Platelet TI	2/14 (14)	0/0	2/14 (14)

n = number of pts with response / N = number of pts eligible for response
*No response assessment available for 1 pt
†CR and PR were investigator-assessed for 19 patients (1 CR, 0 PR)

Summary/Conclusions: CC-486 was generally well tolerated in these MDS pts, even in the LowPlt group with median pretreatment platelet counts bordering on severe thrombocytopenia. Grade 3-4 bleeding events were uncommon overall and occurred less frequently in the LowPlt group. Death was more frequent in the LowPlt cohort, consistent with the poor prognosis associated with marked thrombocytopenia. However, more than 20% of pts in the LowPlt group attained CR during CC-486 therapy and pts were equally likely to attain HI whether in the LowPlt or HiPlt group.

S130

THROMBOPOIETIN RECEPTOR AGONIST ELTROMBOPAG FOR ADVANCED MDS OR AML AND SEVERE THROMBOCYTOPENIA: 12-WEEK, RANDOMIZED, PLACEBO-CONTROLLED, PHASE 2 ASPIRE STUDY

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Background: Thrombocytopenia is a serious life-threatening complication in patients with advanced myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Eltrombopag (EPAG), an oral thrombopoietin receptor agonist, is approved for treatment of chronic immune thrombocytopenia, hepatitis C virus-related thrombocytopenia, and severe aplastic anemia. Preclinical studies showed that EPAG has potential antileukemic effects (Roth M *et al. Blood*. 2012;120:386–94). A randomized, placebo-controlled, Phase 1/2 study in advanced MDS/AML demonstrated an acceptable safety profile at EPAG doses up to 300 mg daily, with no disease progression, and a trend toward improved platelet pharmacodynamics (Platzbecker U *et al. Lancet Haematol*. 2015;2: e417–26).
Aims: To determine the effect of EPAG on reducing the number of clinically relevant thrombocytopenic events (CRTE) in patients with MDS or AML who have Grade 4 thrombocytopenia (platelets <25 Gi/L).
Methods: After 8 weeks of open-label, dose-defining EPAG treatment (Part 1 [Mittelman M *et al. Blood*. 2012;120(21): Abs 3822]), adult patients with advanced MDS or AML were randomized 2:1 in a double-blind fashion (Part 2) to 12 weeks of supportive care plus once daily EPAG (dose range 50–300 mg over the course of treatment) or placebo. Patients were stratified by baseline platelet count (<10 Gi/L *vs* ≥10 Gi/L) and disease severity (International Prognostic Scoring System intermediate-2/high-risk MDS *versus* AML). Eligibility included 10–50% baseline bone marrow blasts and a baseline platelet count of <25 Gi/L. The primary endpoint was reduction in CRTEs (a composite of platelet counts <10 Gi/L, platelet transfusions, and Grade ≥3 World Health Organization [WHO] bleeding scale events) during Weeks 5–12. Secondary endpoints included safety, platelet transfusion independence, maximum WHO bleeding, hematologic improvement, and MDS progression (increased blast percentage or leukemic transformation).
Results: At baseline, age ranged from 29–85 years (mean 72.3; n=98) in the EPAG group *vs* 44–87 (mean 70.6; n=47) in the placebo group. More patients had abnormal (53% *vs* 34%) or poor (34% *vs* 17%) karyotypes in the EPAG group. Fewer EPAG patients had baseline platelets <10 Gi/L (34% *vs* 45%) than placebo patients. Other baseline characteristics were similar. Efficacy results are described in the Table. EPAG-treated patients showed significantly lower CRTE than placebo (54% *vs* 69%; odds ratio=0.202, *P*=0.03). Proportionately fewer patients on EPAG than placebo experienced independent reviewer-assessed disease progression (42% *vs* 60%). The most frequent adverse events in this study were petechiae (42% *vs* 23%), epistaxis (28% *vs* 23%), pyrexia (24% *vs* 28%), diarrhea (21% *vs* 17%), and fatigue (25% *vs* 9%) on EPAG *versus* placebo, respectively. More EPAG (31.6%) than placebo (14.9%) patients discontinued due to AEs. During Part 2, 35% of EPAG and 28% of placebo patients died (*P*=0.287). The primary cause of death for both groups was the disease under study (EPAG 27%; placebo 23%).

Table 1. Efficacy results of 12-week, randomized, double-blind treatment in the ASPIRE study.

	Eltrombopag (n=98)	Placebo (n=47)	Statistics
Patients with weekly CRTE during Weeks 5–12, % (95% CI)	54% (43%, 64%)	69% (57%, 80%)	OR=0.202 (95% CI: 0.05, 0.87); *P*=0.03
Platelet transfusion independence,* days (SD)	26.3 (21.5)	25.4 (19.7)	—
Maximum WHO bleeding grade, n (%)			
WHO Grade 3	5 (5%)	3 (7%)	—
WHO Grade 4	1 (1%)	3 (7%)	—
Any hematologic improvement,† n (%)	10 (10%)	4 (9%)	OR=1.26 (95% CI: 0.37, 4.30); *P*=0.718
Disease progression,‡ n (%)	41 (42%)	28 (60%)	—
Progression from MDS to AML, n (%)	31 (62%)	16 (73%)	—
Overall survival, months	4.24	4.60	HR=0.971 (95% CI: 0.631, 1.496)

AML, acute myeloid leukemia; CI, confidence interval; CRTE, clinically relevant thrombocytopenic event; HR, hazard ratio; MDS, myelodysplastic syndromes; OR, odds ratio; SD, standard deviation; WHO, World Health Organization.
*Platelet transfusion independence was defined as the duration of time between two consecutive platelet transfusions.
†Hematologic improvement was defined as an improvement of >20 Gi/L and 2× baseline for patients with baseline platelets of <20 Gi/L or ≥50 Gi/L and 2× baseline for patients with baseline platelets of ≥20 Gi/L; ≥100% increase and absolute increase >0.5 Gi/L over baseline in neutrophils; and hemoglobin increase by ≥1.5 g/dL over baseline and red blood cell transfusions (given for hemoglobin <9.0 g/dL) reduced by ≥2 per 4 weeks from baseline.
‡Protocol-specific, modified International Working Group response criteria for MDS were applied to define disease progression in patients with MDS or AML.

Summary/Conclusions: Treatment of patients with advanced MDS or AML with the thrombopoietin receptor agonist EPAG *versus* placebo for 12 weeks resulted in fewer CRTEs and did not result in an increase of disease progression. Rates of WHO Grade 3/4 bleeding were lower with EPAG. EPAG did not demonstrate overall hematologic improvement in this study. This study (NCT01440374) was sponsored by GlaxoSmithKline; however, as of March 2, 2015, eltrombopag became an asset of Novartis AG.

S131

LUSPATERCEPT INCREASES HEMOGLOBIN AND REDUCES TRANSFUSION BURDEN IN PATIENTS WITH LOW-INTERMEDIATE RISK MYELODYSPLASTIC SYNDROMES (MDS): LONG-TERM RESULTS FROM PHASE 2 PACE-MDS STUDY

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Background: Splicing factor mutations in MDS patients (pts), notably SF3B1, correlate with bone marrow ring sideroblasts (RS) and ineffective erythropoiesis (IE). Luspatercept (ACE-536), a fusion protein containing modified activin receptor type IIB, is being developed for treatment of anemia due to IE in MDS. Luspatercept binds GDF11 and other TGF-β superfamily ligands to promote late-stage erythroid differentiation and increase hemoglobin (Hgb) levels (Suragani R, Nat Med and Attie K, Am J Hematol, 2014).
Aims: This is an ongoing, phase 2, multicenter, open-label, dose-finding study followed by a long-term extension study to evaluate the effects of luspatercept in pts with low-intermediate risk MDS. Endpoints included erythroid response (IWG HI-E), RBC transfusion independence (RBC-TI, ≥8 weeks), duration of HI-E, pharmacodynamic and iron metabolism biomarkers, safety, and pt-reported QOL.
Methods: Inclusion criteria included age ≥18 yr, Hgb <10 g/dL (if <4U RBC/8 weeks), ESA refractory or EPO >500 U/L, no prior HMA, and no current lenalidomide or ESA. Luspatercept was administered SC every 3 wks for up to 5 doses in the base study, including 7 dose escalation cohorts (n=27 total, 0.125 to 1.75 mg/kg) and an expansion cohort (n=31, starting dose 1.0 mg/kg, max. 1.75 mg/kg). An amendment to the base study allows for additional pt subgroups (n=~50). A 2-year extension study (n=32 to date) is ongoing.
Results: Data (as of 31 Aug 2015) were available for 58 pts. Of these, 39 pts received ≥4U RBC/8 weeks (high transfusion burden, HTB) and 19 pts <4U RBC/8 weeks (low transfusion burden, LTB). Median age was 72 yr (range 27-90 yr) and 66% had prior ESA. Median RBC transfusion burden was 6U/8 weeks (range 4-18 units, HTB pts) and median Hgb was 8.7 g/dL (range 6.4-10.1 g/dL, LTB pts). 82% pts were RS+ (≥15% RS in bone marrow), including 19% RARS and 50% RCMD-RS.
LTB: IWG HI-E was achieved in 8/17 (47%) pts in the higher dose groups, compared with 0/2 in the lower dose groups in the base study. In the extension study, 9/13 (69%) pts achieved HI-E. Mean Hgb increase at Week 12 was 2.9 g/dL in HI-E responders and 1.3 g/dL in HI-E non-responders. 3/3 LTB pts who had 2U RBC/8 weeks at baseline became RBC-TI.
HTB: IWG HI-E was achieved in 16/32 (50%) in the higher dose groups (≥0.75mg/kg), compared with 2/7 (29%) in the lower dose groups, and 8/32 (25%) became RBC-TI in the base study. In the extension study, 13/19 (68%) achieved HI-E and 8/19 (42%) became RBC-TI (duration up to 50+ wks). IWG HI-E was achieved in 22/40 (55%) RS+ pts and 2/7 (29%) RS- pts. Response rates were 64% for EPO <200 U/L, 36% for EPO 200-500 U/L, 57% for ESA-naïve, and 46% for those refractory to prior ESA treatment. 18/30 (60%) pts with SF3B1 mutation responded; other potential predictors of response are being explored. Luspatercept was well tolerated, with 3 possibly related grade 3 adverse events of myalgia, worsening of general condition, and blast cell count increase. The most common possibly related AEs in the base study were diarrhea, fatigue (3 pts each), injection site erythema, bone pain, myalgia, and hypertension (2 pts each).
Summary/Conclusions: Luspatercept treatment was well tolerated and led to erythroid response in ~50% of low-intermediate risk MDS pts. Higher response rates were observed in RS+ and SF3B1mut pts, and responders included pts who were both ESA naïve and ESA-refractory, or with EPO up to 500 U/L. A Phase 3 study of luspatercept in regularly-transfused RS+ patients with lower-risk MDS according to IPSS-R is ongoing (MEDALIST study; clinicaltrials.gov NCT02631070).

S132

COMPARISON OF 1690 MDS PATIENTS FROM THE EUROPEAN LEUKEMIANET REGISTRY AND REFERENCE POPULATIONS – EVIDENCE FOR A SIGNIFICANT IMPAIRMENT IN QOL IN MDS AND DEFINITION OF PREDICTORS OF DIMINISHED QOL

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Background: Health-related quality of life (HRQoL) has been introduced by authorities, stakeholders and clinicians as a relevant patient reported outcome. Description of HRQoL-profile in MDS-patients is essential for individualized treatment algorithms.
Aims: To analyze the impact of MDS on HRQoL at initial diagnosis as compared to sex- and age-matched reference populations and to define predictors of impaired HRQoL.
Methods: A prospective European Registry (EUMDS) for newly diagnosed IPSS low-/int-1 MDS was initiated by the European Leukemianet. The EQ-5D descriptive system was introduced in EUMDS at initial diagnosis. EQ-5D data from MDS-patients were compared with sex- and age-matched reference populations published by the Euroquol Group.
Results: 1985 EUMDS patients from 140 hematology centers in 15 European countries diagnosed between December 2007 to February 2016 were included. 1690 (85.1%) patients (median age 74; range 18 – 95 yrs) and 61.5% male, completed both EQ-5D descriptive system and EQ VAS at the time of inclusion. Demographic characteristics of patients who completed EQ-5D questionnaire did not differ substantially from the whole cohort. Overall, the HRQoL-data in the sample analyzed were likely missing at random. A significant proportion of MDS-patients were characterized by moderate/severe restrictions in the dimensions mobility in 41%, self-care 13.3%, usual activities 36.1%, pain/discomfort 49.5%, and anxiety/depression 37.9%, respectively. Overall, Wilcoxon signed ranks tests showed that in MDS a significantly higher proportion of patients reported problems in mobility (41.0 *vs* 33.5%), usual activities (36.1 *vs* 26.0%) and anxiety/depression (37.9 *vs* 14.9%) than in European norms (p<0.001). Similarly, both EQ-5D index and VAS were more unfavorable in MDS as compared to references (0.74 *vs* 0.76; p<0.05) (69.6 *vs* 71.8; p<0.05) (paired t-tests). Most impairments in HRQoL were significantly correlated with advanced age, female sex, a higher comorbidity burden (defined by HCT-CI or MDS-CI), low Hb-level (<10 *vs* ≥10 g/dl) and red blood cell transfusion need (0 *vs* ≥1))(p<0.001). In contrast the impact of WHO-diagnosis and IPSS-R on HRQoL was only marginal. Comparisons with European reference norms revealed pronounced impairments in MDS-patients in usual activities and anxiety/depression in all age groups (<60 *vs* 60-75 *vs* 75+ yrs) (p<0.001 for each subgroup) and in both sexes (p<0.001). The impact of MDS on mobility was most prominent in male (p<0.001) and elderly persons (60-75 (p<0.01) *vs* 75+ yrs (p<0.001)). VAS was more often diminished in women (p<0.05) and at advanced age (75+ yrs (p<0.001)), as compared to reference cohorts. A hierarchical multilevel analysis was preformed to assess whether there was a significant difference in HRQoL between groups with different demographic and clinical parameters. Based on univariate analyses advanced age, female gender, pronounced comorbidities, low Hb and transfusion need were major determinants, both of EQ-5D index and VAS (p<.001). In multivariate modeling there was a significant loss in HRQoL for elderly (-0.082 for EQ-5D index and -7.33 for VAS for those 75+ yrs), for advanced comorbidities (-0.059; -6.21 for MDS-CI int/high), low Hb-levels (-0.053; -5.56), transfusion need (-0.045; -4.02) and female sex (-0.077; -3.64).
Summary/Conclusions: This study demonstrates profound restrictions in distinct dimensions of the EQ-5D in MDS-patients at diagnosis as compared with age- and sex-matched reference populations and defines major predictors of HRQoL. This analysis forms the basis to address specific needs of MDS-patients and provides data for benchmark analyses.

Red blood cells and iron

S133

GENETIC LOSS OF ERYTHROID TFR2 STRONGLY AFFECTS THE THALASSEMIC PHENOTYPE IN MICE

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Background: Transferrin receptor 2 (TFR2) is a multifaceted protein that in the liver activates hepcidin, the master regulator of iron homeostasis, while in erythroid cells is a component of the erythropoietin (EPO) receptor complex. We have shown that the lack of Tfr2 in the bone marrow (BM) increases the EPO sensitivity of erythroid cells and mimics iron-deficiency (Nai *et al.*, Blood 2015). β-thalassemias are recessive severe disorders of beta-globin gene, characterized by microcytic anemia, ineffective erythropoiesis, splenomegaly and secondary iron overload. Several studies demonstrated that iron restriction partially corrects the β-thalassemia phenotype in the *Hbb$^{th3/+}$* model of thalassemia intermedia.
Aims: In order to verify if the absence of Tfr2, mimicking iron-deficiency, could ameliorate the thalassemic phenotype we generated thalassemic animals with BM selective *Tfr2* inactivation.
Methods: We generated thalassemic mice with (*Hbb$^{th3/+}$*) or without BM *Tfr2* (*Tfr2^{BMKO}*/*Hbb$^{th3/+}$*) by transplanting BM cells from *Hbb$^{th3/+}$* or *Tfr2$^{-/-}$*/*Hbb$^{th3/+}$* mutants into lethally irradiated wild-type mice. Chimerism and hematological parameters were evaluated at 2 months. Mice were sacrificed 4 months after BM transplantation (BMT). At sacrifice blood was collected for hematological analysis, transferrin saturation (TS) and Epo levels; liver, spleen and BM cells were used for gene expression, tissue iron quantification, histology and flow cytometry analysis.
Results: Two months after BMT the engraftment of donor cells was 98-99%. *Tfr2^{BMKO}*/*Hbb$^{th3/+}$* and *Hbb$^{th3/+}$* animals appear viable and indistinguishable from one another. *Tfr2^{BMKO}*/*Hbb$^{th3/+}$* mice have greater red cells count and Hb levels as compared to *Hbb$^{th3/+}$* animals. On the contrary, 4 months after BMT *Tfr2^{BMKO}*/*Hbb$^{th3/+}$* mice are smaller and more anemic than *Hbb$^{th3/+}$*controls, have increased spleen size, TS and hepatic iron, but reduced spleen iron content. The alteration of body iron homeostasis is likely due to their lower hepcidin levels as compared with *Hbb$^{th3/+}$*mice. The ineffective erythropoiesis is exacerbated in the absence of Tfr2 and results in extramedullary spleen and liver erythropoiesis. Anemia is accompanied by increased Epo gene trascription in the kidney and by increased expression of Epo target genes, such as Epor, Bcl-xL and erythroferrone (Erfe) in BM.
Summary/Conclusions: Deleting erythroid Tfr2 in *Hbb$^{th3/+}$* animals has a transient beneficial effect, but is detrimental in long-term. Reasonably the loss of Tfr2 increases the sensitivity of thalassemic erythroid cells to Epo stimulation (as we demonstrated for normal cells), favouring erythroid reconstitution after BMT. However, when erythropoiesis reaches a steady state, increased stimulation becomes deleterious. We propose that this occurs through high and persistent production of erythroferrone, the soluble factor released by the erythroblasts in order to inhibit the hepcidin expression. This would increase iron absorption and release, causing iron overload, further impairing the thalassemic erythropoiesis and generating a "thalassemia major-like phenotype" in *Tfr2^{BMKO} Hbb$^{th3/+}$* mice. Further studies will better elucidate the mechanism of the observed biphasic effect on the phenotype and whether a time-controlled modulation of Tfr2 might improve β-thalassemia.

S134

HIGH SYSTEMIC IRON LEVEL IS A RISK FACTOR FOR CARDIOVASCULAR DISEASE

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Background: Iron accumulates in atherosclerotic lesions but its role in atherogenesis is still debated. In the "iron hypothesis" (1981), Sullivan proposed that iron is detrimental for the cardiovascular system, promoting atherosclerosis progression. So far, epidemiological data and studies in animal models have provided conflicting evidence regarding a role of excess iron in atherogenesis and cardiovascular disease.
Aims: In this study we aimed to investigate the role of iron overload in the development of atherosclerosis.
Methods: To this purpose, a mouse model of type IV Hereditary Hemochromatosis, in which the hepcidin/ferroportin regulatory circuitry is disrupted due

to a point mutation in the iron exporter ferroportin (FPN^{C326S}; Altamura *et al.*, Cell Metabolism 2014), was interbred with ApoE-null mice(ApoE-/-), that show increased susceptibility to atherosclerosis. Plaque formation was analyzed in ApoE-/-FPN$^{wt/C326S}$ mice at 6 and 12 months of age.

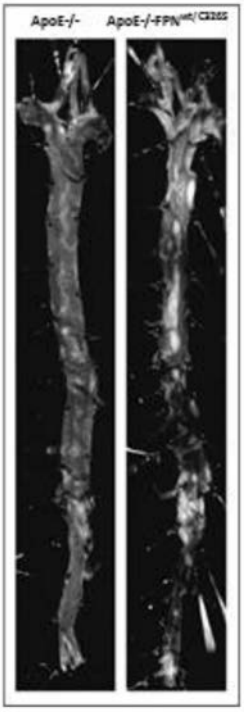

Figure 1.

Results: ApoE-/-FPN$^{wt/C326S}$ mice show high serum iron and cholesterol levels, as expected. Importantly, these mice show strongly increased lesion size and number at both 6 and 12 months of age compared to age-matched Apo-/- mice (6 months: 1.44±0.23 *vs* 5±0.53% aortic lesion area, P=0.0001; 12 months: 10.24±1.21 *vs* 20.44±2.69% aortic lesion area, P=0.0065). The atherosclerotic phenotype positively correlates with higher levels of circulating iron (12 months: 122.2±6.57 *vs* 337±19.22 mg iron/dl serum, P<0.0001) and oxidized LDLs (12 months: 2151±136.8 *vs* 3243±193.9 nmol oxLDL/ml serum, P=0.0002). Iron is deposited in the artery media layer, which correlates with vascular smooth muscle cell senescence, calcifications, vascular oxidative stress (12 months: 2.45±0.2 *vs* 4.33±0.32 nmol MDA/mg aorta protein, P=0.047) and DNA damage. We observe increased vascular permeability (6 months: 0.95±0.2 *vs* 3.11±0.51 mg aorta Evans Blue, P=0.0022), reduced nitric oxide availability and sustained activation and inflammation of the vascular endothelium (P<0.05). Within the atherosclerotic plaques, collagen deposition is reduced (P=0.0023) and lipid content is increased (P=0.0495), indicating enhanced plaque instability and faster disease progression. Plaque macrophages are significantly elevated and correlate with increased iron-induced CCL2 levels (12 months: 155.5±23.27 *vs* 305±38.39 pmol CCL2/ml serum, P=0.01), potentially contributing to increased lesion vulnerability (12 months: plaque vulnerability index P=0.0276). Ecocardiography in ApoE-/-FPN$^{wt/C326S}$ mice reveals an increased left ventricle mass and increased left ventricle area and volume in dyastole, plausibly as an attempt to compensate for increased arterial stiffness. Our mouse model further shows increased fibrinogen and pro-thrombin levels (P<0.05), suggesting a pro-thrombotic role for high systemic iron levels. Prolonged administration of a low-iron diet rescues the severe atherosclerotic phenotype, proving that iron is detrimental for this disease. Experiments are ongoing to test the effect of iron chelation therapy.
Summary/Conclusions: Our data suggest that high circulating iron levels strongly enhance the severity and promote the progression of atherosclerosis, indicating that systemic iron overload is a risk factor for atherosclerosis and predisposes to cardiovascular disease. Our findings have potential implications for those pathological conditions with elevated systemic iron levels, ranging from patients with hemochromatosis to anemic patients dependent on chronic blood transfusions, as well as for individuals subjected to intravenous iron administration (*e.g.* patients undergoing hemodialysis).

S135

THE PYRUVATE KINASE ACTIVATOR AG-348 IMPROVES MURINE B-THALASSEMIC ANEMIA AND CORRECTS INEFFECTIVE ERYTHROPOIESIS
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Background: β-thalassemias (β-thal) are worldwide distributed red cell disorders, characterized by ineffective erythropoiesis and reduced red blood cell (RBC) lifespan. Increased levels of reactive oxygen species (ROS) have been reported to play a key role in anemia of β thal, targeting both erythropoiesis and circulating RBCs. Pyruvate kinase (PK) is an important enzyme in the glycolytic pathway, responsible for conversion of phosphoenolpyruvate to pyruvate, with concomitant formation of the energy carrier adenosine triphosphate (ATP). As mature erythrocytes lack mitochondria, they rely almost exclusively on glycolysis to generate ATP, as well as the interlinked pentose phosphate pathway shunt to generate the reducing agent NADPH. The possible impact of PK activity on erythropoiesis is supported by evidence of ineffective erythropoiesis in human subjects with PK deficiency as well as mouse models of PK deficiency. The PK activator AG-348 has been evaluated in Phase I trials in healthy human subjects (NCT02149966) and is currently in Phase II studies in PK deficiency patients (NCT02476916). In the Phase I studies, AG-348 was shown to decrease levels of an upstream metabolite 2,3-diphosphoglycerate (2,3-DPG) and increased levels of ATP in whole blood, consistent with *in vivo* activation of PK.
Aims: To evaluate the impact of AG-348 on anemia and ineffective erythropoiesis in a mouse model of β thal intermedia.
Methods: Mouse strains C57B6/2J, as wildtype (WT) controls, and Hbb$^{th3/+}$, as a mouse model of β-thal intermedia, were used. Female mice aged between 2-3 months were treated with either vehicle or AG-348 at 50 mg/kg bid by oral gavage. Hematologic parameters, RBC indices, morphology, and reticulocyte count were evaluated at baseline, 7, 14, 21 days of treatment. Mouse erythropoiesis was studied using the CD44/TER119 gating strategy by FACS. ROS levels and the amount of Annexin-V+ cells were evaluated in erythroblast populations. Liver iron accumulation was evaluated by Pearl's staining and expression of liver hepcidin was measured by RT-PCR.
Results: In Hbb$^{3th/+}$ mice, 21 days of AG-348 treatment was associated with (i) a marked amelioration of anisopoykylocytosis; (ii) significantly increased Hb levels, MCV and MCH; (iii) a significant reduction in circulating erythroblasts (Es) and reticulocyte count; and (iv) reduction of ROS levels in circulating RBCs. In addition, AG-348 significantly decreased the amount of membrane precipitated α-globin chains and increased the amount of soluble Hb compared to the vehicle treated Hbb$^{3th/+}$ group. Consistent with these findings, we observed (i) a reduction of extramedullar erythropoiesis as indicated by both a decrease in spleen weight/mouse weight ratio and total Es (CD44TER119 Fsc high cells); (ii) a significant increase in pro-Es and basophilic Es, associated with reduction in orthochromatic Es; (iii) a reduction in ROS levels of Hbb$^{3th/+}$ Es and the amount of apoptotic orthochromatic Es compared to vehicle treated Hbb$^{3th/+}$ mice, suggesting an amelioration of β thal ineffective erythropoiesis. The amelioration of ineffective erythropoiesis was paralleled by a reduction in liver iron overload and up-regulation of hepcidin mRNA in liver from AG-348 treated Hbb$^{3th/+}$ mice.
Summary/Conclusions: Our data show that the PK activator AG-348 beneficially affects ineffective erythropoiesis in a mouse model of β thal and might represent a novel therapeutic tool in clinical management of anemia in β thalassemic syndromes.

S136

RAP-536 (MURINE ANALOG OF ACE-536/LUSPATERCEPT) INHIBITS SMAD2/3 SIGNALING AND PROMOTES ERYTHROID DIFFERENTIATION BY RESTORING GATA-1 FUNCTION IN A MURINE MODEL OF B-THALASSEMIA
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Background: Previously, we reported elevated Smad2/3 signaling in diseases characterized by ineffective erythropoiesis such as myelodysplastic syndromes (MDS) and β-thalassemia (Suragani *et al.* 2014). Luspatercept (modified ActRIIB receptor-Fc fusion protein), a Smad 2/3 ligand trap, has demonstrated efficacy in correcting ineffective erythropoiesis and the resulting anemia in murine models of MDS and β-thalassemia. RAP-536 (murine version of ACE-536/luspatercept) treatment also alleviated disease pathology in β-thalassemic mice.
Aims: In this study, we investigated the molecular mechanism of action of RAP-536 in a murine model of β-thalassemia.
Methods: Wildtype and β-thalassemic mice were used in this study. β-thalassemic mice (*Hbb$^{th3/+}$*) were administered a single bolus of vehicle (VEH) or RAP-536 (30 mg/kg, i.p) (N=2/group). At 16 hours, splenic basophilic erythroblasts (CD71^{+}Ter119^{+}FSC^{high}) were sorted by flow cytometry. RNA was isolated and subjected to genome wide transcriptome profiling using RNA sequencing analysis. Mouse erythroid leukemic (MEL) cells, primary fetal liver erythroid and β-thalassemic erythroid precursors were treated with GDF11 in the presence or absence of ACE-536 as described below.

Results: Transcriptome analysis of β-thalassemic erythroblasts identified 74 genes that were differentially expressed (absolute fold change >1.5, false discovery rate adjusted P value <0.05) in RAP-536 treated samples *vs* VEH treatment. To identify molecular mechanisms, we performed gene set enrichment analysis (GSEA) (Subramanian *et al.*, 2005) on these samples. The analysis depicted significant upregulation of target genes of multiple transcriptional regulators including GATA-1. Previously, multiple studies have established GATA-1 as a master transcriptional regulator of terminal erythroid differentiation. Further GSEA of GATA-1 activator signatures against RAP-536 treatment data revealed a significant up-regulation of 158 activated genes (Normalized Enrichment Score=2.7, P=0) involved in heme biosynthesis (such as Ppox, Fech, and Abcb10) and terminal erythroid differentiation (such as Fog1, Klf1 and Bcl-xl). ACE-536 is known to bind and inhibit Smad2/3 ligands such as GDF8, GDF11 and activin B but not activin A (Suragani et al 2014). Consistent with this data, treatment of MEL and fetal liver erythroid cells with GDF11 (50ng/mL) induced Smad2/3 phosphorylation and ACE-536 co-treatment inhibited the increase in pSmad2/3. In differentiating erythroid cells, GDF11 treatment displayed reduced nuclear GATA-1 protein levels by both western blotting and immunofluorescence studies. Additionally, reactive oxygen species (ROS) levels indicative of oxidative stress were elevated in MEL and primary fetal liver cells following GDF11 treatment. Consistent with the increase in ROS, we found decreased mitochondrial transmembrane potential (Δψm) indicative of unhealthy cells and increased caspase 3/7 activity in erythroid cells treated with GDF11. Importantly, treatment of erythroid cells with ACE-536 and GDF11 decreased ROS, restored Δψm and GATA-1 levels to control levels. Immunofluorescence studies demonstrated decreased GATA-1 levels in the nucleus of erythroid precursors from b-thalassemic cells compared to wild type mice. Treatment of these b-thalassemic erythroid precursors with ACE-536 prevented the decrease in GATA-1 levels.
Summary/Conclusions: Together, these data done with RAP-536 provide a potential mechanistic role for luspatercept as a novel treatment of β-thalassemia. By inhibiting pSmad2/3 signaling, RAP-536 treatment decreases ROS, prevents caspase 3/7 activation and GATA-1 cleavage. Thus by restoring GATA-1 availability and functional activity, RAP-536 treatment causes upregulation of genes involved in promoting terminal erythroid maturation, and consequently corrects anemia in b-thalassemia. Luspatercept currently completed phase 2 clinical trials and has initiated phase 3 studies in patients with MDS and b-thalassemia.

Thrombosis

S137

ETHNICITY AND THE EPIDEMIOLOGY OF VENOUS THROMBOEMBOLISM: A POPULATION-BASED STUDY

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Background: The epidemiology of Venous thromboembolism (VTE) has been largely studied in White populations, with much less known among non-Caucasians. Differences may exist in the biological predisposition to VTE but little is known about the epidemiology of VTE among immigrants or in relation to ethnicity.
Aims: To generate estimates of the incidence of VTE in immigrants to Ontario, Canada, grouped by their geographical region of origin as a proxy for ethnicity, and estimate risk ratios (RR) for different ethnic groups compared to Whites.
Methods: We conducted a population-based retrospective matched cohort study using linked health care and administrative databases in Ontario, Canada. These datasets were linked using unique, encoded identifiers and analyzed at the Institute for Clinical Evaluative Sciences (ICES) according to a pre-specified protocol that was approved by the research ethics board at Sunnybrook Health Sciences Centre (Toronto, Canada). We identified patients who immigrated to Ontario between Jan. 1, 2000 and Dec. 31, 2010. A non-immigrant comparison cohort was matched based on age, sex and place of residence. The main study exposure was an individual's ethnicity based on world geographical region of origin using a previously validated algorithm. Patients were divided in the following ethnic groups: 1) White, 2) Black, 3) Latin American, 4) Arab, 5) East Asian (including Chinese, Japanese, Korean, Filipino and other South East Asian), 6) South Asian, and 7) West Asian. Main study outcome was the occurrence of a first VTE. Secondary outcomes included deep vein thrombosis (DVT) and pulmonary embolism (PE). Patients were followed until the occurrence of an outcome, death, emigration from the province or end of follow up period (Dec. 31st, 2014). We calculated age-and sex specific incidence rates (IR) per 1000 person-years (PY). RR were calculated using Poisson regression models.
Results: We included 1,195,791 people in the immigrant and non-immigrant cohorts respectively, with a total period of observation >17,000,000 PY. Overall, the incidence rate for any VTE was 1.25 per 1000 PY (95% CI 1.23 to 1.27). For DVT the IR was 0.97 per 1000 PY (95% CI 0.96 to 0.99) and for PE it was 0.36 per 1000 PY (95% CI 0.35 to 0.37). The IR for VTE was lower among immigrants (0.87 per 1000 PY; 95% CI 0.85 to 0.89) than non-immigrants (1.59 per 1000 PY; 95% CI 1.56 to 1.61). Compared to white immigrants, the age-specific RR were consistently lower for both men and women of East Asian (RR 0.19-0.33) and South Asian (RR 0.29-0.65) ethnicity across all age groups. For patients of West Asian and Arab ethnicity the age-specific RR were also generally lower except for patients <30 or >80 years old. IR for immigrants of Black and Latin American ethnicity were no different across most age groups. RR for Black women less than 40 years old were higher compared to White immigrants. Among immigrants of Black, Arab and South Asian ethnicity who developed a VTE there was a higher prevalence of a previous delivery or C-section compared to White immigrants.
Summary/Conclusions: Incidence of VTE is lower among immigrants compared to non-immigrants in Ontario. Among immigrants, those of East and South Asian ethnicity have a lower risk of VTE compared to whites. *This study was supported by the Institute for Clinical Evaluative Sciences (ICES), which is funded by an annual grant from the Ontario Ministry of Health and Long-Term Care (MOHLTC). The opinions, results and conclusions reported in this paper are those of the authors and are independent from the funding sources. No endorsement by ICES or the Ontario MOHLTC is intended or should be inferred. AL-L was supported for this study by a grant from the Academic Medical Organization of South Western Ontario Opportunities fund and by the ICES Western Faculty Scholars program.*

S138

DECODING THE RISK OF THROMBOEMBOLIC EVENTS IN LYMPHOMA PATIENTS

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Background: Considering data about increased incidence of thrombosis in lymphoma patients and the impact of thrombosis on the survival of lymphoma patients, the main question was "Which lymphoma patients are candidates for thromboprophylaxis after establishing the diagnosis of lymphoma and during chemotherapy?" Owing to risk of thrombocytopenia due to disease or chemotherapy, patients with hematologic malignancies are often excluded from thromboprophylaxis and those with nonhematologic malignancies are preferred.

Aims: The aim of this study was to develop the prognostic risk score based on individual clinical and laboratory parameters that would allow physicians to designate patients at risk for thromboembolic event.
Methods: We developed prognostic Thrombosis Lymphoma – *ThroLy* score based on the study population including 1820 lymphoma patients, who received at least one cycle of chemotherapy. The study population was divided based on a split-sample random method into the model developing and validation cohorts. The model was developed using data from a derivation, and further assessed in the validation cohort.
Results: 99 patients (5.4%) developed thromboembolic events. The variables independently associated with risk of thromboembolism were: previous venous and/or arterial events, mediastinum involvement, BMI>30 kg/m^2, reduced mobility, extranodal localization, development of neutropenia and hemoglobin level <100g/L. Based on the risk model score the population was divided into the following risk categories: low (score 0-1), intermediate (score 2-3), and high (score >3). For patients in the derivation cohort classified as *at risk* for TE (score >1), the model produced a negative predictive value (probability of not experiencing TE in patients designated low risk) of 98.5%, and the positive predictive value (probability of TE occurring in patient designated at risk) of 25.1%. The sensitivity (probability of being classified as at risk in patients experiencing TE) was 75.4%, and the specificity (probability of being classified as low risk in patients not experiencing TE) was 87.5%. Interestingly, a high-risk score ≥4 had a positive predictive value (probability of TE occurring in patient designated at high risk) of 65.2%. The risk model was then applied to the validation cohort (n=584) in which 34 patients (5.8%) developed TE. Similarly, in the validation cohort, the negative predictive value was 97.6%; the positive predictive value was 28.9%; the sensitivity was 64.7%; and the specificity was 90.2%.
Summary/Conclusions: ThroLy score is more specific for lymphoma patients than any other available score targeting cancer patients. Moreover, it is dynamic, can be changed during the different phases of therapy, does not require non-routine laboratory analysis and is not limited to hospitalized or outpatient settings.

S139

MAJOR BLEEDING IN PATIENTS ON TREATMENT WITH NOAC OR VKA IN REAL-LIFE: CLINICAL PRESENTATION, MANAGEMENT AND OUTCOME

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Background: Limited data are available on major bleeding (MB) occurring during treatment with vitamin K (VKAs) or non-vitamin K antagonist oral anticoagulants (NOACs) outside clinical trials.
Aims: The aim of this study was to compare clinical presentation, management and outcome of MB in patients on treatment with VKA or NOAC in real-life.
Methods: Patients hospitalized for MB while on treatment with VKAs or NOACs were included in a multicenter study to compare clinical presentation, management and outcome of bleeding. The primary study outcome was in-hospital death.
Results: As for September 2015, 806 patients with MB were included in the study, 76% on VKAs and 24% on NOACs. MB was an intracranial hemorrhage in 51% and 21% patients on VKAs or NOACs, respectively (Odds Ratio [OR] 3.79; 95% confidence interval [CI] 2.59-5.54) a gastrointestinal bleeding in 46% and 25% patients on NOACs and VKAs, respectively (OR 2.62; 95% CI 1.87-3.68). In-hospital death occurred in 134 patients (17%), 19% of VKA and 10% of NOAC patients (OR 2.08; 95% CI 1.24-3.49, p=0.005). The rate of in-hospital death was similar in NOAC and VKA patients with intracranial hemorrhage (27% and 26% respectively; OR 1.02, 95% CI 0.49-2.12) and gastrointestinal bleeding (7% and 11%; OR 0.62, 95% CI 0.23-1.64) and lower in NOAC than VKA patients with other MBs (3% and 11%; OR 0.39, 95% CI 0.11-0.39).
Summary/Conclusions: Among patients hospitalized for MB while on treatment with anticoagulants, patients on NOACs are less frequently admitted for intracranial hemorrhage and more frequently for gastrointestinal bleeding as compared with patients treated with VKA. Mortality is lower in patients with MBs on NOACs than VKAs although this finding varies across the different types of MBs.

S140

PODOPLANIN EXPRESSION AND INTRAVASCULAR PLATELET AGGREGATES: THE MISSING LINK BETWEEN CANCER AND THROMBOSIS IN PRIMARY MALIGNANT BRAIN TUMORS

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Background: Venous thromboembolism (VTE) is a common clinical problem in patients with primary malignant brain tumors, and underlying mechanisms are unclear.
Aims: In a prospective observational study, we explored the association of podoplanin, a sialomucin-like glycoprotein that has the ability to induce blood platelet activation and aggregation, with VTE in primary malignant brain tumors. Furthermore, we investigated the ability of primary human glioblastoma cells, isolated from a patient with glioblastoma and thrombotic complications, to activate human platelets *in-vitro*.
Methods: Immunohistochemical (IHC) staining against podoplanin and platelet surface protein CD61 was performed in brain tumor specimens of 213 adult patients (mostly high-grade gliomas [89%]) included in the Vienna Cancer and Thrombosis Study (CATS), a prospective observational cohort study of patients with newly diagnosed cancer or progressive disease. Primary endpoint was symptomatic VTE. *In-vitro*, co-incubation experiments were performed using primary glioblastoma cells isolated from a patient included in CATS who had developed pulmonary embolism (PE) during follow up and whose tumor stained positive for podoplanin in IHC. Activation and aggregation of human platelets upon co-incubation with different concentrations of cancer cells was investigated by light transmission aggregometry and macro- and microscopical visualization.
Results: During 2-year-follow-up, 29 (13.6%) patients developed VTE. In total, 151 (70.9%) tumor specimens stained positive for podoplanin (33 high expression, 47 medium expression, 71 low expression). Patients with podoplanin positive tumors had a lower blood platelet count (Median [25th-75th percentile], G/l: 227 [186-285] *vs* 286 [241-355]; p<0.001) and higher D-dimer levels (mg/ml: 0.85 [0.46-1.92] *vs* 0.42 [0.23-0.79]; p<0.001). Increasing podoplanin-staining intensity was associated with increasing levels of CD61-positive intravascular platelet aggregates in tumor specimens (p<0.001). In Cox regression analysis, high podoplanin expression was associated with increased risk of VTE (hazard ratio [HR] for high *vs* no podoplanin expression: 5.75, 95% confidence interval [CI]: 1.71-19.27; p=0.005). This association was independent of age, sex and tumor grade (HR 5.71, 95%CI: 1.52-21.26; p=0.010). Figure 1 shows cumulative incidence curves of VTE according to podoplanin expression levels. Podoplanin-positive glioblastoma cells induced platelet aggregation, measured by light transmission aggregometry, in a dose-dependent manner, which was not observed in a podoplanin-negative control glioblastoma cell line. Podoplanin-positive glioblastoma cells also induced marked macro- and microscopically visible blood coagulation upon co-incubation with whole blood from a healthy donor, while this was not observed for podoplanin-negative control cells.

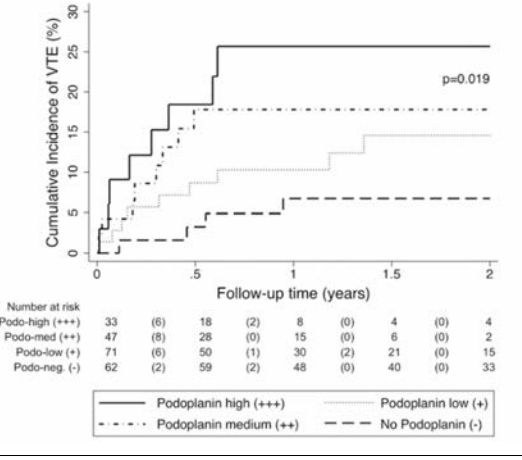

Figure 1. Cumulative incidence of venous thromboembolism (VTE) accounting for competing risk (death of any cause other than fatal VTE) according to expression levels of podoplanin. The probability of VTE significantly increased with increasing levels of podoplanin staining intensity (log-rank p=0.019).

Summary/Conclusions: High podoplanin expression in primary malignant brain tumors, which correlated with intravascular platelet aggregates, lower blood platelet counts and higher D-dimer levels, was associated with increased risk of VTE. *In-vitro*, podoplanin-positive cancer cells isolated from a glioblastoma patient who developed PE induced marked platelet activation and aggregation. Our study might provide a novel mechanistic insight into the pathogenesis of VTE in patients with primary malignant brain tumors.

Stem cells and the microenvironment

S141

HUMAN HEMATOPOIETIC STEM CELL DIFFERENTIATION FOLLOWS A CONTINUOUS WADDINGTON-LIKE LANDSCAPE

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Background: Blood formation is believed to occur via the step-wise progression of haematopoietic stem cells (HSCs) through a tree-like hierarchy of discrete progenitor cell types. Although several recent studies have challenged different aspects of this dogma, a comprehensive model of haematopoiesis and entry of HSCs into lineage commitment is currently lacking. Here, we mapped human bone marrow haematopoiesis by quantitatively integrating flow cytometric, transcriptomic and functional lineage fate data at the single-cell level.
Aims: Reconstruction of how individual HSCs enter lineage commitment by quantitatively integrating transcriptomic and functional single cell data that permits the reconstruction of developmental trajectories during HSC differentiation.
Methods: Healthy human BM HSPCs (Lin$^-$CD34$^+$) were individually sorted and surface marker fluorescence intensities of a panel of FACS surface markers commonly used to characterize these HSPCs were recorded to retrospectively reconstruct immunophenotypes. Index-sorted HSPCs were subjected to RNAseq ("index-omics", 379 cells) to determine their transcriptomes or individually cultured ex *vivo* ("index-culture", 1021 cells) to quantify lineage potential. Subsequently, the functional and transcriptomic data sets were integrated using commonly indexed surface marker expression to identify molecular and cellular events associated with the differentiation of human HSCs at the single cell level.
Results: We found that individual HSCs neither enter lineage commitment at binary branching points nor pass through discrete intermediate progenitor cell stages. In contrast, HSC lineage commitment occurs in a gradual manner best described by a continuous Waddington landscape with initially flat but progressively deepening valleys. Our data determine a detailed model of developmental trajectories within this landscape, as well as their underlying gene expression modules and biological processes.
Summary/Conclusions: Integration of transcriptomic and functional cell fate data at the single-cell level reveals that human hematopoiesis is not organized in a hierarchical "tree" of cell types, but follows a continuous differentiation flow within a Waddington-like landscape with initially flat and gradually deepening valleys.

S142

SINGLE-CELL PROFILING OF HUMAN MEGAKARYOCYTE-ERYTHROID PROGENITORS IDENTIFIES DISTINCT MEGAKARYOCYTE AND ERYTHROID DIFFERENTIATION PATHWAYS

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Background: The conventional haematopoietic hierarchy proposes that megakaryocytes (MK) and erythroid cells (E) derive from a shared progenitor, the MEP. However, the MEP was defined using "bulk" assays and recent studies have suggested that MEP and other myeloid progenitor populations may be heterogeneous. Advances in single-cell techniques now provide the opportunity to dissect cellular heterogeneity within populations and to uncover rare cell types.
Aims: To integrate multiple different single-cell molecular and cell biology approaches to uncover heterogeneity in human MEP and to establish a new FACS strategy to prospectively purify and functionally validate the novel subpopulations.
Methods: Surface immunophenotype (10 markers) and gene expression profiles (GEP) of individual human MEP were correlated using index-FACS and parallel, targeted multiplex PCR using a specifically designed panel of 87 genes. Differentiation potential was tested in novel single-cell differentiation assays (Figure 1).
Results: A total of 681 single Lin-CD34+CD38+CD123-CD45RA-MEPs were analysed by index sorting and GEP. Principal component analysis of GEP data identified three distinct MEP subpopulations. Correlation with cell surface immunophenotype revealed how these novel populations could be prospectively purified using the additional cell surface markers CD44, CD71 and CD41. MEP Population 1 (CD44hi71-41-; ~43.6% of MEP) showed increased expression of CD34, CSF3R and FLT3 and lower expression of GATA1 in single-cell and population-based analyses. Population 2 (CD44mod CD71+CD41-; 37.4%) showed increased E-associated gene expression (*e.g.* KLF1, TMOD1, TAL1, LEF1). Population 3 (CD44mod CD71+CD41+; 5.1%) showed distinct expression of MK-associated genes (*e.g.* FLI1, VWF, NH1B, MPL). Single-cell *in vitro* differential assays demonstrated that the transcriptional profiles corresponded to functional differences in lineage potential: Population 1 (designated Pre-MEPs) contained almost all of the residual myeloid potential within the MEP compartment and showed frequent MK-E bipotent cells. In contrast, populations 2 (E-MEP) and 3 (MK-MEP) showed a marked lineage bias toward E and MK lineage commitment respectively. Pseudo-temporal ordering of the cells along their differentiation trajectory (Monocle analysis) was also compatible with a more primitive phenotype for Pre-MEP, and suggested that CD42 expression correlated with loss of E-associated gene expression, a finding confirmed in functional assays with high MK but absence of E potential in CD71+CD41+CD42+ MEPs, while CD71+41+42- MK-MEP retained residual low level capacity to generate E progeny.

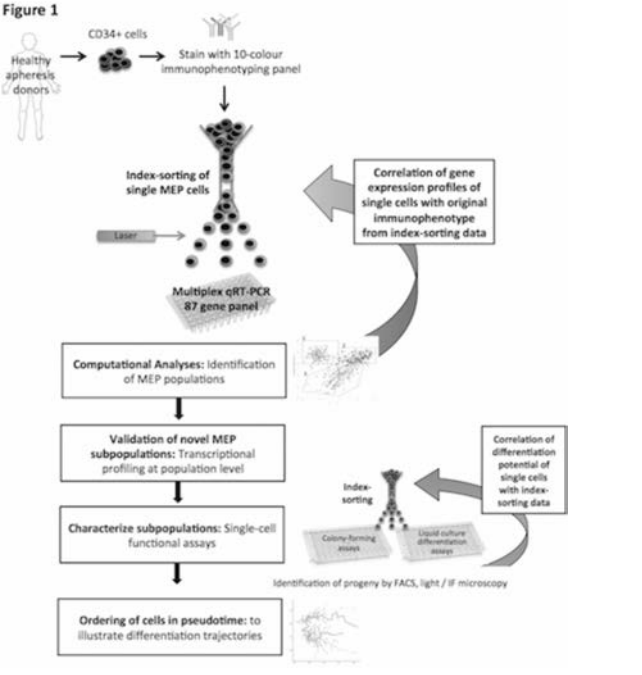

Figure 1.

Summary/Conclusions: This report illustrates the power of combining three single-cell techniques to interrogate cellular heterogeneity within populations, an approach that is applicable to many other systems. We show that classically defined MEP in fact contain three distinct subpopulations: (1)"Pre-MEP", enriched for E/MK progenitors but with residual myeloid differentiation capacity, (2)"E-MEP", strongly biased towards E differentiation, and (3)"MK-MEP", a rare population of bipotent cells that primarily generate MK progeny. Importantly, only a minority of classically defined MEP give rise to mixed E/MK colonies; the majority of the cells are transcriptionally-primed to generate cells of a single lineage. We believe that prospective identification of specific intermediate progenitor populations will allow for in-depth study of disorders of erythro-megakaryopoiesis, including myeloproliferative neoplasms and erythromegakaryocytic leukemias.

S143

INTERFERON ALPHA MEDIATED REMODELING OF THE BONE MARROW STEM CELL VASCULAR NICHE

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Background: Bone marrow endothelial cells (ECs) are a major part of the bone marrow (BM) vascular niche, regulating hematopoietic stem cell (HSC) function and fate. Furthermore, ECs significantly influence the response of an organism to infection, the primary response to which involves synthesis of immune-modulatory cytokines, such as interferon alpha (IFNα). In contrast to the anti-proliferative effect of IFNα on HSCs *in vitro,* we, and others, have shown that IFNα induces cell cycle entry of HSCs *in vivo*. Here we have investigated the result of acute IFNα treatment on the BM vascular niche *in vivo*.
Aims: To investigate the stimulatory effects of acute IFNα treatment on the BM stem cell vascular niche and its role in the chemotherapeutic response.

Methods: To characterize the response of the BM vascular niche to IFNα, wild-type mice were treated with recombinant IFNα or pI:C and were sacrificed 4h or 24h later. BM ECs were subsequently assessed for key inflammatory and EC-stimulatory markers by FACS. IFNα-mediated increased BM vascularity was quantified by *in vivo* labelling using Alexa 633. BM vessel integrity was assessed using the Evans blue assay. VEGF signalling was antagonized using the Anti-VEGF treatment, Avastin. Mice were co-treated with 5-FU and pI:C to evaluate the effect of IFNα-signalling of chemotherapeutic treatment.
Results: IFNα treatment induced a rapid stimulation of BM ECs *in vivo,* resulting in increased bone marrow (BM) vascularity and vascular leakage. IFNα-mediated activation of ECs involved the expression of key inflammatory and EC-stimulatory markers. Abrogation of BM EC activation *in vivo,* using the Anti-VEGF treatment, Avastin, linked VEGF signalling, mediated by BM cell types including HSCs, to IFNα-mediated activation of ECs. Finally, following a period of recovery, IFNα-signalling led to a rapid recovery of 5-FU-mediated cell activation and BM homeostasis.
Summary/Conclusions: Our data shows that IFNα stimulation *in vivo* leads to remodelling of the BM stem cell vascular niche, mediated by VEGF. These data increase our current understanding of the effect of IFNα and anti-VEGF treatment on HSCs and the stem cell niche *in vivo*. IFNα-mediated recovery from 5-FU treatment has obvious implications for therapy, and thus this effect may influence the clinical application of IFNα.

S144

CELL-EXTRINSIC HAEMATOPOIETIC IMPACT OF EZH2 INACTIVATION IN FOETAL LIVER ENDOTHELIAL CELLS

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Background: The cell-intrinsic role of many different epigenetic factors in the regulation of normal haematopoiesis and development of blood cancers is well established. However, the possible role and function of epigenetic regulators in components of the haematopoietic niche remains largely unexplored. Ezh2, is a histone methyltransferase and key component of the polycomb repressive complex 2 (PRC2). Loss of function of PRC2 causes a cell-intrinsic defect in adult haematopoietic stem cells (HSCs) and block in T- and B-lymphopoiesis. Further, both increased and reduced PRC2 activity has been implicated in a range of blood cancers. However, the haematopoietic impact of Ezh2 inactivation in components of the niche has not been explored.
Aims: Recent evidence supports that vascular endothelial cells (VECs) are key components of the haematopoietic niche during foetal development and we therefore aimed to study the haematopoietic impact of Ezh2 depletion in foetal liver (FL) VECs.
Methods: Ezh2 was depleted in haematopoietic cells with or without deletion in VECs using Tie2-Cre (Tie2-KO) or Vav-iCre (Vav-KO) respectively, and the impact on foetal haematopoiesis was explored through phenotypic and functional assays.
Results: At E12.5, both Vav-KO and Tie2-KO embryos showed close to 100% recombination efficiency in haematopoietic cells, whereas only Tie2-KO showed high levels of recombination in VECs ($CD45^-Ter119^-CD31^+$). There was a striking difference in survival, with embryonic lethality at E13.5 in Tie2-KO embryos in contrast to Vav-KO which survived beyond E18.5. Numbers of phenotypic HSCs ($LSKCD48^-CD150^+$) were normal in Tie2-KO at E12.5 and expanded through to E18.5 in Vav-KO embryos, confirmed in functional (*in vivo* reconstitution) assays, excluding an impact of Ezh2 loss on FL HSCs. In contrast, there was a Tie2-KO specific loss of E12.5 FL cellularity (fc=0.44; p=0.0006) and reduction in progenitor cells ($LSKCD48^+CD150^-$; fc=0.33; p<0.0001) that was not present in Vav-KO embryos. Furthermore, Tie2-KO but not Vav-KO E12.5 FLs were severely anaemic, with a marked loss of $CD71^+Ter119^+$ erythroid precursors (fc=0.07; p=0.0010). We reasoned that depletion of Ezh2 in VECs was causing a cell-extrinsic suppression of FL progenitors, particularly those of erythropoietic lineage. This Tie2-KO phenotype was highly reminiscent of that seen in Steel mice, and although KitL mRNA expression was increased (fc=3.62; p=0.0291), immunofluorescence staining demonstrated an almost complete loss of the membrane bound form of KitL (mKL) in Tie2-KO FL. The loss of mKL was not VEC-specific, but also affected Tie2-KO hepatoblasts ($CD45^-Ter119^-CD31^-Alb^+$), which express KitL but are not targeted by Tie2-Cre. As mKL is a known target of Mmp9, and Mmp9 is a known target of Ezh2, we next examined Mmp9 mRNA expression and showed this was upregulated in Tie2-KO FLs (fc=12.41; p=0.0236). In order to confirm the mechanistic role of Mmp9, we demonstrated that Ezh2-depleted VECs from Tie2-KO FL had reduced capability to support erythropoietic colony formation *in vitro* in co-culture assays (fc=0.22; p=0.0036), which could be fully rescued with Mmp9 inhibitor treatment.
Summary/Conclusions: These data demonstrate a marked cell-extrinsic haematopoietic impact of PRC2 inactivation in VECs through loss of mKL expression caused by a marked upregulation of Mmp9 expression. This provides evidence that modulation of epigenetic regulators can have a major cell-extrinsic impact on haematopoiesis, of relevance for the development of epigenetic therapies, including Ezh2 inhibitors.

PRESIDENTIAL SYMPOSIUM

Best abstracts

S145

STOPPING TYROSINE KINASE INHIBITORS IN A VERY LARGE COHORT OF EUROPEAN CHRONIC MYELOID LEUKEMIA PATIENTS: RESULTS OF THE EURO-SKI TRIAL

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Background: The advent of tyrosine kinase inhibitors (TKI) has dramatically improved survival in chronic myeloid leukemia (CML) with a high percentage of patients reaching deep molecular responses (MR; Hehlmann 2014). In several studies, it could be shown that in a substantial part of patients with deep MR, TKI treatment can be safely and successfully stopped (Mahon 2010, Ross 2013). However, exact preconditions for stopping treatment in CML have not yet been defined.
Aims: The EURO-SKI study (European stop TKI study) was set up to define prognostic markers to increase the rate of patients in durable deep MR after stopping TKI. Already in an interim analysis the null hypothesis (relapse-free survival ≤40% at 6 months (m) could be discarded (Mahon ASH 2014, Saussele EHA2014).
Methods: Adult CML patients in chronic phase (CP) on TKI treatment for at least 3 years and in MR4 for at least one year, confirmed by 3 consecutive PCR results during the last 12 m prior to inclusion were eligible. Final MR4 confirmation was performed in a EUTOS standardized laboratory. Patients with a prior TKI failure were excluded. Primary endpoint is the assessment of molecular relapse-free survival after stopping TKI defined as survival without loss of major molecular remission (MMR) at one time point. We here, per protocol, report the results of all patients, with a minimum follow-up of 6 m. A further follow-up after 3 years is planned. Analyses were done on an intention-to-treat basis.
Results: From June 2012 to December 2014, 868 patients in CP CML from 11 countries were included. 96 were excluded (withdrawal of consent n=1, inclusion criteria violation n=23, atypical or unknown transcript n=8, pending cases n=64). Of the eligible 772 patients, 46.6% were female. Median age at diagnosis was 51.9 years (range 11.2 to 85.5); median age at stop was 60.3 years (range 19.5 to 89.9). 10% and 18% were high-risk according to EUTOS and Sokal Score, respectively. One patient decided not to stop therapy after inclusion. 390 patients were mostly pretreated with hydroxyurea prior to TKI therapy, and 87 patients received interferon before TKI. First-line TKI was imatinib in 94%, dasatinib in 2% and nilotinib in 4%. 115 patients switched to second-line TKI due to intolerance, 58 to dasatinib, 7 to imatinib, and 49 to nilotinib (one missing information). Time from diagnosis of CML to first day of stopping TKI varied from 36.7m to 270.7m, median time was 92.7m. Median duration of TKI treatment was 91m (range 36.3 to 170.3) and median duration of MR4 prior to TKI stop was 56.3m (range 12.6. to 159.8). MR4 was reached after a median time of 21.0m (only first-line patients, range 3.0 to 140.0). 717 patients had assessable molecular data for the estimation of molecular relapse-free survival. The median molecular follow-up is 10m. Of these patients, 331 lost MMR, 4 died in remission and 381 are still in MMR at last follow-up (range 1-36 months). This resulted in a molecular relapse-free survival of 62% (95% confidence interval (CI): 58%-65%) at 6m, 56% (CI: 52%-59%) at 12m and 51% (CI: 47%-55%) at 24m (see Fig.1). First univariate analyses in 401 patients with complete records on all prognostic variables confirmed statistically significant influence of duration of TKI treatment and of duration of MR4 on molecular relapse-free

survival up to 6m. Neither sex nor any of the variables part of any of the 2 scores (Sokal, EUTOS) showed a significant association with MMR status at 6m. Data will be completed and multivariate modelling will be presented at the meeting.

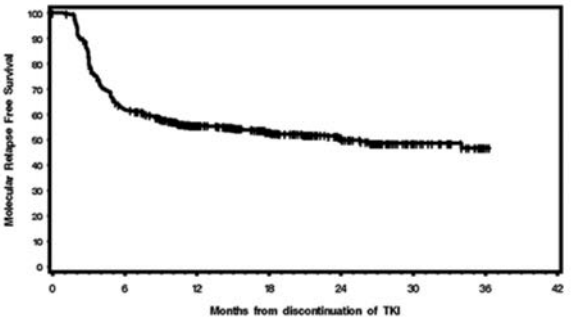

Figure 1.

Summary/Conclusions: In the setting of the EURO-SKI study, high molecular relapse-free remission rates are achievable. First univariate analysis demonstrated the importance of the duration of TKI treatment as well as of MR4 prior to stop. The EURO-SKI trial will further elucidate prognostic factors that improve the rate of patients in durable MMR after withdrawal of TKI.

S146

PERSISTENCE OF DRIVER MUTATIONS DURING COMPLETE REMISSION ASSOCIATES WITH SHORTER SURVIVAL AND CONTRIBUTES TO THE INFERIOR OUTCOMES OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: High-throughput sequencing techniques have shown that AML patients (pts) in complete morphological remission may harbor persistent preleukemic clones, which might be a source of leukemic relapse. Somatic mutations in epigenetic modifiers including *DNMT3A*, *TET2* and *ASXL1* occur in ageing-associated clonal hematopoiesis, and mutations in these genes are frequently present in preleukemic clones of AML pts. Previous studies demonstrated adverse outcomes of pts with persistent leukemia-associated mutations in remission (Klco *et al.*, JAMA 2015; PMID: 26305651).
Aims: We studied persistence of driver mutations in remission and clinical outcomes in a cohort of uniformly treated AML patients.
Methods: We studied 107 adult AML pts (median age, 53 years [y]; range 20-80y) who received intensive induction chemotherapy within the German multicenter AMLCG-2008 trial, and reached either complete remission (CR) (80/107, 75%) or CR with incomplete blood count recovery (CR_i) (27/107, 25%). Most patients (90%) had *de novo* AML, 5% had secondary AML and 5% had therapy-related AML. We analyzed bone marrow (BM) or peripheral blood (pB) specimens obtained at diagnosis (BM, n=96; pB, n=11) and during first remission (BM, n=101; pB, n=6). Ninety-two percent of remission samples were collected within 180 days after the start of induction therapy. We studied 68 genes recurrently mutated in myeloid neoplasms by multiplexed amplicon sequencing (Agilent Technologies), with a mean target coverage of 540x. Sequence alterations with a variant allele frequency (VAF) of ≥2% were classified as known/putative driver mutations, variants of unknown significance, or known germline polymorphisms based on published data including dbSNP, the Catalogue Of Somatic Mutations In Cancer (COSMIC) and The Cancer Genome Atlas (TCGA).
Results: At diagnosis, 426 driver mutations were detected in 42 genes (median, 4 mutations per patient; range, 0-10). In the paired remission samples, 66 mutations in 15 genes were still present (VAF, ≥2%) in 40/107 pts (37%), while 67 pts (63%) had no persistent mutation. Persistence of mutations during morphological remission was most commonly observed for *DNMT3A* (23/37 pts with mutations at diagnosis; 62%), *TET2* (9/13; 69%), *SRSF2* (5/8; 63%) and *ASXL1* (5/12; 42%). Mutations in other genes including *NPM1*, *FLT3*, *WT1*, and *NRAS* were no longer found in the remission samples. Sixty-nine percent of mutations detected in remission had VAFs >10%. Analyses of minimal residual disease (MRD) by flow cytometry or quantitative PCR for *NPM1* mutations or *MLL*-PTD showed similar MRD levels in remission specimens with and without persisting mutations. These findings suggest that mutations detected during remission are present in a pre-leukemic clone rather than in residual leukemic cells. Pts with ≥1 persisting mutation in remission were older (median, 63 years [y]) than pts without any persisting mutation (median, 48y; p<.001). Patients with non-*DNMT3A* persisting mutations tended to be older than those with persisting *DNMT3A* mutations (median, 66.5y *vs* 60y). Persistence of at ≥1 driver mutation in remission, in contrast to complete mutation clearance, associated with shorter relapse-free survival (RFS; median, 14.3 *vs* 58.0 months; p=.009) and shorter overall survival (OS; median, 39.6 *vs* >72 months; p=.005) (Figure A). Similar outcomes were observed for pts with persisting *DNMT3A* mutations and those with other persisting mutations (Figure B). In multivariate analyses adjusting for age, ELN genetic risk groups, and remission status (CR *vs* CR_i), detection of any persisting mutation remained associated with inferior RFS (hazard ratio, 2.2; p=.02) and OS (hazard ratio, 3.0; p=.008).

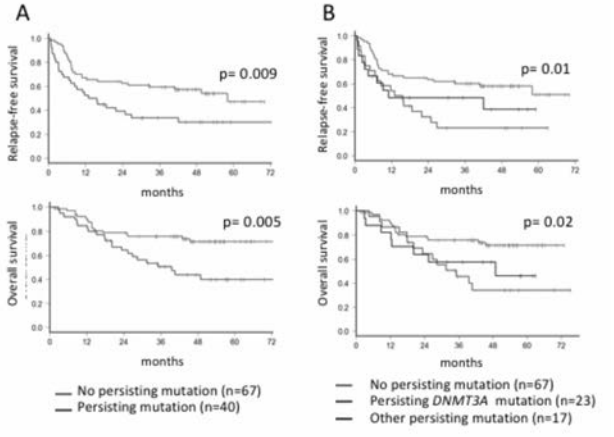

Figure 1.

Summary/Conclusions: Detection of persisting leukemia-associated driver mutations during first CR or CR_i is common in older AML pts and likely indicates persistence of a preleukemic clone. Mutation persistence associates with shorter RFS and OS and might contribute to the inferior outcomes of elderly AML pts.

S147

DISSECTING THE CONTRIBUTION OF UNREGULATED MACROPHAGE IRON RECYCLING AND DIETARY IRON UPTAKE IN GENERATING SYSTEMIC IRON OVERLOAD IN HEMOCHROMATOSIS

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Background: Systemic iron levels are balanced by the hepatic iron hormone hepcidin and its "receptor" ferroportin (Fpn) to prevent the pathological consequences of iron overload or iron deficiency. Hepcidin binding to the iron exporter FPN reduces dietary iron export from duodenal enterocytes and iron recycling from aging erythrocytes in reticuloendothelial macrophages. Mutations as the Fpn(C326S), that disrupt the hepcidin/FPN regulatory loop, cause an uncontrolled iron export from spleen and duodenum resulting in systemic iron overload (Altamura et al, Cell Met. 2014).
Aims: The aim of this study is to quantify the individual contributions of macrophage iron recycling and dietary iron uptake to systemic iron levels. This knowledge is an important prerequisite to develop specific pharmacological strategies to limit iron export in iron-related disorders.
Methods: By applying cre/lox technology, we generated mouse lines expressing the Fpn(C326S) mutation only in duodenal enterocytes (Villin−Cre/FpnC326S) or in macrophages (Lyz−Cre/FpnC326S) to dissect the single contribution of these two iron exporting cell types in generating iron overload. 10−week old C57BL6/J congenic male mice have been analyzed in this study.
Results: Mice carrying the Fpn(C326S) mutation exclusively in the duodenum show identical hematological alterations as found both in hemochromatotic patients and in constitutive Fpn(C326S) mice: increased hemoglobin (Hb), hematocrit (HCT) and mean corpuscular volume (MCV). Serum iron content and transferrin saturation are strongly increased further consistent with the hemochromatotic phenotype. Hepatic iron measurement revealed severe iron deposition that correlates with increased hepcidin expression. Elevated hepcidin levels cause iron retention in reticuloendothelial macrophages of the spleen generating splenic iron overload. Interestingly, Lyz−Cre/FpnC326S mice that show hepcidin independent macrophage iron export failed to show alterations in hematological parameters and serum iron levels. However the spleen is iron depleted, in line with a constitutively expressed iron exporter. By contrast hepatic iron content and hepcidin expression are similar compared to control mice.

Summary/Conclusions: Our results show for the first time that increased duodenal iron export is the major if not the only contributor in the generation of systemic iron overload in hemochromatosis. This finding opens new insights in developing pharmacological strategies aimed to specifically limit dietary iron import for the treatment of primary and secondary iron overload diseases.

S148

ETV6-RELATED THROMBOCYTOPENIA (ETV6-RT): CLINICAL AND PATHOGENETIC CHARACTERIZATION OF AN INHERITED THROMBOCYTOPENIA (IT) PREDISPOSING TO CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Background: ETV6-RT is an autosomal dominant IT recently identified in a few families and suspected to predispose to hematological malignancies.
Aims: To gain information on the clinical and laboratory picture of this new IT, in particular on predisposition to hematological malignancies.
Methods: 130 unrelated and consecutive patients with ITs were enrolled to the study: all of them had no definite diagnosis because they did not fit the criteria for any known IT. *ETV6* mutations were investigated by whole exome sequencing (84 probands) or Sanger sequencing (46 probands): whenever *ETV6* mutations were identified, all available relatives of probands were also investigated. 5 patients from 2 known *ETV6*-RT families already partially described (Noetzli L et al, Nat Genet 2015, 47:535) were also included. Each patient underwent to phenotypic characterization through the following investigations: complete blood count and platelet sizing, flow cytometry of platelet membrane glycoproteins, "*in vitro*" platelet aggregation, platelet activation, platelet adhesion and spreading, differentiation of human megakaryocytes (Mks) and morphological analysis, Mk flow cytometry, evaluation of proplatelet formation by *in vitro* differentiated Mks.
Results: Overall, 20 subjects from 7 families bearing 5 different *ETV6* mutations were identified. The bleeding tendency and the degree of thrombocytopenia were mild, but we found that 4 of 20 patients (20%) had childhood ALL, thus confirming that early leukemic transformation is a major risk of this IT. Moreover, one patient developed JAK2+ polycythemia vera at age 37, suggesting that this disease should be added to the list of malignancies to which the *ETV6*-RT predisposes. Clinical and laboratory findings (platelet aggregation, surface glycoproteins, activation and adhesion) did not identify any peculiar defect that can be used to suspect this disorder. *In vitro* studies revealed that patient Mks have defective maturation and proplatelet formation, while platelets have reduced ability to spread on fibrinogen. At variance with most ITs, platelet size was not enlarged: this finding is shared with ITs due to monoallelic mutations in *RUNX1* and *ANKRD26*, which also have normal platelet size and predispose to leukemia (the familial platelet disorder with predisposition to acute myeloid leukemia, and the *ANKRD26*-RT, respectively). Considering the possibility that patients with ITs have *ETV6*-RT is important both for the risk to develop hematological malignancies, and because this disorder is relatively frequent: in our series of 274 consecutive propositi, *ETV6*-RT was identified in 8 families and had, therefore, a relative prevalence of 2.9% in the whole case series, and of 6.1% in the series of patients with known ITs. In our experience, the frequency of *ETV6*-RT was lower only to that of monoallelic Bernard-Soulier syndrome (BSS, 12.2% in the whole series), *MYH9*-related disease (11.4%), *ANKRD26*-RT (9.4%), and biallelic BSS (5.7%).
Summary/Conclusions: Our study showed that monoallelic *ETV6* mutations cause one of the most frequent forms of ITs and confirmed that affected subjects have little bleeding tendency but high propensity to hematological malignancies, in particular childhood ALL. Since *ETV6*-RT is one of the few autosomal dominant forms of IT without platelet macrocytosis, the screening for *ETV6* mutations is recommended in all patients with these characteristics, along with the screening for *RUNX1* and *ANKRD26* mutations.

S149

BLINATUMOMAB IMPROVED OVERALL SURVIVAL IN PATIENTS WITH RELAPSED OR REFRACTORY PHILADELPHIA NEGATIVE B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA IN A RANDOMIZED, OPEN-LABEL PHASE 3 STUDY (TOWER)

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Background: Blinatumomab, a bispecific T-cell engager antibody construct, binds specifically to CD19 (B cells) and CD3 (T cells) to facilitate lysis of CD19-positive target B-lineage cells. Based on a single-arm phase 2 study, blinatumomab received approvals in the US (accelerated) and EU (conditional) for the treatment of Philadelphia chromosome-negative (Ph–) relapsed/refractory (r/r) B-cell precursor acute lymphoblastic leukemia (BCP-ALL).
Aims: This phase 3, randomized, open-label study investigated the effect of blinatumomab on overall survival (OS) compared with standard of care (SOC) chemotherapy in adult patients with Ph– r/r BCP-ALL.
Methods: Patients with r/r BCP-ALL (if first relapse, within 1 y) were randomized 2:1 to receive either blinatumomab or SOC chemotherapy (investigator's choice of 1 of 4 defined regimens). Randomization was stratified by age, prior salvage therapy, and prior allogeneic stem cell transplant (alloSCT). Blinatumomab was given in 6-week cycles of 4 weeks on (continuous infusion of 9 µg/d in week 1 of cycle 1, then 28 µg/d) and 2 weeks off; dexamethasone was given pre-dose to prevent cytokine release syndrome. Patients in remission after 2 induction cycles were eligible to continue therapy until relapse. OS was the primary efficacy endpoint. Complete remission (CR) and combined CR or CR with partial or incomplete hematologic recovery (CR/CRh/CRi) were secondary efficacy endpoints. The primary analysis was scheduled to occur after 330 deaths had accrued. This prespecified interim analysis for an independent data monitoring committee (DMC) occurred after 248 deaths (75%).
Results: A total of 405 patients were randomized to blinatumomab (n=271) or SOC (n=134) and analyzed for efficacy; 2% and 19%, respectively, elected to receive no study treatment. Baseline characteristics were balanced between treatment groups (blinatumomab, SOC): median age (37y, 37y); median bone marrow blasts (80%, 79%); prior salvage therapy (56%, 52%); and prior alloSCT (35%, 34%). Using results from the DMC analysis, median OS was 7.8 months (95%CI: 5.7, 10.0) for blinatumomab and 4.0 months (95%CI: 2.9, 5.4) for SOC (stratified log-rank test p=.011; hazard ratio=0.71), surpassing the prespecified O'Brien-Fleming boundary p value of 0.0183. Improvement in OS was consistent between subgroups based on age, prior salvage therapy, or prior alloSCT. Response rates were higher for blinatumomab *vs* SOC, including CR (39% *vs* 19%; p<.001) and CR/CRh/CRi (46% *vs* 28%, p=.001). A total of 373 patients received ≥1 dose of blinatumomab (N=266) or SOC (N=107; 47 FLAG±anthracycline; 19 HiDAC-based; 22 high-dose methotrexate-based; and 19 clofarabine-based) and were analyzed for safety; 57% and 25%, respectively, started at least 2 cycles. Safety outcomes were similar between blinatumomab and SOC (table 1).

Table 1. Incidence Rates of Adverse Events (AE), Regardless of Causality.

	Blinatumomab (N=266)	SOC (N=107)
Any AE, % (per 100 patient-months)	99% (631.3)	99% (764.4)
Any grade 3 AE, %	38%	34%
Any grade 4 AE, %	29%	40%
Any grade 5/fatal AE, %	19%	19%
Grade 5 infection, %	11%	12%
Any serious AE, % (per 100 patient-months)	62% (26.4)	45% (38.1)
Infection, %	28%	31%
Blood/lymphatic, %	14%	16%
Nervous system, %	7%	3%
Cytokine release syndrome, %	3%	0%

Summary/Conclusions: This prespecified interim analysis showed that blinatumomab, as compared with SOC chemotherapy, improved the primary endpoint of OS in the phase 3 TOWER study of adults with Ph– r/r BCP-ALL. Remission rates also were higher in the blinatumomab group. AEs in the blinatumomab group were consistent with previous studies. Based on these findings, the DMC recommended stopping the TOWER study for efficacy before the planned final analysis.

POSTER SESSION I

Acute lymphoblastic leukemia - Biology 1

P150

COMPARATIVE ANALYSIS OF GENE REGULATORY NETWORKS IN LYMPHOID LEUKEMIAS USING THE INTERACTIVE HEMAP RESOURCE

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Background: Acute lymphoblastic leukemia (ALL) is a paradigmatic example of a disease that can develop at an early age as a result of genetic lesions that impede lineage commitment. In normal development, cells commit to their lineage and differentiated phenotype state by the activation of transcription factors (TFs), or TF modules, in a series of decision points at which a choice is made between alternative lineage fates. In parallel, epigenetic regulation has emerged as an important mechanism that can corrupt the gene regulatory network.
Aims: We set out to examine whether patient expression profiles could be used to quantitatively detect the blocks to differentiation and to reveal aberrantly active TF loci. We further evaluated how amenable lymphoblastic leukemia cases may be to treatment with drugs acting on epigenetic modifiers.
Methods: We analyzed 1,304 pre-B-ALL, 385 T-ALL and 801 B-CLL transcriptomes as part of a curated set of 9,544 transcriptomes collected across 36 hematological malignancies to facilitate the characterization of mechanisms that can corrupt the regulatory network in lymphoblastic leukemias. Sample similarity was assessed using t-Distributed Stochastic Neighbor Embedding (t-SNE) maps that permitted detecting changes in gene expression at subtype resolution and association with specific TF translocations, epigenetic changes and aberrant enhancers. The expression of genes that encode epigenetic modifiers with existing small molecule drugs (list from ChEMBL) was compared between ALL and chronic lymphoblastic leukemia (CLL) and other hematological malignancies.
Results: We scored gene sets of blood cell type lineage-determining TFs across the transcriptome dataset and found that cancer samples lack the characteristic mutually exclusive lineage-restricted pattern of these TF sets. Analysis of stem cell similarity revealed predominant expression of a subset of hematopoietic stem cell TFs across all acute leukemias. Of these, *ERG* expression was highest in pre-B-ALL and correlated with high activity of a super-enhancer based on DNAse hypersensitivity and enhancer RNA expression. In addition, we found disease cluster-specific activation of transcription-regulating gene loci with low or undetectable expression in normal blood cell types. In the cluster of the common pre-B-ALL subtype carrying the t12;21 translocation (ETV6-RUNX1 fusion), four TFs (*IRX1*, *MYOCD*, *NR3C2* and *SOX11)* had a subtype-specific elevated expression level. We validated with overexpression and silencing of *ETV6-RUNX1* that the observed upregulation of *IRX1* occurs downstream of the fusion TF. Furthermore, high expression of epigenetic modifiers with existing small molecule drugs (available from ChEMBL) was significantly enriched in CLL, T-ALL, one pre-B-ALL and one AML cluster among the 9,544 hematological samples.
Summary/Conclusions: We demonstrate that there are recurrent patterns of aberrant TF expression in leukemia disease clusters. Misregulated expression of ERG in pre-B-ALL appears to be mediated by a stem cell super-enhancer based on the deep sequencing results presented. Moreover, elevated expression of drug targets that function as epigenetic modifiers was enriched in specific leukemia clusters. Their inhibition potentially restores expression of a larger repertoire of misregulated genes. Particularly in CLL, these genes represent promising treatment targets for the reconditioning of blast cells.

P151

MEF2C DYSREGULATION IS ASSOCIATED WITH CDKN1B DELETIONS AND PREDICTS POOR GLUCOCORTICOID RESPONSE IN PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemias (T-ALLs) are aggressive hematologic tumors resulting from the malignant transformation of T-cell progenitors and account for 10 15% of pediatric ALL cases. T-ALL is a heteroge-

neous disease characterized by a number of specific genetic alterations, which define molecular subgroups associated with distinct gene expression signatures, including the *TAL/LMO*, *TLX1*, *TLX3*, *HOXA* clusters as well as the recently identified proliferative and immature subtypes, typically overexpressing *NKX2 1* or *NKX2 2* and *MEF2C*, respectively. The latter, at least in part, overlaps with early T-cell phenotype- (ETP-) like ALL. However, a number of recurrent abnormalities such as *CDKN1B* deletions occurring in about 12% of T-ALL have not as yet been assigned to any specific disease entity.
Aims: In this study we sought to characterize pediatric T-ALL patients with *CDKN1B* deletions, which represent one of the most frequent copy number alterations, in more detail.
Methods: Array comparative genomic hybridization (aCGH) of 102 pediatric T-ALL patients enrolled in the Austrian ALL-BFM 90, 95 or 2000 clinical trials was performed. Cytogenetic analysis and immunophenotyping were done as part of the routine diagnostic work-up. The presence of specific chromosomal abnormalities was furthermore determined by fluorescence *in situ* hybridization (FISH) and/or RT-PCR. Moreover, the samples were subjected to RT-qPCR for *MEF2C* expression.
Results: By aCGH analysis and quantitative genomic PCR we detected the presence of *CDKN1B* deletions in 14 cases. Further genetic characterization of these cases led to their classification into the major T-ALL subtypes: *TAL/LMO* (n=1), *TLX1* (n=1),*TLX3* (n=1), or *HOXA* (n=3). Since many of the cases did not fall into any of these groups, we determined whether they belonged to the immature cluster characterized by *MEF2C* expression and compared them to *CDKN1B* wild-type cases. Remarkably, while only 14% (4/28) of *CDKN1B* wild-type samples showed *MEF2C* dysregulation, this was observed in 54% (6/11) of the *CDKN1B*-deleted cases for which appropriate material for RT-qPCR was available (p=0.017), suggesting an association between loss of *CDKN1B* and *MEF2C* expression. Since *MEF2C* is considered as one of the driving oncogenes of immature/ETP-like ALL, we next evaluated whether the *MEF2C*-dysregulated cases displayed the immunophenotypic characteristics of ETP-ALL: CD1a/CD4/CD8 negative; absent or weak CD5, and expression of one or more myeloid and/or stem cell antigens. Notably, while all cases immunophenotypically defined as ETP-like cases showed upregulation of *MEF2C* expression, only 30% (3/10) of *MEF2C*-dysregulated cases showed an ETP-like phenotype. However, virtually all of them expressed myeloid or B-lineage-specific markers, suggesting a certain lineage plasticity. Furthermore, high *MEF2C* expression levels in primary leukemia samples were associated with a significantly higher proportion of the patients being resistant to glucocorticoid therapy (p=0.0033).
Summary/Conclusions: In summary, our data provide evidence that *CDKN1B* deletions are associated with immature/*MEF2C*-dysregulated T-ALL and that *MEF2C* overexpression, though associated with, is not exclusively found in ETP-like ALL. Furthermore, our results indicate that *MEF2C* expression is associated with or may even be predictive of the response to glucocorticoid treatment.

P152

HEDGEHOG PATHWAY ACTIVATION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PREDICTS RESPONSE TO SMO AND GLI1 INHIBITORS

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Background: The hedgehog signaling pathway contributes to embryonic pattern formation and adult tissue homeostasis as it is important to regulate cell proliferation, survival and differentiation. Aberrant activation of the hedgehog pathway through overexpression of the ligands (SHH, IHH) or mutations in signaling components (PTCH1, SMO, SUFU, GLI1) has been described in various solid tumors. The Hedgehog pathway is important for normal T-cell development, but its potential role in the development of T-cell acute lymphoblastic leukemia (T-ALL) is poorly characterized.
Aims: Our aim was to investigate the role of the hedgehog pathway as an oncogenic factor and a potential target for therapy in T-ALL.
Methods: We analyzed gene expression profiles of human T-ALL samples from two independent cohorts. We determined the effects of hedgehog ligand stimulation, downregulation of critical signaling components or pharmacological inhibition of the pathway in T-ALL cell lines, a mouse T-ALL model and in patient derived xenografts.
Results: Gene expression data analysis of primary T-ALL samples revealed that about 25% of T-ALL cases show ectopic expression of the ligands of the hedgehog pathway. A strong correlation between the ligands SHH and IHH with the main transcriptional factors GLI1 and GLI2 and known GLI target genes was observed, indicative of hedgehog pathway activation through an autocrine loop. Pharmacological inhibition of the hedgehog pathway, or siRNA mediated knock-down of key signaling components caused a decrease of proliferation of a subset of T-ALL cell lines, and sensitized the T-ALL cell lines to chemotherapy. In our JAK3-dependent T-ALL mouse model expression of Shh or Ihh provided a growth advantage to the JAK3(M511I) leukemia cells. Finally, we tested if hedgehog pathway inhibition could inhibit the growth of primary T-ALL cells. For this reason patient derived T-ALL xenograft samples were treated with two hedgehog inhibitors *ex vivo* and *in vivo*. T-ALL samples with high GLI1 expression were sensitive in both *ex vivo* and *in vivo* experimental set up, while T-ALL samples with low or no GLI1 expression were insensitive.
Summary/Conclusions: We demonstrate that the hedgehog pathway is activated in a subset of T-ALL patients and that hedgehog pathway activation affects T-ALL cell proliferation and provides partial protection against chemotherapy. Data from patient derived xenograft T-ALL samples confirms that high GLI1 expression predicts sensitivity of T-ALL samples to hedgehog pathway inhibitors.

P153

IDENTIFICATION OF CANDIDATE ONCOGENES AND CHROMOSOMAL BREAKPOINT SEQUENCING BY TARGETED LOCUS AMPLIFICATION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is characterized by clonal and mutual exclusive chromosomal rearrangements that recurrently activate *TAL1*, *LMO2*, *TLX1*, *NKX2-1*, *TLX3*, *HOXA* or *MEF2C* oncogenes. Most of these translocations or chromosomal rearrangements occur as erroneous D-J or V-DJ rearrangement attempts of T-cell receptor beta (*TCRB*) or TCR alpha/delta (*TCRAD*) genes, positioning oncogenes under the transcriptional control of TCR enhancer elements. Alternatively, oncogenes can also be activated as consequence of *BCL11B* chromosomal rearrangements. Although many oncogenes are known in T-ALL, the driving oncogenic lesion in particular T-ALL cases remains unknown.
Aims: In this study, we aimed to clone reciprocal breakpoint sequences by Targeted Locus Amplification (TLA) to elucidate cellular mechanisms that lead to recurrent *BCL11B-TLX3* chromosomal translocations. Moreover, we want to identify oncogene candidates in various T-ALL patients with *BCL11B*-, *TCRB*- or *TCRAD*-translocations for which the candidate oncogenes have not been identified.
Methods: We used the TLA procedure, a recently developed method that relies on the crosslinking of DNA in live cells, DNA digestion and re-ligation to allow formation of circular DNA ligation fragment. Inverted polymerase chain reaction amplification from specific view-point loci that closely flank chromosomal rearrangement breakpoint followed by next generation sequencing allows for the identification of a 100kb genomic region.
Results: We resolved the nucleotide sequence of chromosomal breakpoints in 30 T-ALL patient samples that were previously characterized by FISH and/or QRT-PCR. For some cases, we confirmed classical chromosomal translocations or rearrangements for *TAL1*, *LMO2*, *TLX1*, *TLX3* or *NKX2-1* oncogenes to *TCRAD*, *TCRB* or *BCL11B* loci. We identified unusual translocations in 8 cases, including *SPI1-BCL11B* t(11;14)(p13;q32), *LMO2-BCL11B* t(11;14)(p13;q32), *TCL1-TCRAD* t(14)(q11;q32), *NKX2.5-BCL11B* t(5;14)(q35;q32), *TLX1-RPP30* t(10)(q32;q24), *TLX3-TCRB* t(5;7)(q35;q34) and *CENPP-TCRAD* t(9;14)(q22;q11). For *TLX3-BCL11B* translocations, most breakpoints were found in the close vicinity of the *TLX3* oncogene and the *BCL11B* enhancer region (major peak) and DNase hypersensitivity sites. However, one *TLX3-BCL11B* sample was not translocated to the major peak, pointing to other enhancer regions that may drive TLX3 expression.
Summary/Conclusions: Identification of chromosomal rearrangements by Targeted Locus Amplification reveals classic chromosomal rearrangements as previously detected by FISH or QRT-PCR in 30 selected patients. By TLA, breakpoint junctions were resolved in 22 patients. In addition, in 8 selected cases from which we were unable to identify a fusion partner by FISH, TLA led to the identification of genomic breakpoints for uncommon translocations. TLA therefore proved a useful tool to identify novel translocation partners from various loci such as the *TCR* or *BCL11B* genes that are recurrently involved in these chromosomal rearrangements in T-ALL. Cloning of molecular translocation breakpoints of diagnostic T-ALL patient samples may further provide excellent minimal residual disease markers for disease monitoring during the course of treatment.

P154

A PLATFORM FOR DETECTION OF FUSION GENES IN ALL FROM TARGET CAPTURE NEXT-GENERATION SEQUENCING OF DNA AND RNA

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Background: Acute Lymphoblastic Leukemia (ALL), the most common leukemia in childhood, is characterized by genomic alterations, such as chromosomal translocations typically affecting a limited number of recurrent genes (targets) with several variable partner genes (partners). Fusion genes can be targeted by chemotherapy and/or new drugs, therefore genomic profiling of ALL has the potential to identify important new prognostic markers and potentially druggable targets.
Aims: Herein, we researched and developed a bioinformatic solution, 'BreakingPoint', for the robust detection of fusion genes. This was implemented in the context of an expert evaluation of Target Capture Next-Generation Sequencing (NGS-TC) protocols both at the DNA and RNA level on ALL samples. The platform will eventually be evaluated for routine clinical diagnosis.
Methods: 'BreakingPoint' has been developed to detect fusion genes from NGS-TC datasets from any sequencing technology and any biological material. Its distinctive feature is that it initially identifies all aligned reads indicating hypothetical breakpoints on the 'targets', then clusters these into longer (thus more informative) consensus sequences, which are then studied across the genome to identify the unknown 'partners'. We tested 'BreakingPoint' on NGS-TC datasets of: (i) DNA material from 10 patients with 7 known gene fusions (Illumina Nextera DNA capture); (ii) RNA material of 6 patients with 8 known gene fusions (NuGen Ovation RNA Fusion Panel); all on Illumina's MiSeq platform. We additionally compared our method to the popular 'Delly' tool (DNA) and the 'TopHat Alignment App' on the 'Illumina BaseSpace Cloud' (RNA).
Results: On the DNA dataset, 'BreakingPoint' identified 6/7 known fusion genes (*e.g.* BCR/ABL1, PAX5/AUTS2) plus one not previously known. The missing P2RY8/CRLF2 gene fusion was most probably due to the low complexity of chromosome X affecting the *in vitro* capture and *in silico* alignment steps. Our method also detected deletions affecting the IKZF1 'target', a gene prone to mutation in ALL patients. 'Delly' identified 5/7 known gene fusions, missing PAX5/AUTS2 and P2RY8/CRLF2. On the RNA dataset, 'BreakingPoint' identified all 8 known fusion genes, including P2RY8/CRLF2, plus a novel gene fusion (PAX5/ZCCHC7). Illumina's 'TopHat Alignment App' detected 5/8 known gene fusions and the novel PAX5/ZCCHC7 rearrangement; false negatives (BCR/ABL1; MLL/AF4;P2RY8/CRLF2) were most likely caused by low read numbers affecting 'targets' coverage. 'BreakingPoint' false positives showed a common pattern of low sequence complexity and few representative reads (less than 2), and in many cases breakpoints fell in long intron non-coding regions (LINC), making them easily recognizable when compared to true positives. Finally, we have implemented a graphical user interface to run 'BreakingPoint' (and other plug-in methods, including 'Delly', if the user so wishes) and browse the results.
Summary/Conclusions: These results indicate that our approach sensitively and reliably detects fusion genes, irrespective of biological material or protocol, even in cases of low reads coverage. This already provides the potential to identify novel and conventional targetable fusion genes. Eventually, after further validation and evaluation, and coupled with the user-friendly interface, we believe that the 'BreakingPoint' platform can be introduced in routine clinical diagnosis.

P155

THE NUP214-ABL1 FUSION COOPERATES WITH TLX1 IN THE DEVELOPMENT OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The episomal NUP214-ABL1 fusion is observed in 6% of T-ALL patients. The resulting NUP214-ABL1 fusion protein is a constitutively activated tyrosine kinase, which activates STAT5 and ERK signalling, thereby stimulating proliferation and survival. Significantly, almost all T-ALL cases with the NUP214-ABL1 fusion also harbour chromosomal translocations resulting in the overexpression of the homeobox transcription factors TLX1 or TLX3.
Aims: To determine whether (i) co-expression of NUP214-ABL1 fusion and TLX1 cooperate together in leukemia development; and (ii) to understand the molecular mechanisms underlying any cooperation at the transcriptional level.
Methods: We have generated a conditional loxP-STOP-loxP NUP214-ABL1 knock-in mouse model, in which NUP214-ABL1 expression can be activated by Cre. We crossed these mice with *Tg(CD4-Cre) mice,* in which Cre is expressed in developing T-cells, and then subsequently with *Tg(Lck-TLX1)* mice, expressing TLX1 under control of the T-cell specific Lck promoter. These crossings resulted in a *Tg(CD4-Cre, NUP214-ABL1, Lck-TLX1)* mouse model. Using RNA-sequencing and ChIP-sequencing, we determined gene expression profiles and binding patterns of STAT5 and TLX1.
Results: The *Tg(CD4-Cre, NUP214-ABL1, Lck-TLX1)* mouse model developed a transplantable CD4+/CD8+ T-ALL with a significantly shorter latency (median survival of 193 days) compared to the *Tg(Lck-TLX1)* mouse model (median survival 385 days) or the *Tg(CD4-Cre, NUP214-ABL1)* mouse model (no disease), indicating true cooperation between NUP214-ABL1 and TLX1 during T-ALL development. Gene expression profiling revealed that there is significant upregulation of the JAK-STAT pathway, the mTOR signalling pathway and the Notch1 signaling pathway in the *Tg(CD4-Cre, NUP214-ABL1, Lck-TLX1)* mouse model, compared to the *Tg(Lck-TLX1)* mouse model. The cooperation between NUP214-ABL1 and TLX1 was also investigated in a human context using the T-ALL cell line ALL-SIL, which carries these two oncogenic alterations. ChIP sequencing results showed that STAT5 and TLX1 have overlapping binding patterns on genes that have been identified as STAT5 target genes. We are currently validating these results in our transgenic mouse model.
Summary/Conclusions: The NUP214-ABL1 fusion and TLX1 overexpression cooperate to drive T-ALL development.

P156

CLINICAL AND BIOLOGICAL LANDSCAPE OF DRIVER MUTATIONS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: B-progenitor acute lymphoblastic leukemia (B-ALLs) accounts for 85% of pediatric ALL and currently categorized into molecular subgroups according to their ploidy and recurrent translocations, such as *ETV6 -RUNX1* (*TEL-AML1*), *TCF3-PBX1 (E2A-PBX1), BCR-ABL1* or *MLL*-rearrangements. In addition, recent genetic studies using high-throughput sequencing have disclosed a number of novel gene mutations in each subgroup. However, the landscape of driver mutations and their relevance to clinical outcome have not been fully investigated in a large cohort of B-ALL patients.
Aims: The purpose of this study is to characterize the distribution of genetic alterations and their clinical relevance in pediatric B-ALLs using high-throughput sequencing in a large cohort of B-ALL patients.
Methods: A total of 524 pediatric B-ALL patients were enrolled in this study, who were uniformly treated according to the Japan Association of Childhood Leukemia Study (JACLS) ALL-02 protocol between 2002 and 2008. These patients were categorized into three risk groups, including standard risk, high risk, and extremely high risk. Infantile, *BCR-ABL1*–positive and Down syndrome–associated ALLs were not enrolled in this cohort. A total of 158 known or putative driver genes in pediatric ALL were analyzed for somatic mutations by targeted-capture deep sequencing using SureSelect (Agilent). Single nucleotide polymorphism-baits were also included, which were designed to allow for genome-wide copy number detection based on deep sequencing.
Results: The median age at diagnosis and observation period were 5.2 years (1-18.5) and 4.2 years (1.8-9), respectively. Sixty-six of the 524 patients (13%) had relapsed diseases and 47 patients (9%) were dead. Real-time RT-PCR and conventional cytogenetic analyses revealed *ETV6-RUNX1*, *TCF3-PBX1*, *MLL* rearrangements, hyperdiploidy (>50 chromosomes) and hypodiploidy (<44 chromosomes) in 113 (22%), 42 (8%), 10 (2%), 95 (18%), and 2 (0.4%) patients, respectively, and the remaining 262 patients (50%) had none of these abnormalities. The mean depth of the targeted sequencing was 569× across the entire cohort. In total, 74% of the patients harbored at least one mutation (median, 2 per patient; range, 0-9), and the mean number of mutations in *ETV6-RUNX1*, *TCF3-PBX1*, *MLL*-rearranged, hyperdiploid, hypodiploid ALLs were 0.63, 1.19, 1, 3.05 and 4, respectively. *CDKN2A*, *KRAS*, *NRAS*, *PAX5*, and *ETV6* were among the most frequently affected genes, which were mutated or deleted in 24%, 21%, 18%, 18%, and 15% of the cases, respectively. In terms of functional pathways, RAS pathway was most frequently affected and altered as many as 50% of the patients, followed by the pathways involved in epigenetic regulation, cell cycle, and B-cell development. Frequency of mutations showed a significant variation among B-ALL subtypes. For instance, RAS pathway mutations were detected in as many as 87% of hyperdiploid ALLs, compared to 3% of *TCF3-PBX1* ALLs. On the other hand, *IKZF1* mutations/deletions were only indentified in those without recurrent translocations, hyperdiploidy and hypodiploidy.
Summary/Conclusions: We revealed the landscape of driver mutations and copy number alterations in pediatric B-ALL and their diversity in B-ALL subtypes. Further analyses are underway to better clarify the prognostic significance of driver gene mutations.

P157

CHARACTERIZATION OF AN *EX VIVO* T-CELL CULTURE MODEL FOR THE STUDY OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia is caused by the combined deregulation of various cellular functions, including NOTCH1 signaling, kinase signaling, and transcriptional regulation. Deregulated kinase signaling is often studied in the Ba/F3 pro-B cell line, but an easy T-cell system is lacking.
Early T cell progenitor cells undergo extensive expansion at the double negative two (DN2) stage *in vivo*, which allows for their expansion and culture *ex vivo*. Importantly, these cells maintain their potential to differentiate into mature T cells *in vivo*. This makes this culture method a suitable model to study T cell development and transformation events in pre-leukemic cells.
Aims: This study aimed to develop and characterize a T-cell model to dissect molecular mechanisms of T cell development and signalling pathways that are implicated in the development of T-ALL. We aimed to identify key transcriptional pathways and regulators during T cell differentiation and to study the effect of oncogenic mutations on these pathways.
Methods: Hematopoietic stem cells were cultured in the presence of SCF and IL7 on delta like ligand 4 (DLL4) coated plates, to induce differentiation into early T cell progenitors. Transcriptional profiling using RNA-sequencing of these pro-T cells was used to identify the key transcriptional pathways and genes induced by IL7, SCF or Notch signalling. Next, dominant oncogenic mutants of AKT, JAK3, NOTCH1 or KRAS were introduced with retroviral vectors, and Pten gene was inactivated using Crispr/Cas genome editing. These cells were analyzed for their possibility to grow in the absence of SCF, IL7 and/or DLL4.
Results: We identified transcriptional signatures, which were specific to IL7, SCF and DLL4 stimulation or induced in a synergistic manner. For example IL7, SCF and DLL4 by themselves were able to induce the expression of the transcription factor Myc. However, only Notch-signalling induced expression of Hey1, Dtx1, Hes1 or CCR7. Using computational tools, we identified transcription factors that mediated expression of these genes, specific to SCF and IL7 stimulation. Furthermore, SCF activated mainly the PI3K-Akt pathway and was unable to induce Stat5a/b phosphorylation. IL7 stimulation, on the other hand, resulted in activation of the Jak-Stat pathway, but provided less activation of Akt. In line with these observations, overexpression of the AKT(E17K) mutant or Crispr/Cas9 mediated Pten deletion resulted in SCF independent growth, whereas cells remained IL7 and DLL4 dependent. Furthermore, some, but not all, JAK3 mutants were able to transform cells to grow in the absence of IL7 and induced Stat5 phosphorylation. KRAS(G12D) mutants allowed the cells to grow in the absence of SCF and IL7. Upon transduction with ligand independent Notch1 mutants, cells became independent of DLL4 stimulation.
Summary/Conclusions: This model system for the SCF/IL7/DLL4 dependent *ex vivo* culturing of primary mouse T-cells enables the study of T-ALL oncogenic events in a T cell context.

Acute lymphoblastic leukemia - Biology 2

P158

FUNCTIONAL RESTORATION OF MUTANT P53 AND CELL DEATH SENSITIZATION IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: In acute lymphoblastic leukemia (ALL), *TP53* alterations are rarely found at diagnosis but in about 12% of patients at relapse. *TP53* mutations (*TP53*mut) have been described to be associated with poor response to therapy and inferior outcome in ALL. Compounds targeting mutated p53 such as APR-246 demonstrated activity in different types of *TP53*mut malignancies. In ALL however, targeting mutant *TP53* has so far not been addressed.
Aims: To evaluate mutant *TP53* as target for directed anti-leukemia therapy in ALL.
Methods: Patient-derived primograft samples established in our NOD/SCID/huALL mouse model were analyzed for *TP53*mut by denaturating high-performance liquid chromatography and validated by Sanger sequencing. Sensitivities of ALL cells in response to different drugs and combinations were assessed analyzing half maximal inhibitory concentrations (IC50) and combination indices (CI). Apoptosis was detected by caspase-3 activation and Annexin-V/propidium iodide positivity. Activation of p53 was analyzed by phosphorylation of p53 (p53pSer15, flowcytometry), expression of p53 downstream molecules and confirmation of phosphorylation was carried out by western blot analysis. p53 deficient cell lines were generated by lentiviral shRNA mediated knock-down.
Results: Of 62 primograft samples, 4 cases with *TP53*mut (6.5%) were identified, corresponding to reported numbers in clinical studies. In parallel, we analyzed 6 BCP-ALL cell lines and identified 2 *TP53*mut and 4 wild type (*TP53*wt) lines. We analyzed the effects of the DNA damaging agent doxorubicin and the p53 targeting small molecule APR-246 (kindly provided by Aprea, Stockholm, Sweden) and observed an insensitivity to doxorubicin in *TP53*mut cell lines and primografts (mean IC50: 100 nM and >65 nM) compared to sensitive *TP53*wt cell lines and primografts (mean IC50: 9 nM and 7.4 nM). In contrast, *TP53*mut ALL cells were highly sensitive to APR-246 (mean IC50: 4.5 µM and 4.4 µM), compared to insensitivity of *TP53*wt cell lines and primografts (mean IC50: 58.3 µM and >90 µM). Increased Annexin-V/PI positivity and caspase-3 activity upon APR-246 exposure indicated apoptosis as mechanism of APR-246 mediated cell death. In order to investigate the specificity of APR-246 for p53, we investigated APR-246 mediated cell death induction in 2 genetically modified, p53 deficient ALL cell lines. No cell death induction could be detected upon p53 absence in contrast to high APR-246 sensitivity in the control transduced cell lines, clearly indicating dependency of APR-246 on the presence of mutant p53. Most importantly, a clear activation of p53 was identified upon APR-246 exposure in *TP53*mut but not *TP53*wt leukemias indicated by increased p53 phosphorylation and increased expression of the p53 target molecules NOXA and PUMA, pointing to restoration of p53 function and induction of downstream signaling in *TP53*mut ALL by APR-246. Moreover, we investigated the ability of APR-246 to re-sensitize *TP53*mut, DNA damage resistant ALL. Upon exposure of *TP53*mut ALL cell lines and primografts to combinations of APR-246 and doxorubicin, a strong synergism and re-sensitization to DNA-damage induced cell death was observed.
Summary/Conclusions: We provide evidence that APR-246 specifically targets *TP53*mut BCP-ALL leading to re-activation of p53, apoptosis induction, and re-sensitization of DNA-damage resistant *TP53*mut ALL cells. Thus, targeting mutated p53 provides a promising novel strategy for therapeutic intervention in this high-risk subtype of BCP-ALL.

P159

DETERMINING THE MUTATIONAL LANDSCAPE OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA REVEALS NR3C1 DELETIONS AND ACTIVATING MUTATIONS IN IL7R SIGNALING AS CAUSE OF STEROID RESISTANCE

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Background: Response to therapy in children with acute lymphoblastic leukemia (ALL) including *in vitro* or *in vivo* steroid response is a strong predictor for survival and cure. T-ALL patients in particular have a high risk to relapse and are refractory to further treatment due to acquired therapy resistance. The mechanisms that underlie steroid resistance are poorly understood.
Aims: This study aimed to investigate the mutational landscape of pediatric T-ALL patients to identify molecular disease mechanisms underlying steroid resistance.
Methods: Whole-genome sequencing (WGS) of 13 T-ALL diagnostic tumor-remission pairs in the pilot group was performed by Complete Genomics to detect chromosomal rearrangements and somatic nucleotide variations. Targeted exome resequencing of 251 genes was performed in a validation cohort of 82 pediatric T-ALL samples, and mutation data was integrated with loss-of-heterozygosity data as obtained by array-comparative genomic hybridization for 53 T-ALL cases. Integrated data was related to clinical outcome and *in vitro* drug responses.
Results: WGS revealed intra-chromosomal (range of 5-25 per patient) and inter-chromosomal (range of 2-11) breakpoint junctions as consequence of deletions, duplications, inversions, translocations or complex rearrangements. Integrated mutation and copy-number data revealed 151 mutated genes (range of 0-51). Deletions in *NR3C1*—which are common in 5q chromosomal deletions in patients with the ALL subtype ETP-ALL (early thymus progenitor ALL)—are associated with steroid resistance. Moreover, we found that mutations in interleukin-7 receptor (IL7R) signaling molecules associated with steroid resistance while leaving cellular sensitivity levels to other chemotherapeutics unchanged. IL7R signaling mutations do not impair NR3C1 function, but activate MEK-ERK and PI3K-AKT pathways and downstream anti-apoptotic proteins BCL-XL and MCL1 while inhibiting GSK3B, a key regulator of steroid-induced pro-apoptotic BIM. Importantly, inhibitors of IL7R signaling reversed steroid resistance in T-ALL cells *in vitro*, whereas a GSK3B inhibitor conferred steroid resistance.
Summary/Conclusions: We revealed the mutational landscape of pediatric T-ALL patients. *NR3C1* deletions and IL7R signaling mutations were identified as cause for steroid resistance. This study supports the recommendation for the clinical application of IL7R signaling inhibitors to restore—or improve—steroid sensitivity of T-ALL cells.

P160

DEVELOPMENT OF A NOVEL FIRST-IN-CLASS ORAL INHIBITOR OF THE NOTCH PATHWAY

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Background: NOTCH signaling is a developmental pathway known to play critical roles during embryonic development as well as for the regulation of self-renewing tissues. Aberrant activation of NOTCH signaling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmarks of cancer. Over activation of NOTCH in human cancers can be a consequence of over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations leading to constitutive activation of the pathway.
Aims: Given the importance of Notch signaling in human cancers, several therapeutic approaches have been utilized to block NOTCH signaling. Two of these strategies are; a) the use of monoclonal blocking antibodies (Mabs) against NOTCH ligands and receptors and b) the use of small molecule □-secretase inhibitors (GSIs). However, these approaches can only be effective if tumor cells express full-length ligand or receptor molecules. On the contrary, in human cancers harbouring NOTCH gene fusion due to chromosomal translocations, the use of Mabs and GSIs will have very limited clinical benefits. A third, yet not fully explored approach could be the blockage of NOTCH signalling by targeting the most downstream event in the NOTCH cascade i,e NOTCH transcriptional activation complex using small molecule inhibitors.
Methods: Here we report discovery and identification of a novel, orally-active small molecule inhibitor, named CB-103, of the NOTCH pathway that blocks NOTCH signaling by targeting the NOTCH transcriptional activation complex in the nucleus.
Results: CB-103 has shown the ability to block NOTCH signalling in human T cell acute lymphoblastic leukemia cancer cell lines, induce neurogenic phenotype in drosophila, induce muscle cell differentiation and inhibit NOTCH dependent cellular processes in mice. Furthermore, CB-103 has shown a remarkable activity in *exvivo* and *invivo* patient derived models of human T-ALL harbouring activation of the NOTCH pathway. In addition, CB-103 exhibit anti-tumor efficacy in a xenograft model of human triple negative breast cancer resistant to GSIs and Mabs against NOTCH ligands/receptors.
Summary/Conclusions: Based on *in vivo* pharmacokinetic/ADME studies, CB-103 has been nominated as development candidate for further preclinical and clinical development.

P161

INTRINSIC AND SYNERGISTIC ACTIVITY OF ABT-199 AND OVERCOMING ABT-199 RESISTANCE BY THE CDK-INHIBITOR DINACICLIB IN B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Therapy resistance and treatment failure in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) are closely associated with deficient cell death pathways. Anti-apoptotic Bcl-2 family proteins are central apoptosis regulators, thereby serving as promising targets for novel, directed therapies. ABT-199 binds selectively to Bcl-2, inhibits its anti-apoptotic function and releases pro-death Bcl-2 family molecules leading to apoptosis induction. ABT-199 demonstrated activity in different hematological malignancies, pre-clinically and in early clinical trials. However, resistance for ABT-199 highlights the need for predictive markers and effective combination treatment strategies.
Aims: We addressed the effectivity of ABT-199 on BCP-ALL including pre-clinical *in vivo* evaluation and addressed resistance mechanisms and strategies to overcome ABT-199 insensitivity.
Methods: The effects of different drugs or combinations on BCP-ALL cell lines (n=6) and patient-derived BCP-ALL primografts (n=16) established by transplantation of primary patient ALL cells onto NOD/SCID mice were investigated analyzing half maximal inhibitory concentrations (IC50) and combination indices (CI). Anti-leukemic activity of ABT-199 was evaluated pre-clinically *in vivo*. Expression of apoptosis regulators was detected by western blot analysis. Mcl-1 deficient cell lines were generated by CRISPR/Cas gene engineering.
Results: A high sensitivity for ABT-199 was identified in 4 of 6 BCP-ALL cell lines with nanomolar IC50 values (mean 212 nM) in contrast to ABT-199 insensitivity in two other lines (IC50 >1μM). Similarly, a high sensitivity for ABT-199 was identified in the majority of ALL primografts (14 of 16, IC50 2 to 156 nM) with two insensitive cases (IC50 >1 μM). Of note, PBMCs from healthy donors also showed ABT-199 insensitivity. We assessed the expression of the apoptosis regulating molecules Bcl-2 and Mcl-1 and found high Bcl-2 levels in ABT-199 sensitive compared to low Bcl-2 levels in resistant ALL. In contrast, ABT-199 resistant leukemias showed high Mcl-1 expression and low levels were found in ABT-199 sensitive cases. Interestingly, CRISPR/Cas9-mediated knockout of Mcl-1 clearly sensitized ABT-199 insensitive cell lines for ABT-199 mediated cell death, indicating the role of Mcl-1 as mediator but also marker for ABT-199 resistance. Most interestingly, the CDK inhibitor dinaciclib induced rapid down-regulation of Mcl-1 at low nanomolar concentrations leading to sensitization for ABT-199 in resistant cell lines and primograft ALL. We also combined ABT-199 with chemotherapeutic agents used for remission induction in current therapy regimens. Co-exposure of ALL cells to vincristine, dexamethasone and asparaginase combined with ABT-199 showed a clear synergistic effect, both in cell lines and primograft ALL. Moreover, in a preclinical setting, recipient animals bearing a high-risk, t(11;19) leukemia were treated with ABT-199 or solvent. Importantly, a clear reduction of leukemia load was observed upon ABT-199 *in vivo* therapy compared to vehicle treated recipients ($p<0.01$).
Summary/Conclusions: Taken together, our data show apoptosis induction and high efficacy of ABT-199 as single compound, both *ex vivo* and pre-clinically *in vivo*, and synergy upon combination with conventional induction chemotherapy. Importantly, Mcl-1 was identified as mediator and indicator for ABT-199 resistance. Most importantly, the CDK-inhibitor dinaciclib was found to down-regulate Mcl-1 leading to sensitization for ABT-199 in previously resistant, Mcl-1 high expressing leukemia cells.

P162

LOSS OF PIGH EXPRESSION FREQUENTLY RESULTS IN A GPI-NEGATIVE SUBCLONE LACKING CD52 MEMBRANE EXPRESSION, CONFERRING ALEMTUZUMAB RESISTANCE TO B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: To improve treatment outcome of patients with B-cell acute lymphoblastic leukemia (B-ALL), several immunotherapeutic approaches have been developed in recent years. *E.g.*, direct targeting of CD19 or CD20 by (bispecific) antibodies or chimeric antigen receptors result in effective control of the disease. In contrast, introduction of alemtuzumab which targets the glycophosphatidylinositol (GPI)-anchored CD52 protein resulted in unsatisfactory clinical efficacy, potentially due to development of escape variants. Indeed, in previous studies (Nijmeijer et al, 2010) we have found outgrowth of CD52-neg-

ative escape variants following alemtuzumab treatment in a mouse model engrafted with human B-ALL. Further analysis showed that these variants expressed normal CD52 mRNA levels, but lacked CD52 membrane expression due to loss of GPI-anchor expression.
Aims: To unravel the mechanism underlying the loss of CD52/GPI expression in B-ALL
Methods: The presence and frequency of CD52/GPI-negative cells was analyzed by flowcytometry in peripheral blood (PB; n=10) and bone marrow (BM; n=8) samples taken with informed consent from B-ALL patients at diagnosis. Diagnosis samples of patients with chronic lymphocytic leukemia (CLL, n= 5), mantle cell leukemia (MCL, n=5) and hairy cell leukemia (HCL, n=6), and PB samples from 5 healthy donors were similarly analyzed in parallel. To investigate the mechanism of GPI loss, gene expression analysis was performed for the 26 genes that comprise the GPI anchor biosynthesis pathway in GPI positive and GPI negative B-ALL populations purified by flowcytometric cell sorting.
Results: To study the presence and frequency of CD52/GPI-negative cells at diagnosis, we analyzed PB and BM samples from B-ALL patients at diagnosis by flowcytometry. GPI-negative cells were present in 6/10 PB and 5/8 BM samples and comprised between 0.01% and 4.98% of the B-cell population. These obvious GPI-negative B-cell populations were not found in CLL (n=5), MCL (n=5), and HCL (n=6) diagnosis samples, or in healthy donors (n=5). To investigate the mechanism of GPI-anchor loss, gene expression analysis was performed for the 26 GPI biosynthesis pathway genes. In contrast to purified GPI-positive B-ALL populations, loss of PIGH mRNA expression, but not of any of the other genes, was found in all GPI-negative populations (n=9). To validate the relevance of this finding, GPI-negative and GPI-positive B-ALL cell cultures were generated from diagnosis material (n=2) and transduced with a retroviral construct encoding PIGH. Restored GPI-anchor expression and coinciding CD52 membrane expression was observed in the GPI-negative B-ALL cultures upon transduction with PIGH, but not empty vector. To explore the mechanism underlying the loss of PIGH mRNA expression in CD52/GPI-negative B-ALL cells, we performed DNA screening and assessed promoter CpG methylation, comparing GPI-negative with GPI-positive B-ALL cultures from the same individual (n=2). These analyses revealed that in GPI-negative cultures both alleles of the PIGH gene were present, unmutated and intact, but with a heavier methylated promoter region compared to the GPI-positive counterparts. Additionally, a 14 day treatment of GPI-negative B-ALL cultures with the demethylating agent 5-Azacitidine resulted in re-expression of the GPI-anchor
Summary/Conclusions: In conclusion, the majority of B-ALL patients presented a CD52/GPI-negative, alemtuzumab resistant, B-ALL population already at diagnosis. These cells lost PIGH expression, a key component in GPI-anchor synthesis. This is not due to genomic instability, but to epigenetic downregulation. Combining epigenetic modulatory drugs with alemtuzumab might be a promising therapeutic strategy to prevent outgrowth of CD52/GPI-negative escape variants in B-ALL.

P163

MUTATIONAL STATUS OF NRAS, KRAS AND PTPN11 GENES IS ASSOCIATED WITH GENETIC/CYTOGENETIC FEATURES IN CHILDREN WITH B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The activating mutations in RAS signaling pathway including *NRAS*, *KRAS* and *PTPN11* genes have been described in B-precursor acute lymphoblastic leukemia (ALL). However, the correlation of RAS pathway gene mutations with recurrent genetic abnormalities was not addressed.
Aims: We aimed to investigate the frequencies, and the association with genetic/cytogenetic abnormalities as well as prognostic relevance of RAS pathway mutations in Taiwanese children with B-precursor ALL, the largest cohort in Asians.
Methods: Between 1995 and 2012, bone marrow samples at diagnosis from 538 children were studied. Mutations at codons 12, 13, and 61 in exons 1 and 2 of *NRAS* and *KRAS* genes, as well as the entire coding region of *PTPN11* were analyzed using PCR-based assay followed by direct sequencing. The mutational status of each gene was correlated with the clinico-hematological features, recurrent genetic abnormalities, and outcomes for those treated with TPOG-ALL-2002 protocol (n=348).
Results: The frequencies of *NRAS*, *KRAS*, and *PTPN11* mutations were 10.5% (56/533), 10.1% (54/533), and 3.4% (18/529), respectively. Mutations of *NRAS*, *KRAS*, and *PTPN11* were mutually exclusive with rare exception, including 4 co-occurrence of *NRAS* and *KRAS* mutations, and 2 co-existence of *KRAS* and *PTPN11* mutations. Together, 23.0% of B-precursor ALL patients had gene mutations involving RAS signaling pathway. No differences in age, Hb level, WBC or platelet counts were observed in patients with or without *NRAS* and *PTPN11* mutations. The frequency of *PTPN11* mutations was significantly higher in girls than in boys (1.4% *vs* 6.0%, *P*=0.006). Patients with *KRAS* mutations presented with significantly younger age (*P*=0.004) and lower platelet counts (*P*=0.007). Only one out of 21*BCR-ABL1* ALL had a mutation (*NRAS*) in RAS pathway. *ETV6/RUNX1* was associated with a lower frequency of *NRAS* mutations (2.2% *vs* 12.3%, *P*=0.002) and a trend of less *PTPN11* mutations (0% *vs* 4.1%, *P*=0.053); none of patients with *TCF3/PBX1* had *KRAS* mutation (*P*=0.024) whereas 2 of the 3 hypodiploid ALL had *KRAS* mutations (*P*=0.028); *KRAS* mutations occurred more frequently in patients with *MLL* rearranged (23.5% *vs* 9.2%, *P*=0.014, but the difference was not significant if we excluded infant ALL (n=30) (20% *vs* 8.7%, *P*=0.223). *NRAS* mutations were more commonly detected in hyperdiploid ALL (18.0% *vs* 9.5%, *P*=0.069). Patients without specific genetic/cytogenetic subtypes had a significantly lower frequency of *NRAS* mutations as compared to those without any of these recurrent genetic abnormalities (7.5% *vs* 13.2%, *P*=0.048) and a higher rate of *PTPN11* mutations (5.0% *vs* 1.6%, *P*=0.051). There were no differences in 5-year EFS and OS with regard to mutational status of *NRAS* (70.7% *vs* 79.0%, *P*=0.575; 89.6% *vs* 82.3%, *P*=0.863, respectively) and of *PTPN11* (80.8% *vs* 77.7%, *P*=0.490; 90.0% *vs* 82.2%, *P*=0.317, respectively). *KRAS* mutations were associated with inferior outcomes (66.0% *vs* 79.7%, *P*=0.039 for EFS and 74.8% *vs* 84.0%, *P*=0.058 for OS) but there was no difference in non-infant ALL (*P*=0.381 for EFS and *P*=0.635 for OS).
Summary/Conclusions: Patients with recurrent genetic abnormalities had rare occurrence of RAS pathway mutations in non-infant pediatric B-precursor ALL. *NRAS* mutations were more frequently detected in patients with hyperdiploidy, whereas *KRAS* mutations were highly associated with infant ALL with *MLL* rearranged. (Grants support: MMH-E-99009, MOST103-2314-B-195-008-MY3, MOST104-2314-B-182-032-MY3, OMRPG3C0021 and grant from Terry Fox Foundation).

P164

BABOON ENVELOPE PSEUDOTYPED LENTIVIRAL VECTORS: A HIGHLY EFFICIENT NEW TOOL TO GENETICALLY MANIPULATE T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA INITIATING CELLS

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Background: T-Acute Lymphoblastic Leukaemia (T-ALL) is a heterogeneous and aggressive hematologic cancer arising from the malignant proliferation of T-cell progenitor cells, arrested at different stages of development. Although recently developed multi-agent chemotherapy protocols significantly improved the clinical outcome of this disease, the prognosis of patients undergoing relapse or therapy resistance remains poor. Since T-ALL chemo-resistance is linked to the activation of pathways that promote and sustain the self-renewal of leukemic initiating cells (LICs), it becomes imperative to develop novel therapies to eradicate these cells. To accomplish this objective it is necessary to perform pre-clinical, functional studies with primary T-ALL patient samples, a challenging task given that tools enabling an efficient genetic manipulation of primary T-ALL cells are still lacking.
Aims: To develop novel strategies to genetically manipulate T-ALL leukaemia initiating cells.
Methods: Fresh or thawed primary T-ALL samples were transduced with lentiviral vectors pseudotyped with a baboon envelop chmeric glycoprotein (BaEV). Two days post-transduction, 500.000-700.000 transduced cells were injected into cohorts of primary NSG(NOD.Cg-Prkdc(scid)Il2rg(tm1Wjll)/SzJ) mice to verify stable gene expression and T-ALL development from the transduced clones. The leukemic engraftment of the transduced cells was than evaluated by FACS analysis, based on GFP, CD45 and CD7 expression in the bone marrow, spleen and peripheral blood. To further assess the leukemic initiating ability of the transduced cells, 500.000-700.000 bone marrow cells issued from primary animals were injected into serial cohorts of NSG mice.
Results: Our work revealed that lentiviral vectors pseudotyped with a Baboon retroviral envelope glycoprotein (BaEV-LVs) are excellent tools to genetically modify T-ALL leukaemia initiating cells. These lentiviral vectors enabled high-level transduction rates (40-80%) at low multiplicity of infection (MOI=10) as they entered primary T-ALL blasts using ASCT1 and ASCT2, two aminoacid transporters that were highly expressed on all T-ALL subtypes. The transduced blasts engrafted cohorts of primary, secondary and tertiary mice, where they infiltrated bone marrow, spleen and peripheral blood, thus demonstrating that BaEV-LVs target leukemic-initiating cells. Importantly, this high performance was not linked to particular oncogenic features of the primary blasts not to a specific stage of maturation arrest. Competitive xenograft experiments, moreover, demonstrated the transduced clones did not acquire a selective growth advantage nor were outcompeted by the not-transduced clones, as evidenced by the relative ratio between GFP positive and GFP negative cells, which remained stable in all the mice serially transplanted. Hence, BaEV-LV mediated transduction does not alter LICs self-renewing pathways. This feature is an

essential pre-requisite to functionally investigate the signalling cascades involved in T-ALL initiation and development.
Summary/Conclusions: BaEV-LV vectors are essential and highly efficient tools to manipulate leukemic-initiating cells and to functionally investigate the molecular pathways involved in T-ALL development and clonal evolution.

P165

HIGH INCIDENCE OF PHILADELPHIA CHROMOSOME-LIKE ACUTE LYMPHOBLASTIC LEUKEMIA (PH-LIKE ALL) IN OLDER ADULTS WITH B-ALL

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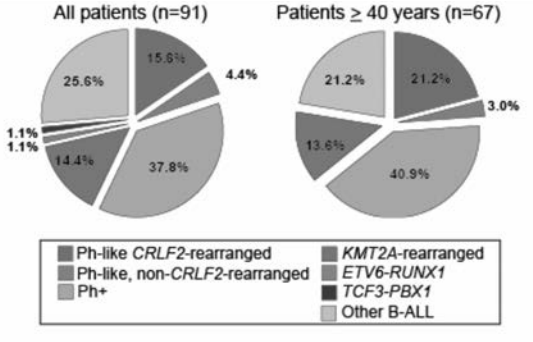
Background: Tyrosine kinase inhibitor (TKI) treatment of patients with Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia (Ph+ B-ALL) improves response and survival in younger adults and has allowed de-escalation of conventional chemotherapy, particularly in older patients. Similar therapeutic approaches may also benefit patients with the Philadelphia chromosome-like (Ph-like) B-ALL subtype recently described in children and adolescents/young adults (AYA), as these leukemias are driven by activating mutations in kinase-associated signaling pathways. The frequency of adult Ph-like B-ALL is largely unknown, however.
Aims: We analyzed diagnostic B-ALL specimens from adult patients (range 18-88 years) by a validated, highly sensitive 15-gene low-density gene expression array (LDA) to determine the frequency of Ph-like ALL.
Methods: We analyzed 90 cryopreserved B-ALL samples from the institutional tissue banks at the University of Pennsylvania and University of Michigan by LDA to identify specimens with a kinase-active gene expression profile, as previously reported (Harvey, *et al. Blood* 2013, 123(21):826a). All samples were tested by reverse transcriptase-polymerase chain reaction (RT-PCR) for *BCR-ABL1* fusion. LDA signature-positive specimens without detected *BCR-ABL1* transcript (Ph-like ALL) were categorized as *CRLF2* (cytokine receptor-like factor 2)-overexpressing or non-overexpressing using LDA quantification of expression levels. *CRLF2*-overexpressing specimens were subsequently assessed for *IGH-CRLF2* and *P2RY8-CRLF2* rearrangements and *JAK1* and *JAK2* mutations via FISH, PCR, or RT-PCR with Sanger sequencing.
Results: By RT-PCR, 37.8% (34/90) of ALL specimens were *BCR-ABL1*+. Additionally, we identified a 20.0% (18/90) incidence of Ph-like ALL with a median age at diagnosis of 43 years (range 19-64). 77.8% (14/18) of the Ph-like ALL specimens harbored *CRLF2* rearrangements, including 7 patients with concomitant *JAK2* mutations. Using multiplexed RT-PCR, the 4 non-*CRLF2*-overexpressing Ph-like specimens were then evaluated for 39 published Ph-like kinase fusions; no recurrent gene fusions were identified. Correlative biology studies confirmed activation of JAK/STAT and/or ABL pathway signaling in primary Ph-like ALL specimens, as well as therapeutic efficacy of JAK or ABL kinase inhibition in adult Ph-like ALL patient-derived xenograft models.



	Ph-Like ALL (n=18)	Ph+ ALL (n=34)	KMT2A-R ALL (n=13)	Other B-ALL (n=25)
Mean WBC (10^3 cells/uL) +/- SEM	69.1 (+/- 29.7)	76.3 (+/- 16.8)	95.1 (+/- 21.7)	35.7 (+/- 10.3)
Median WBC (10^3 cells/uL)	28.6	37.7	90.2	11.6
Mean age in years (range)	43.2 (19-63)	54.6 (22-88)	45.8 (18-71)	42.3 (18-74)
% Male	66.7%	61.8%	36.4%	44 %
% Hispanic/Latino	16.7%	5.9%	0.0%	7.1%

Figure 1.

Summary/Conclusions: We identified a Ph-like gene expression signature in 20% of adults with B-ALL. Importantly, we also demonstrate for the first time that the majority of adult B-ALL is associated with activated kinase signaling. Unlike pediatric/AYA Ph-like ALL, in which kinase fusions occur frequently, *CRLF2* rearrangement is the predominant genetic lesion in adult Ph-like ALL. We propose that adult ALL diagnostic testing algorithms include flow cytometric assessment of surface CRLF2 (thymic stromal lymphopoietin receptor) expression, a surrogate marker for *CRLF2* rearrangement, with subsequent genetic confirmation. Finally, we demonstrate a substantial population of patients ≥40 years of age with non-Ph+ B-ALL who may also benefit from TKI therapies. The efficacy of such treatments should be evaluated via appropriate clinical trials, as clinical translation of our findings will potentially transform therapeutic approaches for adults with Ph-like ALL.

Acute lymphoblastic leukemia - Clinical 1

P166

TOLERANCE, COMPLIANCE AND EFFICACY OF L-ASPARAGINASE DURING INDUCTION PHASE IN ADULT PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA: EXPERIENCE OF THE GRAALL-2005

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Background: In adults aged 18-60 years, many acute lymphoblastic leukemia (ALL) protocols have now incorporated L-asparaginase (ASPA) as part of induction regimen. As in children, adult patients are thus often exposed to pancreatitis, thrombosis, liver injury and allergic reactions. Thus some investigators do not use ASPA at all, like in the Hyper-CVAD regimen. In the GRAALL network, most of the centers started to use ASPA during induction when the pediatric-inspired GRAALL protocol was introduced in 2003.
Aims: The aim of this study was to report the real-life experience of the use of ASPA in the GRAALL-2005 trial and to identify factors associated with 1) main ASPA-related Adverse Events (AEs) and 2) the frequency and the impact of suboptimal ASPA dose administration.
Methods: Between 2006 and 2014, 787 adult patients with newly diagnosed Ph1-negative ALL were included in the multicenter French-Belgian-Swiss GRAALL-2005 trial. After a 7-day steroid prophase, patients received a 5-drug sequential induction including prednisone (PDN), vincristine (VCR), daunorubicine (DNR), cyclophosphamide (CPM) and ASPA (6000 IU/sqm/day for 8 days). Patients with induction failure received a high-dose cytarabine-based salvage course. AEs were assessed according to WHO classification. Four patients were excluded due to the lack of data.
Results: The median age of this cohort of 783 patients (505 BCP- and 278 T-ALL) was 36.1 years (range: 18.1-60.0). Median body mass index (BMI) was 23.6 kg/sqm (range: 15.7-46.3). Initial median WBC was 11.8 G/L (range: 0.4-645) and 55 patients had CNS involvement (7.0%). A complete remission (CR) was achieved in 720/783 (92.0%). A resistance to first induction phase was observed in 36/738 (4.9%) patients evaluable for CR. A grade 3-4 liver toxicity was observed in 272 patients (34.6%), a grade 3-4 pancreatitis in 43 patients (5.5%), and a cerebral venous thrombosis (CVT) in 25 patients (3.3%). An older age was significantly associated with an increased incidence of liver toxicity but a lower incidence of CVT. Pancreatitis was not associated with age. A high BMI was associated with liver toxicity and pancreatitis. In multivariate analysis, age, BMI, CNS involvement, and BCP-phenotype were associated with liver toxicity; BMI and female gender were associated with pancreatitis. During induction phase, patients received 97.8% of ASPA scheduled dose, 99.0% if younger than 45 years old but only 84.8% if older (p<.0001). In comparison, no difference in the administration of myelosuppressive drugs (DNR, CPM) was observed. In multivariate analysis, patient-related variables associated with less than 90% of scheduled ASPA administration (41% of patients) were older age, high BMI, and high ECOG score. In this model, center enrollment volume but also inclusion time period were statistically associated with a higher rate of ASPA administration, suggesting a learning effect. Finally, covariates independently associated with induction failure were higher WBC (OR 1.04 [95%CI, 1.01-1.07]), slower bone marrow clearance at D8 (OR 0.07 [95%CI, 0.02-0.20]), lower ASPA injection number (OR 0.72 [95%CI, 0.60-0.85]), and lower VCR injection number (OR 0.69 [95%CI, 0.55-0.88]), excluding all other induction drug dose variation.
Summary/Conclusions: During ALL induction, most ASPA-related toxicities seen in adult patients are correlated to age and BMI. Experience in the management of ASPA administration may enhance protocol compliance and thus contribute to reduce induction failure risk.

P167

EFFICACY AND SAFETY OF INOTUZUMAB OZOGAMICIN IN OLDER PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA ENROLLED IN THE GLOBAL PHASE 3 RANDOMIZED CONTROLLED INO-VATE TRIAL

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, has demonstrated superior response *vs* standard care for relapsed/refractory acute lymphoblastic leukemia (ALL) in the ongoing phase 3 INO-VATE trial (complete remission [CR]/CR with incomplete hematologic recovery [CRi], 81% [95% CI; 72–88]; minimal residual disease [MRD] negativity in responders, 78% [68–87]; median remission duration [DoR], 4.6 [3.9–5.4] months).
Aims: To assess the efficacy and safety of InO in patients with relapsed/refractory ALL aged ≥55 *vs* <55 years
Methods: Per protocol, the intent-to-treat analyses of CR/CRi included the first 218 of 326 patients randomized (ITT218). The safety population included 139 patients who received ≥1 InO dose (max 1.8 mg/m^2/cycle [0.8 mg/m^2 on day 1; 0.5 mg/m^2 on days 8 and 15 of a 21–28 day cycle for ≤6 cycles]). MRD negativity was assessed by central flow cytometry (<0.01%). Data as of October 2, 2014 are presented (trial ongoing). Informed consent was obtained from all patients.
Results: 109 patients in the ITT218 received InO (median age, 47 [range, 18–78] years; patients ≥55 years, 43 [39%]). Remission rates and DoR were similar whereas MRD-negativity rates in responders were numerically higher in older patients **(Table)**. In the safety population, grade ≥3 adverse events (AEs) were most frequently cytopenias (neutropenia, 46%; thrombocytopenia, 37%; febrile neutropenia, 24%); these grade ≥3 AEs were more common in patients ≥55 (n=53) *vs* <55 years (n=86): thrombocytopenia (49% *vs* 29%), neutropenia (53% *vs* 42%), febrile neutropenia (28% *vs* 21%). Patients ≥55 *vs* <55 years had similar discontinuation rates due to AEs (both 17%). For patients ≥55 *vs* <55 years, any grade hepatobiliary AE rates were similar (both 26%) and included, hyperbilirubinemia (both 15%), and veno-occlusive liver disease (VOD) including post-SCT VOD (both 11%; 2 fatal in patients <55 years [1 after second SCT]).

Table 1.

% (95% CI)*	<55 years (n=66)	≥55 years (n=43)
CR/CRi[b]	80 (69–89)	81 (67–92)
CR	35 (24–48)	37 (23–53)
CRi	45 (33–58)	44 (29–60)
MRD-negativity in responders	74 (60–85)	86 (70–95)
Median DoR, months	4.6 (2.8–5.4)[c]	4.4 (3.6–5.9)[d]

*ITT218; [b]Best response in 1–6 cycles; [c]n=51; [d]n=34

Summary/Conclusions: InO was highly effective in older patients with relapsed/refractory ALL for whom treatment options are currently limited; responses and safety profiles were generally similar to younger patients and the overall study population.

P168

THE COMBINATION OF BORTEZOMIB AND CHEMOTHERAPY IS AN EFFECTIVE TREATMENT TO RE-INDUCE REMISSION IN RELAPSED/REFRACTORY B-CELL PRECURSOR OR T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Achievement of a new complete remission (CR) in relapsed/refractory pediatric acute lymphoblastic leukemia (ALL) is hampered by a low response rate and high toxicity, especially after second or subsequent relapses. Bortezomib, the first proteasome inhibitor approved by Food and Drug Administration for multiple myeloma and relapsed non-Hodgkin lymphoma, showed preclinical activity against ALL blasts. Moreover, a phase II study reported by the Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) study group demonstrated that the combination of bortezomib with vincristine, dexamethasone, pegylated asparaginase and doxorubicin carried acceptable toxicity and good efficacy in patients with relapsed B-cell precursor ALL who failed 2-3 previous regimens (Messinger, Blood 2012).
Aims: In order to improve the CR rate of children with relapsed/refractory ALL, we evaluated the use of bortezomib in combination with vincristine, dexamethasone, pegylated asparaginase and doxorubicin, as a salvage/re-induction

treatment in 24 and 7 children with B-cell precursor and T-cell ALL (2 ETP), respectively. Patient characteristics are reported in Table I.
Methods: Bortezomib (1.3 mg/m^2/dose) was administered intravenously on day 1, 4, 8, and 11. Dexamethasone 10 mg/m^2/day was given orally for 14 consecutive days. Doxorubicin 60 mg/m^2 was given intravenously on day 1. Vincristine 1.5 mg/m^2/dose (2 mg maximum dose) was administered intravenously on day 1, 8, 15, and 22. Pegylated asparaginase 2500 units/m^2/dose was given intravenously weekly for 4 doses. All patients received intrathecal (IT) cytarabine on day 1. Patients without CNS involvement were given IT methotrexate on day 15. Patients with CNS involvement at time of treatment were given IT methotrexate, methylprednisolone, and cytarabine on day 8, 15, and 22.
Results: Between February 2012 and July 2015, 24 pediatric patients with relapsed B-cell precursor and 7 with T-cell ALL were given the treatment detailed above at the Hematology/Oncology Department of the IRCCS Bambino Gesù Children's Hospital, Rome, Italy. Seventeen patients were males and 14 females, median age at diagnosis and at time of last leukemia relapse being 6.7 years (range 1-20.9) and 9.9 years (2.6-24.1), respectively. Four patients were CNS-positive at diagnosis, while 4/31 experienced a combined bone marrow (BM) and extramedullary relapse (2 CNS, 1 kidney and 1 gut, respectively). Fourteen children had previously received allogeneic hematopoietic stem cell transplantation (HSCT, see table 1 for details on the donor). Grade 3 (CTCAE v4.03) peripheral motor and sensory neuropathy developed in 5 (16%) patients. Six out of the 31 patients (19.5%) experienced severe infections (2 sepsis due to *Geotrycum*, 3 to *Candida* and 1 to multidrug resistant *Klebsiella Pneumoniae*). Despite the introduction of broad-spectrum antibacterial and antifungal therapy, 4 of them (13%) died (2 each with *Geotrycum and Candida* infection) without recovering neutrophils. Twenty patients achieved CR (10 with a MRD<0.1%), this leading to a 64.5% overall response rate. Interestingly, in our cohort, not only B-cell precursor patients had a good outcome (15/24, 62.5% CR rate), but also T-cell ALL responded well to this combination of drugs (5/7, 71.4% CR rate). Fifteen of these patients had a long-lasting CR allowing them to receive HSCT resulting in a 2-year Overall Survival (OS) of 37.1% (SE 13.2).

Table 1.

Sex	n (%)
Male	17 (55)
Female	14 (45)
Median age at diagnosis, years (range)	6.7 (1-20.9)
Median age at relapse	9.9 (2.6-24.1)
Lineage	
B-cell precursor ALL	24 (77)
T-cell ALL	7 (23)
CNS involvement at diagnosis	4 (13)
CNS involvement at relapse	2 (6.5)
Lines of therapy before TACL schedule*	
1-2 lines	13 (42)
> 2 lines	18 (58)
Previous HSCT before TACL schedule	
No	17 (55)
Yes	14 (45)
Type of donor	
HLA-identical sibling	5 (36)
MUD	4 (28)
Haplo	5 (36)
Toxicity	
Days to ANC > 500/µL, median (range)	18 (10-56)
Days to platelets > 50 000/µL, median (range)	24 (14-43)
Neuropathy	5 (16)

* front-line therapy is not counted

Summary/Conclusions: In conclusion, the regimen of bortezomib combined with a 4-drug (vincristine dexamethasone, pegylated asparaginase and doxorubicin) re-induction therapy is effective for children with relapsed/refractory B-cell precursor or T-cell ALL. Considering the risk of life-threatening/fatal infections in a such vulnerable population, an intensive antibacterial and antifungal prophylaxis is strongly recommended.

P169

EFFICACY OF FRONT-LINE TREATMENT COMBINATIONS WITH PONATINIB *VERSUS* 1ST- AND 2ND-GENERATION TYROSINE KINASE INHIBITORS FOR PH+ ACUTE LYMPHOBLASTIC LEUKEMIA: A META-ANALYSIS AND META-REGRESSION

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Background: The combination of a tyrosine kinase inhibitor (TKI) and either chemotherapy or corticosteroids is effective induction therapy in *de novo* Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL). Ponatinib is a multi-targeted TKI that is a more potent BCR-ABL1 inhibitor than previous generations of TKIs and selectively suppresses T315I resistance. However, evidence on the comparative effectiveness of ponatinib relative to other TKIs in *de novo* Ph+ ALL has not been well-established.
Aims: This study aims to evaluate the effectiveness, as measured by complete molecular response (CMR) and 2-year overall survival (OS), of front-line treatment combinations with ponatinib *versus* first- and second-generation TKIs (*i.e.*, imatinib, dasatinib, and nilotinib) in Ph+ ALL.
Methods: Nineteen Phase 2 through 4 trials and one retrospective analysis of front-line Ph+ ALL treatments were identified from a 2015 targeted literature review published by the American Society of Hematology. One study used ponatinib with chemotherapy and nineteen studies used a combination of first- or second-generation TKIs with either chemotherapy or corticosteroids. The proportions of patients achieving CMR and 2-year OS were extracted from all study arms and summarized by TKI group (ponatinib *vs* all other TKIs) using pooled estimates with 95% confidence intervals (CIs) from a random-effects meta-analysis. A binomial distribution was assumed to calculate the 95% CIs for the ponatinib trial. Multivariate logistic meta-regressions were conducted to examine the association between TKI groups and CMR rates as well as between TKI groups and 2-year OS, adjusting for age, gender, and in the OS model only, the proportion of patients receiving subsequent hematopoietic stem cell transplantation (SCT). P-values were calculated using non-parametric bootstrapping with 20,000 permutations.
Results: Across the 30 TKI-treatment arms from the 20 included studies, the median (range) age was 46 years (38-69 years), percent of male patients was 53% (40-67%), and percent of patients receiving subsequent SCT was 63% (0-100%). The pooled proportion of patients achieving CMR with ponatinib was 78% (95% CI: 62-90%) *vs* 30% (95% CI: 22-38%) with other TKIs. The pooled 2-year OS with ponatinib was 80% (95% CI: 65-92%) compared to 58% (95% CI: 53-63%) with other TKIs. The odds ratio (OR) for ponatinib *vs* other TKIs from the adjusted meta-regression for CMR (N=18) was 9.12 (95% CI: 1.59-52.21, p=0.025), and was 3.00 (95% CI: 0.63-14.43, p=0.136) for 2-year OS (N=19).
Summary/Conclusions: Our results support the hypothesis that ponatinib in combination with chemotherapy is associated with better clinical efficacy in newly diagnosed Ph+ ALL than combination therapy with older TKIs. In particular, compared to first- and second-generation TKIs, ponatinib was associated with a statistically significant 9-fold increase in the odds of CMR and a higher, though non-statistically significant, odds of 2-year OS. A limitation of our analysis is its reliance on study-level data from a small number of studies with heterogeneous therapy combinations; adjustment for heterogeneity across studies was limited to available covariates only. Nevertheless, our results suggest that ponatinib in combination with chemotherapy may represent a highly effective front-line treatment option for patients newly diagnosed with Ph+ ALL. Further prospective head-to-head clinical trials to confirm these results are warranted.

P170

RISK OF CEREBRAL VEIN THROMBOSIS (CVT) IN ADULT ALL PATIENTS IN FINLAND 1999-2012: SUGGESTION FOR A LEUKEMIA-RELATED PREDICTIVE SCORING MODEL

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Background: Thrombosis causes remarkable morbidity among patients with cancer and is the second leading cause of death after malignancy itself in this patient group. Khorana score estimates the risk of deep venous thrombosis (DVT) in solid tumor neoplasms, and may help physicians to prevent thrombotic complications. However, Khorana score is not applicable in acute leukemias and specific leukemia-related thrombosis risk score is still called for. Previous analyses focusing on thrombosis in ALL patients have mainly been conducted on pediatric study groups. Thrombotic risk profile in adult patients could, however, differ from children and these studies may not be converted straight-forward to adult setting.
Aims: To identify patient characteristics detectable at ALL diagnosis and possibly predisposing to CVT, which often represents with the most drastic consequences in patient's life.
Methods: We performed a population-based retrospective registry analysis on 186 adult-ALL patients treated with a national study protocol ALL2000 between years 1999-2012 in Finland.
Results: Of patients, 118 patients had pre-B ALL, 37 Philadelphia chromosome positive ALL and 31 T-ALL. Thirty-one patients (19%) suffered from a venous thrombosis (VT), whereof 9 (27%) from a CVT. Patients with CVT were thinner (p=0.018 and p=0.010) and had more T-ALL (Pearson Chi-Square values: 10.61, p=0.005 and 9.93, p=0.007), extramedullary leukemia (Pearson Chi-Square values: 5.167, p=0.023 and 5.412, p=0.046), and a higher pre-

chemotherapy CRP (p=0.033 and p=0.123) compared with both patients without VT and with other VTs, respectively (Table 1). A trend for younger age, higher pre-chemotherapy D-dimer, leukocyte and blood blast counts, and a lower platelet count was also detected in patients with CVT in this setting. CVT patients also presented with a higher pre-chemotherapy hemoglobin compared with patients without VT but this distinction did not reach statistical significance. Notably, most CVTs (n=5/9) occurred prior to the introduction of asparaginase, which is known to be the major factor predisposing ALL patients to thrombotic complications. None of the CVT patients had received intrathecal cytostatic treatment during the previous week before the thrombosis was diagnosed. Based on these results we constructed a risk model for CVT, which takes into account seven basic features detectable in patients at ALL diagnosis (Table 1). Patients scoring ≥5 points represented with a 20-fold risk for CVT compared with the low-risk (<5 points) patients (HR=20.841, p<0.0001, CI: 5.208-83.401) in our study group. The scoring model showed a very good specificity and satisfying sensitivity (NPV: 0.982, PPV: 0.375) in this study and is currently under validation in another patient cohort.

Table 1. Clinical and laboratory characteristics recorded at ALL diagnosis in patients obtaining CVT during the first three months of chemotherapy compared with other patient groups.

Variables	CVT vs. Without VT	n-fold	P-value	CVT vs. Other VT	n-fold	P-value
Leukocytes	↑	1.72	0.269	↑	1.16	0.675
Hb *1	↑	1.12	0.307	↔	0.99	0.659
Platelets *2	↓	0.63	0.309	↓	0.61	0.306
Bblast	↑↑	2.91	0.300	↑	1.65	0.497
CRP *3	↑↑	2.32	0.123	↑↑	3.58	**0.033**
D-dimer	↑	3.00	0.318	↑	2.25	0.236
Age *4	↓	0.66	0.166	↓↓	0.62	0.056
BMI *5	↓↓	0.84	**0.018**	↓↓	0.77	**0.010**
T ALL *6	↑↑	3.60	**0.005**	↑↑	4.10	**0.007**
Extramedullary leukemia *7	↑↑	2.50	**0.023**	↑↑	3.10	**0.046**

Parameters used in risk score model: *1 Hb > 100, *2 Platelets < 80, *3 CRP > 20, *4 Age < 41 years, *5 BMI < 24 kg/m2, *6 T-ALL = yes and *7 extramedullary leukemia = yes. A socre ≥ 5 points stands for over a 20-fold risk for CVT compared with patients in low-risk (< 5 points) group.

Summary/Conclusions: Thrombotic events and especially CVTs often have a major impact on patients with ALL during their course of leukemia treatment and after. Here, we present a set of laboratory and clinical characteristics identifiable at ALL diagnosis and associating with a very high risk of CVT during the first months of ALL treatment. These high-risk characteristics (*e.g.* low BMI and young age) are partly in contradiction to the acknowledged features predisposing patients to other forms of VT. This phenomenon hints for a distinctive mechanism of CVT in ALL patients possibly in linkage with ALL biology.

P171

INITIAL RESULTS FROM UKALL60+, A UK/HOVON COLLABORATIVE PHASE 2 STUDY IN ELDERLY PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: The outcome for older adults with acute lymphoblastic leukaemia (ALL) is generally poor. The UKALL12/ECOG2993 study reported a greater incidence of high risk cytogenetic abnormalities, increased toxicity (*e.g.* 18% Induction death rate) and poorer outcomes (CR rate 73% and 5 year overall survival 21%) in those 55- 65 years of age. There is a dearth of studies which focus on patients at the very oldest end of the age spectrum despite an increasing incidence with age.
Aims: We aimed to conduct a pragmatic study intended to establish baseline chemotherapy from which to design widely-applicable studies of novel agents in older people.
Methods: We established UKALL60+ as a collaboration between the UK National Cancer Research Institute Adult ALL Group and HOVON to study treatment choice and outcomes in patients aged 60 years and over (or 55 years and over with co-morbidities) with ALL. The study offers five 'arms' to be decided by investigator and patient choice; Non-intensive (designed to be delivered primarily out of hospital), Intensive, Intensive+, Philadelphia positive (Ph+) and Registration only (in which treatment is at investigators discretion, including no active therapy). Any elderly patient with newly diagnosed ALL is eligible. There are no exclusions for co-morbidities, including prior malignancies. Baseline characteristics of each group including Charlson index, ECOG, Karnofsky and CRASH scores are being collected with the aim of determining why each regimen was allocated. The primary endpoint is the rate of complete remission (CR) after 2 phases of induction. Secondary objectives include determination of MRD status at 3 time points, EFS and OS at 1 year, treatment related mortality and quality of life assessments.
Results: Since activation in December 2012 81 patients (84% of those screened) with a median age of 67 years (Range 55-83) have been enrolled. Median follow up is 9.4 months (Range 1 day to 29.8 months). ECOG performance status was 1 in 33 (40%), 2 in 37 (45%) and ≥3 in 13 (15%). Treatment allocation has been non-Intensive n=11, Intensive n=34, Intensive+ n=7, Ph+ n=18 and Registration only n=11 patients. It is too early to perform a full analysis of the reasons given for choosing each regimen but age appears to be a major factor for Ph-ve patients, with the average age 74 years (Range 64-82) in the non-Intensive arm compared with 66 years (Range 56-76) in the Intensive and Intensive+ arms. A total of 32/80 patients (40%) had high risk cytogenetics including *BCR-ABL1* (n=18), low hypodiploidy (n=10), complex karyotype (n=2) and *KMT2A-AFF1* (aka *MLL-AF4*) (n=1). So far forty-eight patients have completed 2 phases of induction chemotherapy with 37 of these attaining CR (77%). Failure to complete this was very common (n=17); reasons include inadequate response/relapse=8, death=5, clinician or patient decision=4. Baseline MRD samples were received for 62 patients, 43 of which have been evaluated to date by immunoglobulin heavy chain/T cell receptor re-arrangement quantification or BCR-ABL quantification. Only 3 of these patients were MRD negative following the first cycle of induction chemotherapy. Grade 3/4 AEs were seen in 54/58 assessable patients. The most common toxicities were haematological (71%) and infections (38%). To date 28 deaths have been reported; 18 patients died of ALL, 5 infection, 1 cardiac, 1 multi-organ failure and 3 data outstanding. Quality of life data has been collected and the results are anticipated.
Summary/Conclusions: ALL in older patients is difficult to treat with a delicate balance between efficacy and toxicity. Treatment choices currently vary widely. The goal of our trial is to provide a representative view of elderly ALL treatment, to identify a group where the current therapeutic approaches need improvement and to pave the way for older individuals to be at the forefront of development of novel approaches in *de novo* ALL.

P172

RELAPSE OF ACUTE LYMPHOBLASTIC LEUKAEMIA IN OLDER/ELDERLY PATIENTS - A SWEDISH POPULATION-BASED STUDY

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Background: Knowledge about relapsed acute lymphoblastic leukaemia (ALL) in older/elderly patients is scarce.
Aims: To study the outcome of ALL relapse treatment in a population-based cohort of older/elderly patients.
Methods: Out of the Swedish ALL-registry and cause of death-registry patients 55-85 years (y) old diagnosed with B- and T-ALL from 2005 to 2012 were identified. Clinical, laboratory and treatment data were validated and supplemented from medical records. Informed consent was obtained and the study approved by the local ethical committee in accordance with the declaration of Helsinki. Proportions were compared with chi-square test. Overall survival (OS) was estimated by the Kaplan Meier method and distribution compared with log-rank test using IBM SPSS package (v 23). Confidence intervals (CI) of 95% were obtained.
Results: Of 103 patients with B- and T-ALL receiving intensive treatment with achieved first complete remission (CR1), 63 (61%) relapsed (28 males and 35 females). Median age at relapse was 67y (57-83). B-ALL was present in 57/63, T-ALL in 6/63, and Philadelphia-positive (Ph+) disease in 19 of 61 patients (31%) (two with unknown Ph-status). Bone marrow was the sole relapse local in 57 (91%) patients. Median time from CR1 to relapse (TTR) was 9 moths (range: 1-93) and seven relapsed after allogeneic hematopoietic stem cell transplantation (hSCT). Treatment with the goal of a second complete remission (CR2) was applied in 33 patients and the first course consisted of the Swedish protocol ABCDV (*n*=8), FLAG-Asp (*n*=6), FLAG-Ida (*n*=3), MEA (*n*=4), FLAG (*n*=1), Hyper-CVAD (*n*=3), NOPHO adult protocol (*n*=1), nelarabine (*n*=1) or other combinations (*n*=6). Regimens were supplemented with tyrosine kinase inhibitor (TKI) in six patients, DLI in one and CAR-T in one patient. hSCT in CR2 was performed in three patients (of 10 considered for the procedure). Two of them died of relapse and transplant related mortality respectively and one is still alive after seven years. Palliation was given to the 30 remaining patients with CHOP/COP (*n*=5, with rituximab added in 2), other intravenous chemotherapy combinations (*n*=5), TKI [alone (*n*=5), with chemotherapy/cortisone (*n*=3), radiotherapy (*n*=1) and DLI (*n*=1)], cortisone and/or oral chemotherapy combinations (*n*=7), no treatment (*n*=1) and unknown (*n*=2). Remission induction was given predominantly in late (>1y) relapse [18/27 (67%) *vs* 15/36 (42%); *P*=0.049]. In total, CR2 was achieved in 18 patients (29%) - 14/33 (42%) after remission induction and 4/30 (13%) after palliative treatment. Higher proportion of patients attained CR2 in late as compared with early relapse [13/27 (48%)

vs 5/36 (14%); *P*=0.003]. Median survival after relapse was 4 months (range: 0-95). The estimated 1 and 3y OS after relapse was 17 (CI: 7, 27)% and 7 (CI: 0, 14)%. There was no significant difference in OS between patients receiving remission induction and palliation. Age at relapse did not influence OS. Patients with late relapse had improved survival (*P*=0.001). CR2 achievement (as compared with treatment failure) enhanced survival in late [3y OS, CI: 34 (5, 64)% *vs* 7 (0, 21)%; *P*=0.001] but not early relapse (no survivors at 3y; *P*=0.22).

Summary/Conclusions: Intensive treatment can be considered in older ALL patients in case of late relapse. However, for the majority of the patients, the prognosis was poor, which supports alternative approaches such as immunotherapy or novel drugs.

LB173

ADULTS AND CHILDREN (1-45 YEARS) WITH PH-NEGATIVE ALL HAVE ALMOST IDENTICAL OUTCOME IN RISK-STRATIFIED ANALYSIS OF NOPHO ALL2008

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Background: Compared with children, adults with acute lymphoblastic leukemia (ALL) have had inferior survival, when treated with traditional adult ALL regimens.

Aims: The aim of this study was to examine the event free survival (EFS) of children and adults with ALL (1-45 years) diagnosed, risk stratified, and treated uniformly according to the NOPHO ALL2008 protocol.

Methods: We collected information on 1509 patients from Sweden, Norway, Iceland (children only), Finland (children only), Denmark, Lithuania, and Estonia diagnosed 7/2008-12/2014 with Ph-negative ALL and treated according to the NOPHO ALL2008 protocol. Patients were registered in the NOPHO ALL2008 database upon diagnosis and subsequently followed systematically at three months intervals.

Results: Of 1509 patients, 1022 were children 1-9 years (484 female), 266 were 10-17 years (106 female) and 221 were adults 18-45 years (85 females). Adult patients more often had T-ALL (9% (1-9 years), 25% (10-17 years) and 32% (18-45 years), p<0.001), and *KMT2A* (a.k.a *MLL*) rearrangements (*KMT2A*-r, 3% (1-9 years), 5% (10-17 years) and 6% (18-45 years) , p<0.001), but lower median WBC when stratified by lineage (B-lineage 9.9 (1-9 years), 8.1 (10-17 years) and 6.8 (18-45 years) $x10^9$/L, p=0.01; T-lineage 134 (1- 9 years), 71.7 (10-17 years) and 38.8 (18-45 years) $x10^9$/L, p<0.001). Day 29 MRD was significantly higher for adults (p<0.001). Based on immunophenotype, WBC at diagnosis, MRD (discriminator 0.1%, FCM for BCP and PCR for T) at day 29 and 79, and presence of intermediate (dic(9:20) or iAMP21[*RUNX1*] or t(1;19)) or high risk cytogenetics (*KMT2A*-r or hypodiploidy (modal number <45)), patients were stratified into 4 risk groups (SR, IR, HR, and HR+hSCT, Table 1). Only MRD d29 ≥5% or d79>0.1% stratified to hSCT in 1st complete remission (CR1). Older patients were skewed towards higher risk group (Toft, Eur J Haematol 2013), but for each treatment arm severe toxicities (except for thrombosis and osteonecrosis) and intervals between treatment phases were almost identical for children and adults (Toft, Eur J Haematol 2015). After a median follow up for patients in CR1 of 4.0 years (75% range: 2.4-5.9 years), 16 patients (3 patients 18-45 years) had died during induction (induction failure), and 50 patients (12 patients 18-45 years) had died in first remission. A total of 123 patients relapsed (36 patients 18-45 years), and 12 children and 1 adult developed a second malignancy. The overall 5y-EFS for patients 1-9, 10-17 and 18-45 years was 88% (95%CI: 86; 90), 79% (95%CI: 73; 85) and 73% (95%CI: 67; 79), respectively, p <0.001. However, when stratified by risk group, the poorer EFS for adults was significant only for patients with intermediate risk ALL (Table 1).

Table 1.

Age group (Median age)		Total	1-9 (3 years)	10-17 (14 years)	18-45 (26 years)	p
Patients		N=1509	N=1022	N=266	N=221	
BCP/T		N=1278/231	N=929/93	N=199/67	N=150/71	<0.001
Induction failure		N=16 0.01(±0.01)	N=10 0.01(±0.00)	N=3 0.01(±0.01)	N=3 0.01(±0.01)	0.9
Death in 1. Remission		N=50 0.03(±0.00)	N=23 0.02(±0.00)	N=15 0.06(±0.02)	N=12 0.06(±0.02)	0.002
Relapse		N=123 0.10(±0.01)	N=60 0.08(±0.01)	N=27 0.12(±0.02)	N=36 0.19(±0.03)	<0.001
Event Free Survival (SE)						
EFS overall		0.84(±0.01)	0.88(±0.01)	0.79(±0.03)	0.73(±0.03)	<0.001
Standard risk (SR)	BCP & WBC<100K & d29 MRD <0.1%	N=673 0.92(±0.01)	N=546 0.92(±0.01)	N=83 0.90(±0.04)	N=44 0.87(±0.07)	0.20
Intermediate risk (IR)	BCP & WBC<100K & d29 MRD <0.1% or T a/o WBC≥100K & d29 MRD <0.1% or dic(9;20), t(1;19), iAMP21[RUNX1]	N=542 0.86(±0.02)	N=345 0.90(±0.02)	N=103 0.82(±0.04)	N=94 0.78(±0.05)	0.002
High risk (HR)	T a/o WBC≥100K & d29 MRD ≥0.1% or KMT2A-r hypodiploidy (<45 or DI<0.85)	N=176 0.66(±0.04)	N=85 0.65(±0.05)	N=46 0.68(±0.07)	N=45 0.66(±0.08)	0.9
High risk + hSCT (HR+hSCT)	d29 MRD ≥5% a/o d79 MRD (≥0.1%)	N=100 0.71(±0.05)	N=35 0.80(±0.08)	N=30 0.74(±0.09)	N=35 0.61(±0.09)	0.12

Table 1. Cumulative risk adjusted for competing events and event free survival.
16 patients with Induction failure and 2 patients not risk grouped due to severe toxicity (1-9 and 10-17 years) were excluded in survival analysis.
N number of patients, EFS event free survival. SE standard error, SR standard risk, IR intermediate risk, HR high risk, hSCT hematopoietic stem cell transplantation.

Summary/Conclusions: The EFS for adult ALL patients has improved markedly with NOPHO ALL2008 treatment compared with historical data. Although adult patients more often have higher risk ALL, their overall cure rates are close to those of children when stratified by risk group.

Acute myeloid leukemia - Biology 1

P173

MICRORNA-155 AND MICRORNA-708 ARE OPPOSING MODULATORS OF HOXA9 ACTIVITY IN ACUTE MYELOID LEUKEMIA

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Background: In order to identify microRNAs (miRNA) relevant in acute myeloid leukemia (AML), we profiled global miRNA expression in a murine AML progression model based on Hoxa9 and Meis1 overexpression.
Aims: We found miR-155 and miR-708 as the most significantly upregulated miRNAs in leukemic Hoxa9/Meis1 cells compared to the preleukemic Hoxa9/ctrl cells (both p<0.01). Subsequent analysis of primary AML cases (n=38) comprising various AML subtypes showed significantly elevated levels of miR-155 and miR-708 in all samples compared to total bone marrow from healthy donors, indicating potential oncogenic roles for these miRNAs. We further investigated, *in vivo*, the role of miR-155 in AML by retroviral overexpression with Hoxa9 in murine bone marrow (mbm) cells followed by syngeneic transplantation. Overexpression of miR-155 in conjunction with Hoxa9 (Hoxa9/miR-155) caused a significantly accelerated onset of a myelomonocytic leukemia (p<0.001), but still a less aggressive course of disease compared to mice transplanted with Hoxa9/Meis1 (p<0.001). In order to assess if miR-155 is dispensable for the onset of AML, we transformed miR-155-/- and miR-155+/+ mbm with Hoxa9/Meis1 followed by syngeneic transplantation. No difference in onset of AML was observed. Further analysis revealed that absence of miR-155 impaired homing of Hoxa9/Meis1 cells but did not impact their proliferation rate, which eventually compensated for impaired engraftment in this AML model.
Methods: We then hypothesized that the combination of miR-155 and miR-708 could further replace the oncogenic potential of Meis1. Therefore, mbm cells were retrovirally transduced with Hoxa9, miR-155 and miR-708 (Hoxa9/miR-155/miR-708) or Hoxa9 and miR-708 (Hoxa9/miR-708) and functionally analyzed *in vitro* and *in vivo*. To our surprise, miR-708 abrogated the leukemogenic effect of Hoxa9, alone or in combination with Hoxa9/miR-155 *in vivo* (p=0.0117, p<0.0001), with little or no engraftment. Transcriptome analysis revealed that miR-155 and miR-708 have opposite effects on Hoxa9-induced transcription.
Results: To further understand why miR-708, a potent tumor suppressor miRNA, is upregulated in the highly aggressive Hoxa9/Meis1 AML cells, we explored the role of miR-708 in leukemia initiating cells (LIC). Hoxa9/Meis1 cell subpopulations enriched for LIC based on c-kit, Mac-1 and Gr-1 expression were FACS-sorted and transplanted into syngeneic mice. The c-kit$^+$Gr-1$^-$Mac-1$^-$ cells caused a significantly shorter survival of transplanted mice compared to the other sorted subpopulations (p=0.0072 and p=0.0021, respectively), with the c-kit$^-$Gr-1$^+$Mac-1$^+$ subpopulation resulting in the longest survival. This was mirrored by lower expression of miR-708 in the LIC-enriched c-kit$^+$Gr-1$^-$Mac-1$^-$ subpopulation compared to bulk (p=0.032), whereas there was no difference in miR-155 expression. Similar findings were made in human AML samples (n=7) sorted into LIC-enriched subpopulations using CD117, CD34 and CD38. Together, these results highlight the role of miR-708 as an orchestrator of the leukemic hierarchy through its tumor suppressor activity.
Summary/Conclusions: In conclusion, we demonstrate for the first time a functional role for miR-155 in homing of AML cells. In addition, we propose as a novel concept where miR-708, a tumor suppressor miRNA, stratifies the leukemic hierarchy.

P174

VENTX INDUCES EXPANSION OF PRIMITIVE ERYTHROID CELLS AND CONTRIBUTES TO THE DEVELOPMENT OF ACUTE MYELOID LEUKEMIA IN MICE

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Background: Homeobox genes are key factors in the development of acute leukemias. So far, little is known about the role of non-clustered homeobox genes such as *VENTX*. The Vent-like homeobox gene *VENTX* is a member of the Vent gene family in mammals and is the mammalian homolog of the *Xenopus xvent* gene. Our group has previously shown that the homeobox gene VENTX shows high and aberrant expression in human acute myeloid leukemia (AML) characterized by the AML1-ETO (AE) fusion (Rawat *et al.*, PNAS 2010).
Aims: With this study, we wanted to test the functional relevance of VENTX in the development of AE positive AML in mice.
Methods: To address this, mice were transplanted with bone marrow progenitor cells retrovirally engineered to express VENTX or AML1-ETO alone or in combination.
Results: Whereas none of the AML1-ETO mice developed any disease, all the recipients of AML1-ETO/VENTX double transduced cells succumbed to AML after a median latency of 334 days (range 19-403 days) post transplantation. Of note, induced AML cases were positive for erythroid markers such as CD71 and Ter119. Strikingly, VENTX alone induced a massive expansion of the primitive erythroid compartment in all transplanted mice. Furthermore, this massive perturbation in differentiation converted into an acute erythroleukemia in a fraction of mice. All leukemias in the AE/VENTX and the VENTX experimental arms were rapidly transplantable with a median latency of 40 days until leukemia induced death, characterized by high blast counts in the bone marrow (83%, range 55% - 92%). *Ex vivo*, leukemic cells grew permanently generating AE/VENTX positive cell lines. RNA-Seq analyses from CD34$^+$ cord blood transduced with VENTX documented 279 differentially expressed genes compared to the empty vector control, comprising pathways belonging to the categories “viral carcinogenesis”, “cytokine-cytokine receptor interaction”, “JAK-STAT signaling”, “cancer”, “signaling pathways in cancer”, “transcriptional misregulation in cancer” and “Huntington’s disease” in the KEGG analysis. Genes necessary for terminal erythroid differentiation such as the EPO-receptor and GATA1 were downregulated by constitutive VENTX expression. In line with the observation of VENTX induced primitive erythroid expansion in mice, VENTX was highly expressed in patients with primary human erythroleukemias and polycythemia vera. Knockdown of VENTX in the erythroleukemic HEL cell line significantly blocked growth *in vitro* and engraftment in NSG mice. Overexpression of VENTX impaired expression of genes linked to erythroid differentiation in human stem and progenitor cells.
Summary/Conclusions: In summary, these data indicate that aberrant expression of VENTX induces expansion of primitive erythroid cells and collaborates with AML1-ETO in inducing AML in mice.

P175

ABNORMAL INTRAGENIC TRANSCRIPTIONAL ACTIVATION IN MLL-AF9 AND KAT6A-CREBBP ACUTE MYELOID LEUKEMIA (AML)

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Background: The mutated transcription factors AML1-ETO, CBFB-MYH11 and CEBPA cause unique pathogenic gene expression profiles (GEPs) in AML. Previously, we identified an AML subgroup with a unique GEP characterized by outlier high expression of the *BRE* gene. Most cases with this unique GEP harbor the oncogenic transcriptional regulator MLL-AF9. However, since ~50% of adult *MLL-AF9*$^+$ AML cases does not exhibit this unique GEP nor outlier high *BRE* expression, we postulated that another pathogenic mechanism besides the MLL-AF9 translocation causes this unique GEP.
Aims: We aimed at characterizing outlier high *BRE* expression and the associated unique GEP in *MLL-AF9*$^+$ AML, whether these phenomena were exclusive to *MLL-AF9*$^+$ AML and whether they associated with known oncogenic pathways.
Methods: AML samples were subjected to RNA-sequencing (n=4), H3K4me3 (n=7) and/or H3K27ac (n=3) ChIP-sequencing. Informed consent was obtained from all subjects prior to this study. 5’ Rapid Amplification of cDNA Ends (RACE) was performed to characterize the 5’ end of BRE transcripts. *BRE* gene expression was measured by qRT-PCR. Expression of an alternate BRE transcript was normalized to PBGD expression and a control sample without outlier high *BRE* expression. A normalized value >500 was considered high. Ingenuity pathway analyses were performed on the top 200 genes positively associated with *BRE* expression in MLL-AF9$^+$ AML.
Results: RNA-seq and qRT-PCR of *MLL-AF9*$^+$ patient samples showed a marked increase in *BRE* expression starting from exon 5, exclusively in samples with outlier high *BRE* expression. H3K4me3 and H3K27ac ChIP-sequencing revealed clear marks in *BRE* intron 4, 3 kb upstream of *BRE* exon 5, only in AML cases with outlier high *BRE* expression. To demonstrate that this region relates to active transcription, we performed 5’ RACE. A new transcript starting in *BRE* intron 4 near the H3K4me3/H3K27ac marks was identified, with sequences spliced to *BRE* exon 5. A qRT-PCR specific for the alternate BRE

transcript showed that it was frequently expressed in adult *MLL-AF9* samples and in samples with the *KAT6A-CREBBP* fusion gene (18/35, median expression high 8580, low 0.6). This is in line with published data showing that part of *MLL-AF9*$^+$ and *KAT6A-CREBBP*$^+$ samples share a common GEP. Also childhood *MLL*-rearranged and *KAT6A-CREBBP*$^+$ AML samples frequently expressed the alternate BRE transcript (31/43, median expression high 8172, low 26.9). Alternate *BRE* expression was generally low in patients with other types of AML (n=42, 1.4) and non-AML hematological malignancies (n=10, 0.4) or normal CD34$^+$ and bone marrow cells (n=14, 0.3). A polymorphism in BRE intron 5 revealed that alternate *BRE* transcription initiation is bi-allelic. Importantly, analysis of genome-wide H3K4me3 marks and RNA-seq data uncovered 98 potential intragenic transcriptional activation sites, several of which are in genes correlating with high *BRE* expression. Ingenuity pathway analyses using the top 200 genes positively associated with *BRE* expression, identified potential pathogenic pathways including p53 and protein kinase A signaling (p<0.03).
Summary/Conclusions: Alternate transcription initiation in the ALK gene was recently identified as oncogenic mechanism causing melanoma (Wiesner *et al.*, Nature, 2015). Our data show genome-wide alternate transcription initiation as new phenomenon in *MLL-AF9*$^+$ and *KAT6A-CREBBP*$^+$ AML cases that exhibit a unique GEP. Potential oncogenic pathways were identified in these AML subgroups. Further studies investigating the molecular mechanisms contributing to alternate transcription initiation in AML are warranted.

P176

HHEX TRANSFORMS PROMYELOCYTES AND COOPERATES WITH FACTOR INDEPENDENCE TO CAUSE PROMYELOCYTIC LEUKEMIA IN MICE

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Background: The transcription factor Hhex (Hematopoietically-expressed homeobox gene) is important for lymphoid commitment and causes T-cell leukemia when overexpressed. We have recently found that Hhex is overexpressed in human myeloid leukemias and maintains leukemia stem cell self-renewal in mouse models of AML via repression of Cdkn2a. However, whether Hhex overexpression also affects hematopoietic differentiation is not known.
Aims: The aims of this study are to investigate the effects of high levels of Hhex expression in growth factor independent hematopoietic progenitor cells and the roles of the functional domains of Hhex.
Methods: To study the effects of Hhex on haematopoietic progenitor differentiation, we immature Lin$^-$Sca$^+$Kit$^+$ (LSK) hematopoietic progenitors were transduced with retroviruses expressing wild-type and mutant forms of Hhex.
Results: Hhex overexpression in LSK cells caused serial replating of myeloid progenitors and to the rapid establishment of IL-3-dependent promyelocytic cell-lines. Structure function analysis demonstrated a requirement of the DNA binding domain and the N-terminal repressive domains of Hhex in promyelocytic transformation. This included a PML protein interaction domain, although loss of PML failed to prevent Hhex-induced promyelocyte transformation *in vitro*. RNA-Seq analysis showed that Hhex overexpression leads to repression of myeloid developmental genes including MPO. To test the leukemic potential of Hhex, Hhex-transformed promyelocyte lines were rendered growth factor-independent using a constitutively active IL-3 receptor common β subunit (βcV449E). The resultant cell-lines caused rapid promyelocytic leukemia in mice.
Summary/Conclusions: In addition to its role in repressing tumor suppressor pathways in myeloid leukemias, overexpression of Hhex causes a differentiation blockade and contributes to promyelocytic leukemia *in vivo*. As such, Hhex overexpression may contribute to human myeloid leukemias via multiple pathways.

P177

A SOMATIC MUTATION OF GFI1B IDENTIFIED IN LEUKEMIA ALTERS CELL FATE VIA A SPI1 (PU.1) CENTERED GENETIC REGULATORY NETWORK

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Background: Establishing a link between leukemia mutations and the malignant process requires functional assessment of their biological impact in the context of appropriate normal and malignant primary cells. Against this backdrop, we have identified a mutation (D262N) in the erythroid/megakaryocytic affliated transcriptional repressor GFI1B in acute myeloid leukemia (AML) and explored its biological properties.
Aims: Establishing a link between a *GFI1B* mutation and leukemia in the context of normal and malignant primary cells.
Methods: Human material and clinical information was obtained with informed consent and approval of institutional ethics committee from 69 patients with AML. Point mutation analysis: *GFI1B* coding regions were analyzed by DHPLC. Next generation sequencing performed with Hiseq2000 (Illumina) allowed the identification of a novel mutation. Cloning: *GFI1B* cDNA was amplified, cloned in pGEM-T easy vector and sequenced. Point mutation was introduced by PCR. Wild-type or mutated coding cDNA was cloned into pHRSINCSGW lentivirus vector, expressing green fluorescent protein (GFP). The *SPI1* hairpin was cloned into the SLX vector, with or without wild-type or mutant *GFI1B*. Enrichment of hematopoietic stem and progenitors: Enrichment of human CD34+ cells from mobilized peripheral blood was by magnetic selection (Miltenyi Biotec). Transduced GFP/CD34 positive cells were sorted. Methylcellulose Colony-Forming Cell (CFC) assays: CFC assays were performed with MethoCult H4436 (StemCell Technologies), 15 days. Liquid differentiation assays: CD34+ cells expressing mutant or wild type *GFI1B* were maintained under liquid culture conditions that support multi-lineage differentiation: Myelocult® H5100 with human cytokines, SCF, FLT3L (50 ng/ml); IL-3, GM-CSF, M-CSF (10 ng/ml); G-CSF (0.1 MUI); EPO (100 ng/ml) for 5-6 days. MDS cell culture: CD34+ cells were cultured in MyeloCult® H5100 with 10% (v/v) HS-5 conditioned medium, human SCF and IL-3 (both 50 ng/ml). AML patients gene expression profiling and data analysis: This has been published (www.ncbi.nlm.nih.gov/geo, GSE1159).
Results: We identified a *GFI1B* new mutation (D262N) in an AML patient with antecedent myelodysplastic syndrome (MDS). The GFI1B-D262N mutant functionally antagonizes the transcriptional activity of wild-type GFI1B. GFI1B-D262N promoted myelomonocytic *versus* erythroid output from primary human hematopoietic precursors and enhanced cell survival of both normal and MDS derived human precursors. Re-analysis of 285 AMLs transcriptome data revealed a significant subset of patients in which expression of wild-type *GFI1B* was inversely correlated with that of *SPI1* (*PU.1*). In delineating this GFI1B-SPI1 relationship we show (I) SPI1 is a direct target of GFI1B, (II) expression of GFI1B-D262N produces elevated expression of SPI1, (III) SPI1-knockdown restores balanced lineage output from GFI1B-D262N-expressing precursors, (IV) we also observed that *GFI1B* produced an increase in *MLLT3*, while expression of mutant *GFI1B* reduced *MLLT3* expression.
Summary/Conclusions: In conclusion, our data links GFI1B to both the myeloid and erythroid transcriptional networks by repressing SPI1 and increasing MLLT3 expression, helping to understand its role in lineage specification and its potential in promoting blood malignancy. In fact, our clinical findings and experimental data show that the GFI1B D262N mutant plays a role in AML in humans and does so primarily through the agency of master transcriptional regulator SPI1, reflecting GFI1B physiological function in *SPI1* regulation.

P178

COMPARATIVE FUNCTIONAL ANALYSIS OF THE COMMON INTERACTORS OF 7 MLL FUSION PROTEINS

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Background: The Mixed Lineage Leukemia gene (MLL) is a frequent target of chromosomal rearrangements in human malignancies. Balanced translocations result in the fusion of the MLL gene to over 65 different fusion partner genes, leading to the production of novel chimeric proteins. Critical effectors of distinct MLL fusion proteins have previously been identified, and some of them were shown to hold great potential for targeted therapies. However, it is not clear if these effectors are conserved among all MLL fusion proteins or if different molecular mechanisms of transformation exist for distinct MLL fusion proteins.
Aims: We aimed to identify common critical effectors of 7 selected MLL fusion proteins (MLL-AF1p, MLL-AF4, MLL-AF9, MLL-CBP, MLL-EEN, MLL-ENL, MLL-GAS7), which have been presumed to employ different molecular mechanisms of oncogenic transformation.
Methods: Stable cell lines allowing for inducible expression of 7 different affinity-tagged MLL fusion proteins were prepared and transgene expression was verified by qRT-PCR and Western Blotting. Affinity purification coupled to mass spectrometry (AP-MS) identified novel interactors of the 7 MLL fusions. Functional annotation of the resulting interactome revealed significant enrichment of a large number of protein complexes. To assign functional information to a subset of 128 interactors, which were found to bind to ≥5 MLL fusions, we employed a RNAi screening approach. The MLL-AF9-positive MOLM-13 cell line was transduced with pools of viral vectors allowing for the expression of 6 shRNAs targeting the same candidate gene. We established a screening methodology that is suitable for positive and negative selection readouts. To confirm and further validate selected high-confidence candidates we performed additional RNAi screens in other MLL-rearranged- and MLL-wild-type leukemia cell lines and characterized RNAi-knock down efficiencies in various human and murine cell systems.
Results: Advanced statistical filtering using a novel, improved algorithm developed by us yielded a densely connected protein-protein interaction network of >950 proteins around 7 MLL fusions. Many protein complexes previously shown to be associated with MLL were significantly enriched, such as PAF-, SWI/SNF-

and the RNA Pol II containing coactivator complexes. 128 proteins were found to interact with ≥5 of the 7 MLL-fusions. This list of conserved MLL-interactors is highly enriched for proteins with known functions in chromatin metabolism and transcriptional control. Several RNAi screens in different human leukemia cell lines were used to functionally dissect the conserved MLL-fusion interactome. Our screening methodology identified known MLL interacting proteins such as Menin, DPY30 and HCFC1 as critical effectors of MLL fusion proteins, validating the approach. In addition, we selected a small set of novel interactors of MLL fusion proteins for further validation experiments, including a protein involved in cytoskeletal organization.
Summary/Conclusions: In conclusion, we developed a robust experimental pipeline allowing for the functional characterization of cellular effects of MLL fusion proteins in a comprehensive and comparative manner, which will contribute to further clarify the molecular mechanisms of MLL-fusion dependent leukemogenesis.

P179

TRANSFORMING ACTIVITY OF THE NUP98-MLL FUSION IN A CONDITIONAL TRANSGENIC MOUSE MODEL

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Background: Genes encoding for mixed-lineage leukaemia (MLL) and nucleoporin 98 (NUP98) are both involved in multiple recurrent chromosomal rearrangements associated with haematological malignancies. Inv(11)(p15q23), present as a sole cytogenetic abnormality in patients with myelodysplastic syndromes (MDS) or acute myeloid leukaemia (AML), was reported to lead to the expression of a fusion containing the FG-repeats of NUP98 and almost the entire open reading frame of MLL but lacking the N-terminal menin/LEDGF interaction domain, known to be critical for transformation of all currently known MLL fusions through expression of critical downstream targets like the HOXA gene cluster.
Aims: To address the transforming potential of the NUP98-MLL fusion we established conditional transgenic mice.
Methods: The human NUP98-MLL fusion ORF of 13kb was integrated into the *Hprt* gene locus under the control of a *tetracycline (Tet) responsive element* in mouse ES cells harbouring a *reverse Tet transactivator* (*rtTA*) in the *Rosa26* locus and allowing conditional expression of the transgene by doxycycline (DOX) administration. The effects of conditional NUP98-MLL expression on the haematopoietic system were analysed.
Results: *Ex vivo* expression of NUP98-MLL in lineage-marker depleted bone marrow (BM) cells increased colony formation and replating in methylcellulose. In liquid cultures, NUP98-MLL increased proliferation and impaired differentiation of the cells shown by cell morphology and flow cytometric analysis for Gr-1, Mac-1, CD34 and c-Kit expression. *In vivo* induction of NUP98-MLL led to an MDS-like disease with progression to an AML phenotype after a median latency of 78 weeks. The pre-leukemic phenotype was characterized by the presence of dysplastic myeloid cells in the periphery, decreased Mac-1/Gr-1 expression in the BM, and extensive extramedullary haematopoiesis, especially erythropoiesis in the spleen. These mice also exhibited an increased number of haematopoietic stem and progenitor cells (HSPCs) that provided a significant competitive repopulation advantage when transplanted into irradiated syngeneic recipients. So far, 4 mice have progressed to AML characterized by increased white blood counts, presence of blasts in the periphery with infiltration of the BM, spleen, and other organs. The disease was transplantable into irradiated recipients and depended on NUP98-MLL expression since propagation of the disease was significantly impaired in the absence of DOX. As the fusion lacks the menin/LEDGF interaction domain we addressed the sensitivity to small molecules targeting this interface and expression of the HOX gene cluster. Interestingly, in contrast to cells immortalized by the MLL-AF9 or MLL-ENL fusions, NUP98-MLL expressing cells were resistant to the small molecule Mi2-2 menin inhibitor and did not express high levels of the HOX-A-B-C genes. In addition, treatment with the bromodomain inhibitor JQ-1 did not induce cell cycle arrest or significant cytotoxicity but increased the fraction of cells in >G2 phase suggesting alternative transforming mechanisms of NUP98-MLL implicating the cell cycle checkpoint control.
Summary/Conclusions: We show that transgenic NUP98-MLL expression in mice resulted in an "MDS-like" pre-leukemic state progressing to AML after a long latency. The transforming mechanisms of NUP98-MLL fusion differed from other MLL-fusions resulting in relative resistance to small molecules that are currently being explored for targeted AML therapy.

P180

CONDITIONAL DELETION OF THE HOXA CLUSTER IN MLL-AF9 IS INCOMPATIBLE WITH LEUKEMIA MAINTENANCE

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Background: Hox gene expression is high in hematopoietic stem/progenitor cells (HSPCs), decreases during normal differentiation but remains elevated in leukemia subtypes. Polycomb repressor complexes and histone modifiers, *e.g.* Mixed Lineage Leukemia (MLL), are key regulators of Hox expression. MLL rearrangements, frequent in acute leukemia, are associated high HOXA expression. However, necessity for the HoxA cluster in MLL-leukemia maintenance is not fully elucidated.
Aims: Determine the criticality of the HoxA cluster in MLL-AF9 leukemia maintenance.
Methods: Ectopic overexpression of MLL-AF9 (MA9) in HSPCs in conditional compound transgenic mouse backgrounds MxCre^{+}/HoxA$^{flox/flox}$ (MAFF) or HoxA$^{flox/flox}$ (AFlox) models resulted in increased colony formation and growth in liquid culture. Transformed colonies, serially re-plated (n=5) in methylcellulose and transplanted into sub-lethally irradiated recipient mice, resulted in primary leukemia. Initially, MAFF-MA9 leukemias were used to examine *in vivo* deletion of the HoxA cluster using intraperitoneal injections of Poly(I:C) to initiate an interferon response. To further examine the necessity for the HoxA cluster in disease maintenance, AFlox-MA9 leukemias were treated e*x vivo* with Cre-recombinase (MSCV-Cre-GFP) or vector control (MSCV-GFP), sorted based on GFP expression and used for gDNA-PCR, gene expression (Illumina NextSeq) and transplantation into sub-lethally irradiated recipient mice (500 cGy).
Results: Generation of MLL-AF9 leukemias in the MAFF background (MAFF-MA9) resulted in deletion of one HoxA cluster allele (HoxA$^{+/-}$), validated by genomic PCR and gDNA sequencing from expanded single colonies, presumably due to viral-induced activation of the Mx1 promoter. Poly(I:C) treatment of these mice resulted in a modest extension in survival (1-2 days) compared to controls. Direct treatment of MAFF-MA9 cells with interferon-α (*in vitro*) resulted in further deletion of the HoxA cluster (HoxA$^{-/hypo}$) and significant reduction in colony formation compared to controls. Although non-leukemic MAFF HSPCs retained colony forming ability after complete HoxA cluster deletion (HoxA$^{-/-}$) no HoxA$^{-/-}$ colonies were recovered from the interferon-α treated MAFF-MA9 cultures. Cre-recombinase-induced deletion of the HoxA cluster from AFlox-MA9 leukemia cells was confirmed by gDNA-PCR and sequencing. Transplantation of Cre-treated AFlox-MA9 cells resulted in significant increased survival ($P<0.002$) by up to 74 days in recipient mice, compared to controls. Further examination of the leukemias that developed from these Cre-treated AFlox-MA9 cells demonstrated retention of one allele of the HoxA cluster, as a result of escapees. To gain insight into the molecular mechanisms underlying the HoxA requirement for MLL-AF9 maintenance, matched Cre- or control treated AFlox-MA9 samples used for the transplantation were further examined for differential gene expression by Illumina NextSeq analysis. Gene expression signatures obtained from this analysis were submitted to the LINCS database to identify candidate small molecules that can mimic the HoxA deletion in MLL-AF9 leukemia.
Summary/Conclusions: Together these data support a fundamental role for the HoxA cluster in MLL-AF9 maintenance indicating dependency for this leukemia subtype which may be exploited for therapeutic benefit.

P181

TRIB2 TRANSFORMED GMP AS THE MYELOID LEUKEMIA INITIATING CELL

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Background: TRIB2 is a member of the mammalian Tribbles family of serine/threonine pseudokinases. When ectopically expressed in hematopoietic stem and progenitor cell (HSPC) enriched bone marrow cells, TRIB2 was shown to induce acute myeloid leukemia (AML) in a murine transplant model dependent on C/EBPα degradation, showing it to be a potent myeloid oncogene. The cell of origin or leukemia initiating cell (LIC) refers to the cell from which a specific leukemia normally arises, and it is hypothesised that the LIC may influence the progression, disease phenotype and response to therapy. It remains unclear whether the LIC is a HSC or a more committed progenitor cell in TRIB2-driven AML.
Aims: Our current study focuses on identifying the LIC in TRIB2-driven AML and characterizing its role in disease potency and maintenance.
Methods: FACS sorted CD45.2^{+} stem and progenitor cell populations (HSC, MPP, CMP, GMP and MEP) transduced with a lentiviral vector encoding TRIB2 were cultured in methocult supplemented with cytokines that support myeloid cell growth and differentiation. Following the first plating (P1), GFP^{+} cells were sorted and serially replated. Cells which still formed colonies by the third replating (P3) implied acquisition of self-renewal ability and increased proliferation characteristic of myeloid transformation. These CD45.2^{+} cells from P3 were transplanted into sublethally irradiated CD45.1^{+} C57BL/6 recipient mice and chimeric animals were monitored for 1 year. Chemoresistance experiments were performed on the bulk bone marrow population (CD45.2^{+} cells

>95%) from the TRIB2 AML mice. Cells were treated with a range of concentrations of daunorubicin (DNR), followed by trypan blue cell counts to assess viability.
Results: Our study identified that while lentivirally transduced TRIB2 can transform all stem and progenitor cell populations of the hematopoietic system with variable efficiencies *in vitro*, the GMP subpopulation was identified as the LIC of TRIB2-driven AML. TRIB2 transformed GMP cells generated a more potent AML with complete penetrance and shortened latency compared to all other HSPC populations analysed. Indeed, phenotypically different diseases were propagated from TRIB2 expression in the HSC and GMP, with the former having a weakly penetrant, longer latency AML with a mixed lineage phenotype, whereas the later was a dominantly myeloid disease phenotype with a short latency. We next addressed the chemotherapeutic response of TRIB2 positive AML cells. We show that GMP-TRIB2 AML and bulk TRIB2 AML cells are chemoresistant. TRIB2 overexpression decreases DNR induced apoptosis, and knockdown of TRIB2 expression in AML cells leads to an increase in apoptotic gene expression. Our studies illustrate that TRIB2 expression is key in mediating the anti-apoptotic signals following DNR treatment.
Summary/Conclusions: We identify the GMP as the LIC in TRIB2 driven AML. Our findings are further supported by our previous work showing that degradation of C/EBPa is required for TRIB2-driven AML and the GMP population expresses the highest level of C/EBPa in hematopoiesis. We provide evidence for TRIB2 role in chemoresistance, and that the TRIB2 LIC is a highly chemoresistant cell. Our findings provide insight into the molecular events contributing to AML, and provide potential for novel avenues for therapeutic targeting.

P182

TRIB2 REGULATES THE CELL CYCLE UNDER STRESS CONDITIONS IN A MURINE CELL MODEL OF LEUKEMIA

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Background: *TRIB2* is a known oncogene in different leukemias (AML, T-ALL) and can interact with different signalling pathways promoting the modulation of transcription factor and kinase proteins activation and expression in cancer. The expression level of TRIB2 is low in the myeloid lineage, but can be aberrantly increased in AML and other cancer types by different transcription factors (*e.g.* NOTCH1, MEIS1, E2F1, C/EBPα p30, PITX1 and TAL1). The tumorigenic mechanism of deregulated TRIB2 in myeloid cells has been elucidated, and involves degradation of the tumor suppressor C/EBPα p42. Far less is known about TRIB2 role in other AML oncogenic pathways. A link between TRIB2 and other key AML oncogenes has been documented, where *TRIB2* is one of the targets of Meis1 in NUP98/HOXD13/MEIS1-AML cells and HOXA9 has been shown to cooperate with TRIB2 to accelerate Trib2 driven AML. The mechanism of TRIB2 oncogenic cooperativity in AML is currently not known.
Aims: To assess the role of TRIB2 in the AML oncogenic pathways. In particular, we aimed to unravel the link between Trib2 and cell cycle progression after genotoxic stress.
Methods: To study the function of TRIB2, we utilised hematopoietic stem and progenitor cells (HSPCs) from WT and Trib2$^{-/-}$ mice. We generated an *in vitro* model of transformed leukemic cells by retrovirally expressing the fusion gene NUP98/HOXA9 (NH9) in murine HSPC and expanding them continuously in presence of IL3. Immortalized WT and Trib2$^{-/-}$ NH9 cell lines were challenged with the chemotherapeutic drug Daunorubicin (DNR) and apoptosis, cell cycle progression and DNA damage signalling pathways were analysed and compared between WT and Trib2$^{-/-}$ transformed cells using standard FACS, WB and qPCR techniques.
Results: Sensitivity to DNR is comparable between WT and Trib2$^{-/-}$ NH9 cells, as shown by AnnexinV-DNA double staining. Despite this, analysis of cell distribution within the cell cycle phases (GO, G1, S-G2-M) in the presence of DNR showed that Trib2$^{-/-}$ NH9 cells do not arrest in GO in response to the treatment as WT NH9 cells do, but rather continue to progress through to G2/M cell cycle phases. Moreover Trib2$^{-/-}$ NH9 cells exhibit higher expression of the mitotic marker Phospho-Histone H3 after the DNR treatment. Gene expression analysis revealed that Trib2$^{-/-}$ NH9 cells treated with DNR show evidence of DNA damage signalling pathways activation (with upregulation of p21, p16 and GADD45a). This response was higher than in the WT counterpart, suggesting a stronger or unresolved damage signalling in the Trib2$^{-/-}$ NH9 cells. MAPK p38 has a role in the activation of cell cycle check points after DNA damage, and is involved in cell cycle arrest to allow cells to repair the DNA before re-entering the cell cycle. WB analysis suggests that Trib2$^{-/-}$ NH9 cells have impaired phosphorylation of p38 in response to DNA insult consistent with the absence of a cell cycle arrest. Consistent with the absence of cell cycle checkpoint, p21 protein levels are also reduced in Trib2$^{-/-}$ NH9 cells compared to the WT cells after DNR treatment. Evidence for γH2Ax modification in Trib2$^{-/-}$ NH9 cells confirms that the damage was present and accumulated unresolved.
Summary/Conclusions: Our results show that Trib2 plays a role in the cell cycle checkpoint in transformed cells following DNA damage. The absence of Trib2 results in the loss of checkpoint controls including p38 MAPK and p21 activation and downstream activation of protective cell cycle mechanisms. These data suggest that the expression of Trib2 in AML may protect cells from genotoxic stress by preventing the accumulation of DNA damaged cells.

P183

CHROMOTHRIPSIS: A NEW MECHANISM OF CANCER INITIATION AND PROGRESSION IN ADULT ACUTE MYELOID LEUKEMIA

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Background: Chromosomal abnormalities are predictive of response in Acute Myeloid Leukemia (AML) and guide therapeutic strategies. Chromothripsis, a one-step catastrophic mechanism of genomic instability, could represent a driving force in the development and progression of hematological diseases.
Aims: To discover the mechanisms underlying the pathogenesis, chromosomal instability and heterogeneity of AML, we used single-nucleotide polymorphism (SNP) microarrays to study chromothripsis in our cohort of patients (pts).
Methods: We performed classical cytogenetic karyotyping and microarray analysis using Genome-Wide Human SNP Arrays 6.0 or Cytoscan HD Arrays (Affymetrix) in 303 AML pts at diagnosis (both *de novo* and secondary). SNP Array data were analyzed by Nexus Copy Number™ v7.5 (BioDiscovery, v.7.5). The survival analysis was performed with Kaplan-Meier method using Mantel-Cox test.

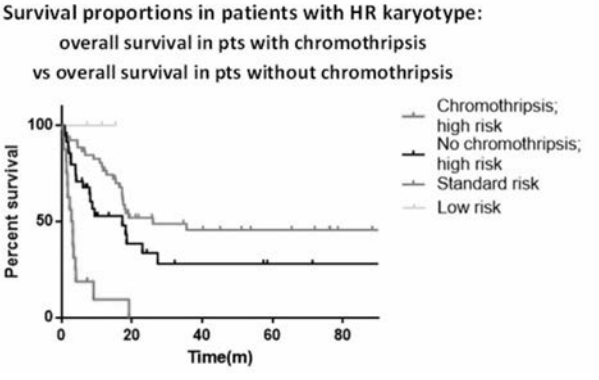

Figure 1.

Results: Twenty-three out of 303 pts (7.6%) showed chromothripsis events involving different chromosomes (2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17 and 20), reflecting the random distribution of the one-step catastrophic event. By Nexus Copy Number we defined chromothripsis as a 2-3 variation of copy number (CN) state with at least 5 subsequent rearrangements, including CN gain and loss, interspersed with regions of diploid state and variable number of breakpoints. The pts affected by chromotripsis had a median age of 67.5 years, complex karyotype and high risk (HR) disease according to ELN definition. This group was screened for *FLT3*, *NPM1*, *IDH1*, *IDH2* and *TP53* mutational status. *TP53* was evaluated in 21/23 pts, displaying that the majority (17/21) of the pts with chromothripsis harbored a *TP53* mutation, while 4/21 pts were wild type for *TP53* but 2 of those had a single-copy loss of the gene. *TP53* mutation has a significantly higher incidence in chromothripsis pts than in others without chromothripsis ($p<0.001$). Chromothripsis defines a group of pts with poor prognosis compared with other in the cohort ($p<0.001$), with a median survival of 2.9 months and 19.1 months respectively. Remarkably, chromothripsis defines the group with the worst prognosis even if compared with pts harboring HR[JM1] karyotype features without chromothripsis ($p<0.001$) (Fig.1). Median survival was 2.9 months in pts with chromothripsis, 17.3 months in HR pts without chromothripsis, and 26.0 months for pts with standard risk features. Moreover, by comparing pts with (23/303) and without (280/303) chromothripsis, we identified several genes differentially altered between the 2 groups ($p<0.001$). The statistically most common deleted genes in chromothripsis are *RAD50*, *MARCH3*, *PRDM6*, *SSBP2*, *CDC23*, *HDAC3*, *CHD1*, *TBCA*, *LMNB1*, *JMY*, with 5q being the main altered chromosomic region. While the significant amplifications included *ZDPM2*, *RUNX2*, *RUNX1T1*, *FLT3*, *ERG*, *TTC3* and *GPC6*. The most significantly affected pathways in the chromothripsis group of pts are: regulation and extension of axon, canonical Wnt signaling pathway involved in regulation of cell proliferation, positive regulation of mitotic cell cycle, chromatin organization, telomere formation and maintenance ($p<0.001$).
Summary/Conclusions: Chromothripsis is a recurrent event in adult AML and independently defines a group of pts with poor prognosis. It is strongly associ-

ated with *TP53* mutations and losses, highlighting the importance of *TP53* for maintaining genomic stability in adult AML.
Acknowledgements: ELN, AIL, AIRC, PRIN, Progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

Acute myeloid leukemia - Clinical 1

P184

AN EVALUATION OF THE TYROSINE KINASE INHIBITOR PACRITINIB IN PATIENTS WITH RELAPSED FLT3-MUTATED ACUTE MYELOID LEUKAEMIA (THE UK NCRI AML17 STUDY)

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Background: Pacritinib (formerly SB1518) is a third generation tyrosine kinase inhibitor with activity against a number of targets of relevance to acute myeloid leukaemia (AML). Kinases with IC_{50} <50nM to pacritinib include JAK2 (6nM), JAK2V617F (9nM), FLT3 (15nM), interleukin-1 receptor-associated kinase (IRAK1) (14nM) and c-FMS / colony-stimulating factor-1 (CSF1R) (40nM). FLT3 remains of intense interest as a therapeutic target in AML; activating mutations of *FLT3* are associated with early relapse after chemotherapy and poor survival. Efficacy against JAK2 has motivated study of pacritinib in myelofibrosis. Although *JAK2* mutations are rare in AML, the JAK-STAT pathway is frequently activated and may represent a mechanism of resistance to FLT3 inhibitors. There is also pre-clinical evidence that activity against IRAK1 and CSF1R may overcome microenvironmental resistance mechanisms. For these reasons we undertook a preliminary assessment of pacritinib as part of the UK NCRI AML17 Trial (ISRCTN55675535).
Aims: To assess the safety and efficacy of pacritinib monotherapy in *FLT3*-mutated AML patients who had relapsed following chemotherapy.
Methods: Patients commenced pacritinib at an oral dose of 200mg bid with the option, if well-tolerated, of increasing to 300mg bid after 14 days of treatment. Clinical assessments included optional bone marrow at day 14 and formal response assessment including blood and bone marrow examination at day 28. Toxicity was assessed using NCI-CTC Version 3.0. Treatment beyond day 28 was at the investigator's discretion. Patients were permitted to continue on pacritinib for up to 12 months and/or proceed to salvage chemotherapy with or without allogeneic SCT.
Results: A total of 30 patients received pacritinib therapy. Median age was 54 years (16-68), median presenting WBC was $35.6x10^9$/l (1.2-182) and all had cytogenetically intermediate risk disease. 26 patients (87%) had *FLT3*-ITD mutations (median *FLT3*-ITD allelic burden 30% [7-94]) and 4(13%) had *FLT3*-TKD point mutations; 16 patients (53%) had concomitant mutated *NPM1*. Four patients had received previous TKI therapy, all with lestaurtinib (CEP701) alongside first-line chemotherapy. Eight patients had received prior allogeneic SCT. 28 of the patients had relapsed disease; median duration of first CR was 6.5 months (1-65). 23 patients were in first relapse (including 1 molecular relapse) and 5 in second relapse. Two patients had primary refractory disease. Patients received a median of 64 days pacritinib (3-200); all were dosed at 200mg bid with 1 patient escalating to 300mg bid. Most toxicities were minor (grade1/2). The most common toxicities were nausea/vomiting (53%), diarrhoea (33%) and raised ALT (30%). One patient stopped pacritinib after 3 days due to nausea (grade 2). 6 further patients were considered non-evaluable either due to early death (2 patients; infections), inadequate response evaluation sampling (2 patients) or the addition of salvage chemotherapy within the 28-day evaluation period (2 patients). In evaluable patients, the overall rate of response to pacritinib monotherapy at the day 28 assessment was 17% (4/23) including 3 CRi (2 CRp) and 1 PR; 3 of these patients were successfully bridged to allogeneic SCT with the fourth patient relapsing at day 60. 3 additional patients (13%) achieved >50% reduction in bone marrow blasts without evidence of peripheral count recovery, 2 going on to receive salvage chemotherapy with FLAG-Ida. 16 patients (70%) were considered non-responders. The 7 clinical responders included 6 patients treated in first relapse and 1 with primary refractory disease; all had *FLT3*-ITD mutations.
Summary/Conclusions: This is the first clinical experience of pacritinib in AML. Tolerability was encouraging and, in the challenging setting of relapsed/primary refractory FLT3-mutated AML where FLT3-directed monotherapy has seldom achieved complete remission, clinical responses were seen in one-third of evaluable patients. Importantly, several patients were successfully bridged to potentially-curative allogeneic SCT. Further clinical evaluation of pacritinib in this setting is warranted.

P185

ORAL THROMBOPOIETIN RECEPTOR AGONIST ELTROMBOPAG TREATMENT DURING INDUCTION CHEMOTHERAPY FOR ACUTE MYELOGENOUS LEUKEMIA (AML): RESULTS OF A RANDOMIZED, DOUBLE-BLIND, PHASE 2 STUDY

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Background: Thrombocytopenia is a significant problem for patients with AML due to the disease process and the side effects of its treatment. Eltrombopag is an orally bioavailable, small molecule, thrombopoietin receptor agonist that stimulates platelet production by a mechanism similar but not identical to endogenous thrombopoietin. A randomized, placebo-controlled, Phase 1 study suggested a benefit of eltrombopag treatment of up to 300 mg daily in patients with advanced myelodysplastic syndromes or AML (Platzbecker U, *et al. Lancet Haematol*. 2015;2:e417-26).
Aims: To assess the safety and tolerability of daily treatment with eltrombopag *versus* placebo in patients with AML receiving anthracycline-based induction treatment; these included rates of adverse events (AEs), changes in left ventricular ejection fraction (LVEF), and clinical laboratory parameters including effects on cytopenias.
Methods: In this randomized, double-blind, Phase 2 study, adult patients with AML of any subtype (except M3 and M7) (n=148), and who had not received previous treatment for AML, received standard induction chemotherapy with daunorubicin on Days 1-3 and cytarabine on Days 1-7 (Table 1), and eltrombopag 200mg (100mg for East Asians) or placebo daily, starting on Day 4. Study drug could be titrated to 100-300mg based on platelet counts. Randomization was stratified by antecedent malignant hematologic disorder (yes/no) and age (18-60 *vs* >60 years). Safety was the primary endpoint. The trial began in September 2013, enrollment is completed, and all patients completed induction/reinduction. Follow up for survival continues. This primary analysis represents results through June 2015.

Table 1. Primary safety and efficacy results of a randomized, placebo-controlled, Phase 2 study of the thrombopoietin receptor agonist eltrombopag in patients with AML receiving anthracyclines-based induction chemotherapy.

	Eltrombopag 100–300 mg Daily (Starting D4)* Daunorubicin 90 mg/m² IV D1–3 (60 mg/m² for patients >60 years) Cytarabine 100 mg/m² IV D 1–7 (n=74)		Placebo Daily (Starting D4) Daunorubicin 90 mg/m² IV D1–3 (60 mg/m² for patients >60 years) Cytarabine 100 mg/m² IV D1–7 (n=71)†	
Safety, n (%)				
Any AE	72 (97%)		66 (93%)	
Any serious AE	24 (32%)		14 (20%)	
Deaths	23 (31%)		13 (18%)	
Cause of death				
MI	1 (1%)		0	
Hemorrhage‡	5 (7%)		1 (1%)	
Cancer	12 (16%)		7 (10%)	
AML	12 (16%)		6 (8%)	
Other	0		1 (1%)	
Sepsis	3 (4%)		3 (4%)	
Pneumonia	1 (1%)		2 (3%)	
Respiratory failure	1 (1%)		0	
Missing	1 (1%)		1 (1%)	
LVEF event§	5/57 (9%)		7/62 (11%)	
Grade 3/4 laboratory AE	Baseline n/N (%)	Post-baseline n/N (%)	Baseline n/N (%)	Post-baseline n/N (%)
Thrombocytopenia	36/74 (49%)	74/74 (100%)	35/71 (49%)	71/71 (100%)
Neutropenia	38/71 (54%)	72/74 (97%)	44/69 (64%)	71/71 (100%)
Anemia	15/74 (20%)	68/74 (92%)	20/71 (28%)	66/71 (93%)
Estimated Efficacy	HR or OR (EPAG/PBO)	95% CI	*P* value	
Time to platelet recovery ≥20 Gi/L	HR=0.84	(0.28, 2.48)	0.746	
Time to platelets >100 Gi/L	HR=1.10	(0.74, 1.63)	0.618	
Overall response¶	OR=0.87	(0.40, 1.89)	0.712	
Overall survival	HR=1.52	(0.80, 2.89)	0.193	

AE, adverse event; D, day; CI, confidence interval; EPAG, eltrombopag; HR, hazard ratio; IV, intravenous; LVEF, left ventricular ejection fraction; OR, odds ratio; MI, myocardial infarction; PBO, placebo.
*EPAG could be increased up to 300 mg once daily (100 mg starting dose for those with East Asian heritage, which could be increased to 150 mg) based on platelet count <100 Gi/L until a platelet count of at least 200 Gi/L is achieved, until remission is assessed by bone marrow biopsy, or for a maximum of 42 days from the start of the chemotherapy induction cycle.

Results: The eltrombopag *versus* placebo groups, respectively, were well matched at baseline: overall median (range) age 58.5 (23-77) *versus* 59.5 (21-75); median (range) platelet counts 51.5 (5-241) Gi/L *versus* 50.0 (9-232) Gi/L; 51% *versus* 42% females; and 22% poor karyotype in both groups. The frequency of AEs did not differ between treatment groups, but frequencies of serious AEs and deaths were greater in the eltrombopag group (with more deaths due to hemorrhage and cancer, which included disease under study and other malignancies) (Table 1). Deaths due to hemorrhage included pulmonary and intracranial/cerebral/brain hemorrhages, and occured during periods of thrombocytopenia. Thromboembolic events were similar between groups (7% *vs* 6% for eltrombopag *vs* placebo). Reductions in LVEF (a potential effect of drug-drug interaction between eltrombopag and daunorubicin) did not differ between groups (Table 1). Increases from baseline in Grade 3/4 laboratory AEs for thrombocytopenia, neutropenia, or anemia did not differ significantly between treatment groups. Time to platelet recovery, time to platelets >100 Gi/L, overall disease response, and survival also did not differ significantly between groups (Table 1).
Summary/Conclusions: The thrombopoietin receptor agonist eltrombopag did not significantly affect rates of thrombocytopenia during induction therapy with daunorubicin and cytarabine or disease response in adults with AML compared with placebo in this primary analysis. No statistically significant difference in LVEF events between eltrombopag and placebo was observed. *Funding:* This study (NCT01890746) was sponsored by GlaxoSmithKline; however, as of March 2, 2015, eltrombopag became an asset of Novartis AG.

P186

SAFETY STUDY OF CRENOLANIB, A TYPE I FLT3 INHIBITOR, WITH CYTARABINE/DAUNORUBICIN OR CYTARABINE/IDARUBICIN INDUCTION AND HIGH-DOSE CYTARABINE CONSOLIDATION IN NEWLY DIAGNOSED FLT3+ AML

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Background: Combining tyrosine kinase inhibitors (TKIs) of FLT3, such as midostaurin, with chemotherapy in FLT3+ mutant AML has improved CR rates (59% *vs* 53%) as well as overall survival (OS). Crenolanib is a novel, type I, oral pan-FLT3 TKI demonstrating encouraging single-agent activity against multiply relapsed FLT3-ITD and tyrosine kinase domain (TKD) mutant AML. We here report interim data from a Phase II trial to evaluate the safety and efficacy of crenolanib in combination with upfront chemotherapy (cytarabine plus idarubicin or daunorubicin) in patients (pts) with newly diagnosed FLT3+ AML.
Aims: To assess the safety and tolerability of crenolanib (100 mg TID) with induction and consolidation chemotherapy in newly diagnosed FLT3+ AML. Secondary objectives are to assess CR rate and relapse-free survival following crenolanib and chemotherapy.
Methods: Patients >18 years (y), with newly diagnosed FLT3+ AML (ITD and/or TKD mutants including secondary AML) are eligible. Crenolanib, 100 mg TID, is given daily from day (d) 9 following 7+3 induction (cytarabine 100 mg/m^2/d and either daunorubicin d1-3 (<60y: 90 mg/m^2; ≥60y: 60 mg/m^2) or idarubicin 12 mg/m^2 d1-3). Re-induction is permitted for inadequate leukemia cytoreduction on days 15-28. Consolidation with 6 doses of high dose cytarabine (HiDAC) (<60y: 3 g/m^2; ≥60y: 1g/m^2) is given with daily crenolanib (100 mg TID) starting d7. Eligible pts can proceed to allogeneic SCT. Crenolanib maintenance can be given for 1y post HiDAC or SCT. Crenolanib is held if total bilirubin is abnormal.
Results: 19 pts with newly diagnosed FLT3+ AML have so far been enrolled (11 females, 8 males) with a median age of 55y (range 23-74y). Median baseline WBC was 27,740/µL (range 1,760-248,800; 4 pts had WBC >100,000/µL). 13/17 (76%) of pts had normal karyotype, 2 pts had trisomy 8, 1 pt was pseudodiploid and 1 pt had complex karyotype. Majority of pts, 79% (15/19), were FLT3-ITD+ve with 10/15 also carrying NPM1 mutation. 4/19 (28%) pts were FLT3-D835+ve with 1 pt also carrying NPM1 mutation and 1 pt carrying D835Y and N841T mutations. 11/19 pts were tested for other mutations present at baseline: DNMT3A in 6/11 pts (55%), SF3B1, IDH1, TET2 and RUNX1 each in 2/11 pts (18%), and IDH2, MLL, SRSF2 and WT1 each in 1/11 pts (9%). All 19 pts have received induction chemotherapy: 13 received daunorubicin (8 pts at 90 mg/m^2, 5 pts at 60 mg/m^2) and 6 received idarubicin (12 mg/m^2) No pt has required a second induction. 2 pts died prior to starting crenolanib during induction 1 (due to sepsis and respiratory failure) and are not evaluable. Crenolanib 100 mg TID has been well-tolerated in combination with cytarabine/anthracycline induction with only 2 pts requiring dose reductions (due to rash and periorbital edema, respectively). Common AEs are consistent with what is seen with crenolanib monotherapy, including grade 1 or 2 nausea, vomiting, diarrhea and rash. Crenolanib did not appear to delay count recovery; median count recovery for WBC was 28d (range 16-43d) and platelet was 27d (22-46d). A high CR rate was seen in the 14 pts who are currently evaluable: 13/14 pts (93%) achieved CR with full count recovery, and all pts became FLT3-ve. Only 1 pt was refractory after first cycle of induction therapy and was taken off study. To date, 9 pts have received a total of 13 cycles of consolidation with HiDAC and crenolanib. 2 pts have undergone allogeneic SCT.
Summary/Conclusions: Crenolanib can be safely administered at full doses with cytarabine/anthracycline induction chemotherapy and HiDAC consolidation. Initial CR rates after just one cycle of induction with 7+3 and crenolanib are high. To date, no patients have relapsed. Accrual to this trial continues.

P187

PHASE II STUDY OF CLADRIBINE AND LOW-DOSE ARAC ALTERNATING WITH DECITABINE IN OLDER PATIENTS WITH AML

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Background: Treatment of AML in older and unfit patients (pts) with intensive chemotherapy is complicated by increased toxicity and high early mortality. Additionally, many of these pts are not ideal candidates for stem cell transplant (SCT). Lower intensity approaches using hypomethylating agents (HMAs) are currently used for these pts, but more effective therapies are needed. Cladribine, which itself has hypomethylating properties, has been shown to improve survival in AML in combination with standard-dose araC (Holowiecki JCO 2012).
Aims: We developed a low-intensity, prolonged-maintenance treatment protocol studying the combination of cladribine and low-dose araC (LDAC) alternating with decitabine (DAC) in patients aged ≥60.
Methods: Pts with adequate organ function and newly diagnosed AML (excluding APL), including secondary- (s-AML) and therapy-related AML (t-AML), and high risk MDS were eligible. Induction was cladribine 5 mg/m^2 IV over 30 minutes on days 1-5 followed by araC 20mg SQ BID on days 1-10. Consolidation/maintenance consisted of 2 cycles of cladribine 5 mg/m^2 IV over 30 minutes on days 1-3 + araC 20 mg SQ BID on days 1-10 alternating with 2 cycles of DAC 20 mg/m^2 on days 1-5, for a maximum of 18 cycles. One cycle was 4 weeks and up to 2 cycles of induction were allowed.
Results: A total of 86 pts have been enrolled with a median age of 69 years (range, 49-85), including 37 pts (43%) ≥age 70. 49 pts (57%) had s-AML or t-AML and 16 pts (19%) had therapy for an antecedent hematologic disorder. Pt characteristics are listed in Table 1. Of the 86 pts evaluable for response, there were 52 CR (60%), 5 CRp (6%), 2 CRi (2%) and 1 PR (1%) for an overall response rate (CR/CRp/CRi) of 69% (59/86). Of the 49 pts who achieved a CR/CRp and had minimal residual disease (MRD) testing by flow cytometry at day 29, 24 pts (49%), achieved MRD negativity. However, MRD negativity at day 29 did not correlate with improved OS. 13 of the 57 pts (23%) who achieved a CR/CRp went on to SCT. With a median follow-up of 24+ months (m), the median OS is 13.8 m (16.5m in responders) and the median CR duration is 21.1m. (Figure 1a) The 1-year OS estimate is 57%. All pts had cytogenetic and molecular characterization of their AML prior to treatment, allowing correlation with outcomes (Figure 1b). Responses and median OS among different subgroups are shown in Table 1. The 4-week mortality was 1%. Grade ≥3 infections occurred in 16% of pts. There were no treatment-related grade 3/4 non-heme adverse events (AEs). Most common non-hematologic AEs were elevated bilirubin, nausea/vomiting, rash/itching, diarrhea and mucositis.

Table 1. Patient Characteristics (N=74).

Characteristic	N (%)	Outcomes by Genetic Subset (N)	CR/CRp	Median OS (m)
Median age [Range]	69 [49-85]	Diploid karyotype (27)	88%	16.4
Cytogenetics		Adverse karyotype (35)	46%	12.5
Diploid	27 (31)	Complex [> 2 abnl] karyotype (23)	43%	5.3
Adverse	35 (41)	TP53 mutated (12)	50%	16.2
Misc. other	18 (21)	DNMT3a mutated (9)	89%	12.6
Insufficient	6 (7)	FLT3-ITD (8)	100%	14.2
Median BM Blast [Range]	32 [8-95]	JAK2 mutated (7)	57%	7.5
Median WBC [Range]	2.7 [0.5-72.3]	NPM1 mutated (14)	100%	15.2
Median Platelets [Range]	41 [4-772]	RAS mutated (18)	61%	14.3
Median Creatinine [Range]	0.89 [0.46-1.94]			
Median Bilirubin [Range]	0.6 [0.2-2]			

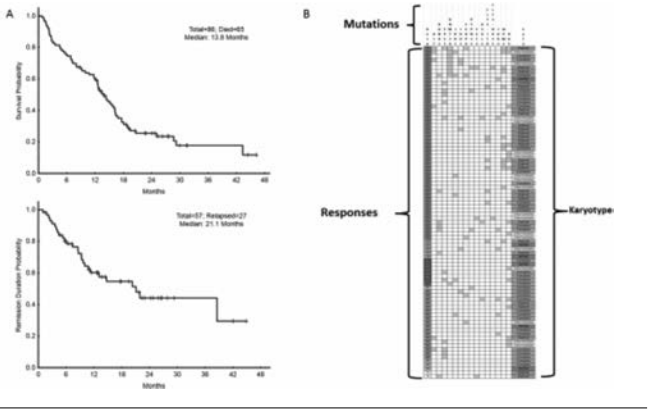

Figure 1.

Summary/Conclusions: The low intensity program of cladribine + LDAC alternating with DAC produces durable responses and is a well-tolerated ambulatory regimen for older patients, including those with unfavorable-risk features.

P188

DIFFERENTIATION RESPONSE TO GILTERITINIB (ASP2215) IN RELAPSED/REFRACTORY FLT3 MUTATED ACUTE MYELOID LEUKEMIA PATIENTS IS ASSOCIATED WITH CO-MUTATIONS IN NPM1 AND DNMT3A

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Background: FLT3 inhibitors show substantial clinical activity in relapsed/refractory *FLT3-ITD* mutated acute myeloid leukemia (AML). A subset of patients show a clinical response manifest by terminal differentiation of bone marrow blasts into granulocytes that contain *FLT3-ITD*. From clinical trials of the FLT3 inhibitor quizartinib (AC220), we observed this differentiation response predominantly among patients with a normal karyotype and mutated *NPM1* and/or *DNMT3A* (Nybakken, *et al.* Leukemia 2015).
Aims: To confirm whether observations from quizartinib trials reflect a common response pattern to FLT3 inhibitor treatment.
Methods: We performed analysis of the local institutional cohort of patients enrolled on a phase 1/2 study of gilteritinib (ASP2215) for relapsed/refractory AML (NCT02014558). All subjects were screened for 33 AML-associated mutations at study entry by next generation sequencing and all available histomorphologic, and cytogenetic material underwent an independent pathologist's blinded review.
Results: We restricted our analysis to the 25 *FLT3*-mutated subjects who received single-agent gilteritinib at ≥80 mg/day, as correlative pharmacodynamic data showed that this dose consistently generated continuous, potent FLT3 target inhibition (Levis MJ, *et al.* ASCO abstracts 2015). 2 subjects were withdrawn prior to the first treatment response assessment (refractory leukemia and lethal infection); 2 subjects had technically insufficient marrows. The remaining 21 subjects were evaluable for marrow response. Subjects had a median age of 61 years (range 22-86 years) and were relapsed or refractory after a median of 2 prior lines of chemotherapy (range 1-7). Subjects received gilteritinib at a median dose of 200 mg (range 80-300) for a median of 154 days (range 38-642). Baseline karyotype was normal in 9/20 (45%). 19/21 patients had *FLT3-ITD* mutation (3 had both *FLT3-ITD* and *FLT3-TKD*), and 2 had *FLT3-TKD* only. Gene mutations seen in >10% of subjects included *NPM1* (13, 62%), *DNMT3A* (11, 52%), *WT1* (8, 38%), *TET2* (7, 33%), and *IDH1/2* (4, 19%). All subjects treated for >28 days showed complete or near-total eradication of peripheral blood blasts. Thirteen subjects (62%) experienced a differentiation response defined by markedly left-shifted clonal granulocytic hyperplasia with persistent *FLT3-ITD:WT* allelic ratio, ≥50% reduction in overall blast percentage from baseline, and peripheral blood neutrophil recovery. 11/13 (85%) subjects with differentiation response had mutated *NPM1*, but only 6/12 (50%) had a normal karyotype. While 2/3 *NPM1* mutated subjects with WT *DNMT3A* did experience differentiation response, 10/11 subjects with *DNMT3A* mutation also had *NPM1* mutations, confounding our ability to associate differentiation response and *DNMT3A* mutation status alone. 3/21 subjects demonstrated both undetectable leukemic blasts from the marrow along with marked allelic ratio reduction or elimination of detectable *FLT3-ITD*, suggesting alternate mechanisms of treatment response (*e.g.* cytotoxicity or clonal selection without differentiation). Additionally, 5/21 evaluable subjects showed minimal to no reduction in marrow blasts. Considering the 8 non-differentiation responses plus the sole patient with growing AML prior to day 28, only 2/9 (22%) had an *NPM1* mutation, and 5/8 had abnormal karyotype.
Summary/Conclusions: Differentiation response to gilteritinib is strongly enriched among relapsed/refractory *FLT3*-mutated patients with *NPM1* and *DNMT3A* mutations. Our data inform response interpretation and may promote future trial enrichment strategies.

P189

PHASE IB/II STUDY OF NIVOLUMAB IN COMBINATION WITH 5-AZACYTIDINE (AZA) IN PATIENTS (PTS) WITH RELAPSED ACUTE MYELOID LEUKEMIA (AML)

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Background: PD1+ T-cells are significantly increased in the bone marrow (BM) of pts with relapsed AML as compared to healthy adult donor BM (*Daver et al., AACR 2016*). Epigenetic therapy upregulates PD1 and PD-L1 in MDS/AML and upregulation of these genes may be associated with the emergence of resistance and inferior survival (*Yang et al., Leukemia 2013*).
Aims: This single-center phase Ib/II study was conducted to determine the recommended phase 2 dose (RP2D), efficacy and safety of nivolumab in combination with AZA in pts with relapsed AML.

Methods: Pts are eligible if they have AML and failed prior therapy, have adequate performance status (ECOG ≤2), and organ function. The first six pts received AZA 75mg/m^2 days 1-7 with nivolumab 3mg/kg on Day 1 and 14. Courses were repeated approximately every 4-5 weeks. One of six pts had a dose limiting toxicity (grade 3 pneumonitis) and this dose was established as the RP2D. 24 additional pts have been treated at the RP2D.
Results: To date, 30 pts (15 *de novo*, 15 secondary AML) with a median age of 72 years (range, 45-76) have been enrolled. They included 9 (30%) pts with diploid, 14 (47%) with adverse, and 7 (23%) with miscellaneous cytogenetics. Median number of prior therapies was 2 (range, 1-4), including hypomethylating agent-based (n=17), high dose cytarabine-based (n=15), intermediate dose cytarabine-based (n=7), and targeted therapies (n=5). Median bone marrow blast%, WBC, hemoglobin, and platelet counts were 43% (13-90), 2.3x10^9/L (0.5-81), 9.3g/dL (7.4-12.7), and 28x10^9/L (8-145), respectively. Baseline bone marrow samples from all pts were analyzed for common myeloid mutations by next generation sequencing. The most frequently detected mutations were *DNMT3A* (n=9), *ASXL1* (n=6), *CEBPA* (n=4), *RAS* (n=4), *TP53* (n=3), and *TET2* (n=4). 22 pts are evaluable for response (Fig 1): 4 (18%) achieved complete remission (CR)/ complete remission with insufficient count recovery (CRi), 2 (9%) had hematologic improvement (HI) with blast reduction, 5 (23%) had HI only, and 5 (23%) had ≥50% BM blast reduction only. 6 pts had stable disease (n=2) or progression (n=4). 8 pts are too early for response assessment at this time. Median number of cycles to any response was 2 (1-4). The median follow-up is 3.6 months (1.5-8.7). Grade 3/4 and Grade 2 immune toxicities were observed in 6 (20%) and 5 (17%) pts, respectively. These included 6 pneumonitis, 2 nephritis, 1 skin rash, and 2 transaminitis. Time to onset of toxicities ranged from 4 days to 3.5 months. All toxicities responded rapidly to steroids and all pts were successfully rechallenged with nivolumab. No pts came off study due to immune toxicities. No association between toxicities and response was identifiable. The median progression free and overall survival for 22 evaluable pts was 3.5 and 5.9 months, respectively. These results compare favorably to a historical cohort of 99 relapsed AML pts treated on other hypomethylating agent combination protocols at our institution between 2005-2015 (Table 1). Identification of baseline and dynamic biomarkers of response and survival by sequential immunohistochemistry, flow-cytometry, and RNA-sequencing is currently ongoing.

Table 1. AZA+nivolumab *versus* historical HMA-combinations at MDACC

	AZA+nivolumab N=22	Hypomethylator-combination protocols N=99	*P*-value
CR/CRi	18%	11%	0.36
8-week mortality	5%	20%	0.08
Med PFS	3.5 m	2.2 m	<0.001
Med OS	5.9 m	4.1 m	0.25

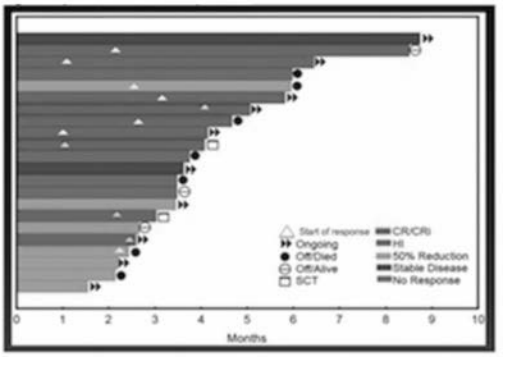

Figure 1. Response in 22 evaluable patients.

Summary/Conclusions: Combination of AZA and nivolumab is effective in pts with relapsed AML. Immune mediated toxicities occur and may be adequately managed with early recognition and systemic steroids.

P190

CLINICAL RESPONSE IN RELAPSED/REFRACTORY AML PATIENTS CORRELATES WITH LEUKEMIC BLAST MOBILIZATION AND DIFFERENTIATION INDUCED BY BL-8040, A POTENT CXCR4 ANTAGONIST; RESULTS OF A PHASE IIA STUDY

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Background: The bone marrow (BM) niche protects acute myeloid leukemia (AML) cells from chemotherapy. BM retention of AML cells is dependent on CXCR4 and its ligand CXCL12 (SDF-1); high CXCR4 expression correlates with poor survival in AML patients. Blocking CXCR4 disrupts the interaction of AML blasts with the BM and augments the anti-leukemic effect of chemotherapy. BL-8040 (BKT140) is a high affinity CXCR4 inhibitor with long receptor occupancy. Recent clinical trials have demonstrated BL-8040's robust hematopoietic cell mobilization from the BM. BL-8040's anti leukemic effect is mediated through robust leukemic blast mobilization, induction of leukemia cell differentiation and apoptosis. Here we report results of a phase 2a study evaluating BL-8040 in combination with cytarabine (Ara-C) for the treatment of relapsed/refractory AML patients (NCT01838395).
Aims: To assess the safety, efficacy and PK/PD parameters of BL-8040 in combination with Ara-C in relapsed/refractory AML patients.
Methods: The study included a dose escalation phase (3+3 design) followed by an expansion phase. Each patient received a once daily SC dose of BL-8040 as monotherapy on days 1-2 followed by the same dose of BL-8040 plus Ara-C (1.5g/m^2 for patients ≥60; 3g/m^2 for patients <60) on days 3-7. Six BL-8040 doses (0.5-2.0mg/kg) were tested in the escalation phase with 1.5 mg/kg selected for the expansion phase. PD parameters such as extent of mobilization, induction of differentiation and apoptosis, CXCR4 expression and receptor occupancy were assessed by BM biopsy on day 3 and throughout the study. Remission was determined by BM biopsy on day 30.
Results: Forty five patients (median age, 61y; range, 23-75y) were treated with BL-8040 (including 3 patients treated on compassionate use basis). BL-8040 was escalated up to 2.0 mg/kg without reaching the MTD. Combination with Ara-C was safe and well tolerated at all doses. Three SAE's (Sweet's Syndrome, PCP pneumonia and allergic type reaction) were reported by the investigators as possibly related to BL-8040. Primary BL-8040 related AEs were transient, mild to severe injection site and systemic reactions, none of which were considered as DLT. The available composite complete remission (CR+CRi) rate is 38% in patients receiving BL-8040 doses of 1 mg/kg and higher (n=39). While baseline BM disease burden was comparable between responders (R) and non-responders (NR) (38% *vs* 40% BM blasts, respectively), responders demonstrated significantly lower levels of circulating blasts at baseline compared to non-responders (2.9% *vs* 19.3%, respectively). Furthermore, response to treatment was associated with higher mobilization of AML blasts following 2 days of BL-8040 monotherapy (CR=7.0, CRi=4.2, PD=1.1, SD=0.4, fold change) and with induction of granulocytic differentiation in the BM (R=3.2 *vs* NR=1.4, fold change). PD analysis further confirmed BL-8040's long receptor occupancy and its ability to induce apoptosis.
Summary/Conclusions: The results demonstrate that, in difficult to treat AML patients, sustained blockade of CXCR4 with BL-8040 combined with Ara-C may improve the clinical response rates achieved historically with Ara-C. In addition the data, for the first time, may suggest that better clinical responses are seen in patients with more efficient CXCR4 inhibition (reflected by higher mobilization and induction of granulocytic differentiation) and lower peripheral circulating blasts (despite comparable marrow blasts) at baseline. This finding may serve as a biomarker for patient selection in future BL-8040 studies in AML.

P191

RESULTS OF A PHASE I STUDY OF GMI-1271, A NOVEL E-SELECTIN ANTAGONIST IN COMBINATION WITH INDUCTION CHEMOTHERAPY IN RELAPSED/REFRACTORY AML: A NOVEL, WELL-TOLERATED REGIMEN WITH A HIGH REMISSION RATE

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Background: The treatment of patients with relapsed and refractory acute myeloid leukemia (AML) remains a significant challenge with poor outcomes primarily due to low remission rates as well as short remission duration. Although cytotoxic chemotherapy remains the standard approach for the treatment of patients with relapsed or refractory (R/R) AML, novel agents are urgently needed to improve clinical outcomes. The regimen consisting of mitoxantrone, etoposide, and cytarabine (MEC) is commonly used for patients with R/R AML, with remission rates of 25-30%. Binding of leukemic blasts to E-selectin, an adhesion molecule expressed constitutively in the bone marrow endothelium, activates leukemic cell survival pathways, thereby contributing to chemotherapy

resistance. GMI-1271 is a novel antagonist of E-selectin. Here we report initial results from a phase 1/2 trial of GMI-1271 with MEC for the treatment of patients with R/R AML.
Aims: To evaluate the safety, tolerability, pharmacokinetics (PK), and antileukemic activity of the combination of GMI-1271 plus MEC.
Methods: An open-label phase 1/2 trial enrolled patients with R/R AML receiving MEC induction chemotherapy. Eligible patients (ECOG 0–2) must have received ≤2 prior induction regimens, have no active CNS disease and adequate renal and hepatic function. Adjunctive treatment with GMI-1271 at increasing doses was administered concurrent with chemotherapy (24 hours prior, throughout, and 48 hours post MEC); MEC consisted of mitoxantrone 10 mg/m^2/d, etoposide 100 mg/m^2/d, and cytarabine 1000 mg/m^2/d IV for 5 days and supportive care given as per institutional guidelines. Dose limiting toxicity (DLT) was defined as either persistent neutropenia and/or thrombocytopenia beyond day 42 in the absence of disease or any grade 3 non-hematologic toxicity that did not resolve to Grade 2 by day 42.
Results: Two dose level cohorts have been completed, with 13 subjects treated (GMI-1271 at 5 mg/kg (N=6) and10 mg/kg (N=7)). The median age was 51 (range 26 –74) and 9 were male (69%). Seven patients (54%) had adverse cytogenetic risk (SWOG) and 6 patients (46%) had intermediate cytogenetic risk; none were favorable. Eight patients (62%) had relapsed disease and 5 (38%) were refractory to primary therapy. Two patients had relapsed within a year of hematopoietic cell transplant (HCT), 2/13 were FLT3-ITD mutated, 1 patient had extramedullary disease (EMD). All subjects tolerated GMI-1271+MEC well and completed study treatment without dose reduction or interruption; 30 day mortality was 0%. Mucositis developed in 5 subjects (4 at 5 mg/kg), of whom 2 required IV nutrition (both at 5 mg/kg). No DLTs were observed. Initial population PK analysis showed 28% lower clearance than healthy adults and overall similarity in PK profile. Seven of 13 subjects achieved CR (54%); one achieved CRi (transplanted before CR documented); one achieved morphologic leukemia-free state (inadequate count recovery); and four had persistent disease. CR/CRi rate was 8/13 subjects (62%). Of 5 with data post CR/CRi, 1 proceeded to HCT, one relapsed at 90 days, 3 remain in CR (4, 4, and 2 months duration). Responders include refractory AML (3), relapsed FLT3-ITD mutated (1) and EMD (1).
Summary/Conclusions: In early clinical assessment of the novel E-selectin antagonist GMI-1271, we report in a group of R/R AML patients a CR/CRi rate of 62%, higher than expected given the high risk cytogenetic features in this group. No DLTs have been observed. Enrollment in a third cohort is ongoing and will be reported. A Phase 2 expansion cohort is planned in both R/R AML as well as elderly *de novo* AML.

P192

RESULTS OF A PHASE 1B STUDY OF VENETOCLAX PLUS DECITABINE OR AZACITIDINE IN UNTREATED ACUTE MYELOID LEUKEMIA PATIENTS ≥65 YEARS INELIGIBLE FOR STANDARD INDUCTION THERAPY

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Background: Venetoclax (VEN) is a potent, orally bioavailable BCL-2 inhibitor with single-agent activity in relapsed/refractory acute myeloid leukemia (AML) patients (pts), displaying synergistic activity with hypomethylating agents in preclinical studies. This trial evaluates VEN plus decitabine (DEC) or azacitidine (AZA) in treatment (Tx)-naive AML pts ≥65 y (NCT02203773).
Aims: The objectives of the study include safety, preliminary efficacy, and biomarker evaluations.
Methods: Tx-naive pts (ECOG PS ≤2, ≥65 y, intermediate- or poor-risk karyotype) not eligible for standard induction therapy received DEC (Arm A: 20 mg/m^2 iv) daily on days (D) 1-5 or AZA (Arm B: 75mg/m^2; subcutaneous or iv) daily on D 1-7 of each 28-D cycle in combination with once-daily continuous oral VEN. VEN dose escalation follows a 3+3 design; 1200 mg is the final dose level.
Results: As of 11/28/15, 39 pts (49% male; median age 74 y [65-85 y]) have been enrolled in Arm A (n=20) and Arm B (n=19). Median time on study is 111 D (6-375 D); 16 pts (41%) remain on therapy. Biomarker analysis and response evaluations have been completed in 34 pts with 400-mg and 800-mg VEN doses. As of 9/19/15, overall response rate (ORR; complete response [CR]/CR with incomplete marrow recovery [CRi]/partial remission [PR]) within this population was 76% (CR: 13/CRi: 11/PR: 2; 26/34 pts). Poor-risk cytogenetics and IDH1/2 mutations were reported in 24% (8/34) and 32% (11/34) of pts; ORR was 88% (7/8) and 82% (9/11), respectively. Median time to CR/CRi was 29.5 D (24-112 D). Most common treatment emergent adverse events (TEAEs) were nausea (54%), febrile neutropenia (41%), diarrhea (44%), decreased appetite (33%), and peripheral edema (31%). No dose-limiting toxicity was reported. Febrile neutropenia (41%) and neutropenia (33%) were the most common Grade 3/4 TEAEs. Most frequent serious AE was febrile neutropenia (28%). Four relapses occurred, all on Arm A. Six deaths occurred (3 disease progression, 1 sepsis, 1 respiratory failure, 1 bacteremia). MTD has not been reached.
Summary/Conclusions: Tx with VEN plus DEC or AZA shows a tolerable safety profile, with high response rates observed in Tx-naive AML pts ≥65 y, including those with adverse biologic disease features.

P193

A PHASE I TRIAL OF A PHARMACODYNAMICALLY-CONCEIVED DECITABINE/THIOGUANINE COMBINATION IN PATIENTS WITH ADVANCED MYELOID MALIGNANCIES

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Background: Using a chemosensitivity screening assay, we previously demonstrated that decitabine and thioguanine combinations can rescue therapeutic efficacy in primary leukemia cells isolated from patients with relapsed and refractory acute myeloid leukemia (AML). Although decitabine and thioguanine have single-agent anti-leukemic activity, they have not previously been used concurrently.
Aims: To test the safety and preliminarily assess additive/synergistic activity of this combination, we performed a Phase I dose escalation trial of thioguanine given with standard dose decitabine.
Methods: Patients with AML >60 yrs not candidates for standard induction, relapsed/refractory AML, advanced MDS, and CMML were eligible. Two thioguanine dose levels were evaluated: 80 and 120 mg/m2/day, given on Days 1-12 of induction and Days 1-7 of maintenance. Decitabine at 20mg/m2 was administered on Days 3-12 during induction and on Days 3-7 during maintenance. Standard safety measures, clinical outcomes, and activity of decitabine and thioguanine was assessed by patient-specific pharmacodynamics including an *in vitro* chemosensitivity assay, genome-wide analysis of DNA methylation changes, and BH3 profiling in order to measure the degree to which samples were primed to undergo apoptotic cell death.
Results: Twelve patients (median age, 67) with denovo AML (1), transformed AML (6), relapsed/refractory AML (4), and CMML (1) were treated. DLT's were seen in two patients. One developed acute renal failure requiring hemodialysis (80mg/m2) and another persistent grade 4 leukopenia/thrombocytopenia (120mg/m2). Grade 3 infectious complications were common: neutropenic colitis, bacteremias, and pneumonias. 10 of 12 patients completed therapy with 4 undergoing Allogeneic-BMT (3 AML, 1 CMML), 4 removed for disease progression, and 2 for DLT. One patient relocated after induction, and another declined therapy after induction. Median cycles given was 3. Bone marrow blast reductions to <5% were seen in 6 of 7 evaluable patients with AML (1 CR and 3 CRi). The overall response rate in this high-risk patient population was 67%. The patient who achieved a CR (relapsed AML) received 4 previous cycles of single-agent decitabine, demonstrating that the decitabine and thioguanine combination regimen can rescue previous hypomethylating agent failure. The chemosensitivity assay results for thioguanine on pretreatment mononuclear cells directly correlated with clinical outcome in both response to therapy and clinical resistance. BH3 profiling was also performed on pre-treatment myeloblasts. Significant apoptotic priming corresponded to good initial clinical response. In addition, in the single patient who responded and subsequently relapsed, decreased apoptotic priming was observed in the relapsed sample, suggesting that the thioguanine/decitabine regimen applies selective pressure for reduction in apoptotic priming.
Summary/Conclusions: The decitabine and thioguanine combination was well-tolerated and showed surprising anti-leukemic activity. Importantly, chemosensitivity and BH3 profiling accurately predicted responses and confirmed the etiology of the loss of therapeutic response. A muti-center Phase II clinical trial is being planned.

Microenvironment and signaling in CLL

P194

GENOMIC CHARACTERIZATION OF HIGH-COUNT MBL INDIVIDUALS INDICATES THAT EARLY DETECTION OF DRIVER MUTATIONS AND SUBCLONAL EXPANSION ARE PREDICTORS OF ADVERSE CLINICAL OUTCOME

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Background: High-count monoclonal B-cell lymphocytosis (MBL) is an asymptomatic expansion of clonal B-cells with less than $5x10^9$/L B-cells in the peripheral blood and without other manifestations of chronic lymphocytic leukemia (CLL). Yearly, 1% of individuals with MBL evolve to CLL requiring therapy; thus it is critical to develop a better understanding of the biologic events that determine which MBLs progress to intermediate/advanced CLL stages. High-count monoclonal B-cell lymphocytosis (MBL) is an asymptomatic expansion of clonal B-cells with less than $5x10^9$/L B-cells in the peripheral blood and without other manifestations of chronic lymphocytic leukemia (CLL). Yearly, 1% of individuals with MBL evolve to CLL requiring therapy; thus it is critical to develop a better understanding of the biologic events that determine which MBLs progress to intermediate/advanced CLL stages.
Aims: We performed a longitudinal sequencing analysis on a cohort of high-count MBL, assessing the mutation profile, clonal heterogeneity and clonal dynamics over time and their impact on disease progression.
Methods: We performed targeted deep sequencing (TDS) on 48 high-count MBL individuals and explored the mutation status of 21 driver genes recurrently mutated in CLL. We analyzed the clonal evolution in 45 of these 48 MBLs by screening 2-4 sequential samples (average time between samples 56 months, range 10-119 months). At the last follow-up, 19 cases (39%) had progressed to Rai>0, and 10 cases (20%) required treatment. We analyzed 152 DNA samples from 48 cases (104 tumor CD5+/CD19+ samples and the 48 germ line CD5-/CD19- controls) using semiconductor-sequencing technology (IonTorrent PGM). Genetic information was integrated with relevant clinical and biological parameters. Subsequently, we evaluated the role of mutations and clonal evolution with progression to intermediate/advanced stage CLL (Rai stages I-IV) and time to treatment (TTT).
Results: We found somatic non-synonymous mutations in 25 MBLs (52%) at the initial time-point analyzed, including 13 (27%) with >1 mutated gene. Recurrent mutations were found in *SF3B1* (10% of cases), *BIRC3, DDX3X, CHD2* (8% each), *NOTCH1* (6%), and *ATM, BRAF, EGR2, FBXW7, KRAS, MED12, MYD88,* and *ZMYM3* (4% each). Furthermore, *BCOR*, *ITPKB, POT1, RIPK1* and *SAMHD1* were each mutated in one case. No mutations were found in *HIST1H1E, TP53* and *XPO1*. In cases that subsequently progressed to intermediate/advanced stage CLL, mutations were detected 41 months (median time) prior to progression. Except for *NOTCH1, TP53 and XPO1,* which have shown a lower incidence in MBL, driver genes were mutated with a similar prevalence to CLL, indicating the early origin of most driver mutations in the MBL/CLL continuum. Subclonal expansion of mutated drivers was found in 6 out of 10 (60%) cases who progressed to require treatment, compared with only 5 of 35 (14%) non-treated cases. Individuals with clonal expansion had both significantly shorter PFS (HR: 5.3 95% CI: 1.3-20.9; p=0.02), as well as significantly shorter TTT (HR: 6.4 95% CI: 1.8-23.2; p=0.005).
Summary/Conclusions: The findings of this study suggest that mutations in driver genes are an early event in the MBL-CLL continuum and predict progression to clinically significant CLL. Clonal heterogeneity and subclonal expansion is already found in individuals with high count MBL and its presence predicts an earlier development of CLL. Sequential genetic evaluation of individuals with MBL provides insights that add to our understanding of the events that contribute to progression from MBL to CLL and have potentially profound clinical implications.

P195

IDENTIFICATION OF NOVEL B CELL RECEPTOR ANTIGENS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: B cell receptor (BCR) signaling is one of the main pathways activated in the Chronic Lymphocytic Leukemia (CLL) microenvironment. Nurselike cells (NLC), an important monocytic component of this microenvironment, spontaneously outgrow *in vitro* from high density CLL-PBMC cultures, and can expose proteins on their surface, which can be recognized by the CLL-BCRs and activate BCR signaling. CLL cases whose cells carry unmutated immunoglobulin heavy variable (*IGHV*) genes (U-CLL) generally experience an aggressive disease course, and U-CLL BCRs are highly polyreactive towards a variety of self-antigens, most of them still incompletely characterized.
Aims: The aim of this study was to characterize BCR signaling in the CLL-NLC co-culture system and to identify novel CLL-BCR antigens expressed by NLC.
Methods: CLL-PBMCs were cultured *in vitro* for 14 days until outgrowth of NLC, which was confirmed by phase contrast microscopy. CLL cells were then removed from the NLC co-culture and tested for BCR expression by flow cytometry and activation of BCR signaling by Western Blot. Chemokine secretion was tested in co-culture supernatants by ELISA. Concomitantly, NLC were harvested and lysed, followed by immunoprecipitation of protein lysates with recombinant monoclonal antibodies obtained by cloning of 4 U-CLL BCR sequences, all of them carrying 100% identity with the germline *IGHV* sequence. Protein lysates from the human mesenchymal stromal cell line hTERT were also tested, as control. Immunoprecipitated proteins were analyzed by label-free mass spectrometry followed by bioinformatic data analysis with the softwares Max Quant and Perseus.
Results: Down-modulation of IgM-BCR surface levels was observed after 14 days of co-culture of 10 CLL cases onto NLC. IgM levels recovered after 48 hours following *in vitro* culture in the absence of NLC, reaching pre-exposure levels after 72 hours. Activation of AKT and ERK kinases and induction of MYC protein were observed in 10 CLL cases after 14 days of CLL-NLC co-culture, and phosphorylation/expression levels of these proteins were reduced when CLL cells were removed from the NLC support. We also detected high levels of the BCR-related chemokines CCL3 and CCL4 in 18 CLL-NLC supernatants, in particular in the ones derived from U-CLL cases. Immunoprecipitation of 3 NLC lysates and the hTERT control with 4 recombinant U-CLL BCRs, followed by label-free mass spectrometry and bioinformatic data analysis, allowed the identification of 6 clusters of commonly recognized proteins (Figure 1). One cluster (Cluster 5) included 11 putative CLL-BCR antigens that were expressed by all 3 NLC samples, but not by hTERT, and included cytoskeletal proteins and membrane-associated proteins, some of them known autoantigens in other diseases.

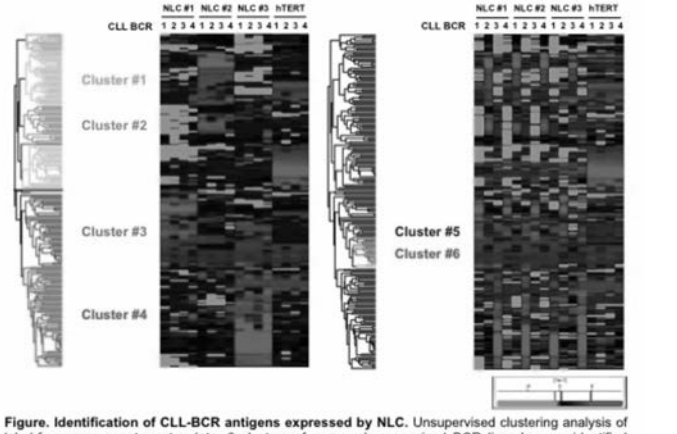

Figure. Identification of CLL-BCR antigens expressed by NLC. Unsupervised clustering analysis of label-free mass spectrometry data. 6 clusters of commonly recognized BCR ligands were identified through immunoprecipitation of NLC lysates derived from 3 CLL patients (NLC #1, NLC #2, NLC #3) or hTERT cells with 4 different U-CLL BCRs.

Figure 1.

Summary/Conclusions: The BCR signaling pathway is activated following co-culture of CLL cells with NLC, which can expose CLL-BCR ligands on their surface. By applying a novel mass-spectrometry based approach, we identified a number of putative CLL-BCR antigens expressed by NLC, which will be further validated for BCR binding and signaling activation properties, as well as for expression and localization in CLL-NLC co-cultures and lymph node sections from CLL patients. Overall, these results will allow a better characterization of the molecular mechanisms regulating BCR signaling in the CLL microenvironment and the identification of novel therapeutic targets for CLL therapy.

P196

CXCL12 ANTAGONISM BY THE L-RNA APTAMER OLAPTESED PEGOL IS EFFECTIVE AS A MOBILIZING AND THERAPEUTIC AGENT IN A MURINE CHRONIC LYMPHOCYTIC LEUKEMIA MODEL

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Background: The pathophysiology of chronic lymphocytic leukemia (CLL) depends on the interactions of the malignant B cells with the lymphoid tumor

microenvironment. The chemokine stromal cell-derived factor 1 (SDF-1, CXCL12) is constitutively secreted and presented by stroma under normal as well as malignant conditions. In CLL, binding of CXCL12 to its over-expressed classical receptor CXCR4 regulates cell migration and increases cell survival and cell adhesion-mediated therapy resistance, making this signaling axis a therapeutic target. Olaptesed pegol (NOX-A12), a PEGylated L-stereoisomer RNA aptamer, is a novel high-affinity CXCL12 antagonist. Using short term adoptive transfers of human leukemia cells into immune deficient mice, we recently demonstrated that olaptesed pegol antagonizes CLL cell homing to bone marrow.
Aims: Here, we aimed to determine the potential of olaptesed pegol to mobilize CLL cells from lymphoid organs and to antagonize disease progression *in vivo* using the murine Tcl transgenic (tg) CLL transplantation model.
Methods: Splenocytes from Tcl1 tg mice with overt leukemia were transplanted into the peritoneal cavity of C57BL/6J wildtype mice. Engrafted recipients with active leukemia (defined by a peripheral blood CD5+/CD19+ tumor cell concentration ≥10%) were randomized to treatment with olaptesed pegol or the CXCR4 antagonist AMD3100. CLL cell mobilization into peripheral blood was analyzed via flow cytometric detection of CD5+/CD19+ cells before substance application, and 1 h, 3 h, 6 h, and 24 h post application. Treatment studies of engrafted animals were performed using either olaptesed pegol or fludarabine, or ibrutinib followed by olaptesed pegol compared to ibrutinib alone. Readout parameters were tumor cell concentration and overall survival of mice.
Results: Olaptesed pegol induced a substantial mobilization of CLL cells into peripheral blood with an approx. 2-fold increased leukemic cell count compared to pre-application levels. Raised CLL cell counts in blood were observed within 1 hour, reached a peak upon 6 hours and were still visible at 24 hours post treatment while AMD3100 treatment did not result in consistent CLL cell mobilization. In long-term treatment experiments, olaptesed pegol prolonged overall survival of mice as efficient as fludarabine. Whereas ibrutinib followed by olaptesed pegol reduced blood tumor burden to a comparable extent as ibrutinib monotherapy, the combination had a stronger effect on median survival which was prolonged by 36% *vs* vehicle control and 18% *vs* ibrutinib alone.
Summary/Conclusions: Our data demonstrate the potential of olaptesed pegol as a CLL cell mobilizing and therapeutic agent. Further studies corroborating its efficiency as a single treatment using primary Tcl transgenic animals are ongoing.

P197

THE PAN-PIM KINASE INHIBITOR LGB321 TARGETS APOPTOTIC PATHWAYS AND MICROENVIRONMENTAL INTERACTIONS IN CLL
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Background: In recent years, the emergance of kinase inhibitors like Ibrutinib has drastically altered treatment strategies and improved outcomes in CLL patients, but lack of cure and resistance to therapy still remain serious problems. PIM kinases are involved in various important disease mechanisms in CLL, with PIM1 regulating CXCR4 surface expression impacting its interaction with the microenvironment, and PIM2/3 affecting the apoptotic machinery by regulating BAD. The Pan-PIM kinase inhibitor LGB321 (Novartis) targets all 3 PIM kinases and therefore affects both, CLL apoposis and its interaction with the microenvironment.
Aims: In the study presented here, we aimed to investigate the effect of the Pan-PIM inhibitor LGB321 on CLL *in vitro* and *in vivo*.
Methods: CLL patient samples: Peripheral blood samples were obtained with informed consent in accordance with the declaration of Helsinki from B-CLL patients who were either untreated or off therapy for at least 6 months. Immunoblotting: Protein expression of PIM1, PIM2, PIM3, CXCR4, pCXCR4, pBAD, BAD and beta-Actin was determined after Pan-PIM inhibitor treatment and was analyzed using the ImageJ software. Apoptosis Assay: PBMCs were plated with or without support of the murine stromal cell line M2-10B4 and treated with LGB321. After 24 and 48 h of incubation, cells were stained with a CD5-V450 and a CD19-APC antibody, followed by AnnexinV/7-AAD staining (BD) according to the manufacturer´s instructions. CLL cell homing into NOG mice: NOG mice were transplanted at 8 weeks of age. Primary human CLL cells were treated with LGB321 for 12 hours. CLL cells were resuspended in HBSS and mice received transplants via teil vein injection. Mice were analyzed 4 hours after transplantation. LGB treatment of CLL-Xenograft mice: PBMCs were transplanted using both retro-orbital and intraperitoneal application. Treatment started one week after transplantation and LGB321 was applied using oral gavage. After 2 weeks of treatment, mice were sacrificed, and spleen and bone marrow cells were harvested and stained for human CD5, CD19 and CD45 (BD).
Results: LGB321 was highly effective in inducing apoptosis in primary CLL cells, independent of risk factors like 17p deletions or the mutation status. Apoptosis induction correlated with reduced pBAD and BAD levels. Furthermore, LGB321 was also effective in the presence of protective stromal cells and could completely overcome the stroma protective effects. Mechanistically, we found that LGB321 treatment blocked the CXCR4/CXCL12 axis by dephosphorylating the CXCR4 receptor on Ser339, by reducing total CXCR4 protein levels and by blocking the externalization of the CXCR4 receptor. Concordantly, PIM inhibition blocked CXCR4 functions like migration towards a CXCL12 gradient (P<.0001), and reduced homing of LGB321-pretreated primary CLL cells towards the bone marrow (P=.0001) of NOG mice *in vivo*. *In vivo* experiments comfirmed the efficacy of LGB321 in 4 different CLL xenograft studies. Transplantation of primary human CLL cells into NOG mice and treatment with LGB321 for 2 weeks strongly reduced WBC counts, spleen size and spleen infiltration with human CLL cells (P=.0295) in all four CLL cases, and blocked BAD as well as CXCR4 phosphorylation also *in vivo.*
Summary/Conclusions: Our results demonstrate, that the Pan-PIM kinase inhibitor LGB321 might be an effective treatment option for CLL patients by impairing PIM2/3 mediated CLL-cell survival, and by blocking the PIM1/CXCR4-mediated interaction of CLL cells with their protective microenvironment *in vitro* and *in vivo*. Future clinical trials should be performed to validate its efficacy in human CLL.

P198

***IN VIVO* CHARACTERIZATION OF LYN KINASE IN THE PATHOGENESIS OF CHRONIC LYMPHOCYTIC LEUKEMIA**
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Background: The introduction of therapeutics targeting B cell receptor (BCR) signaling such as the inhibitors of BTK and PI3K-δ has been changing the field of CLL therapy formidably. Inhibition of LYN, a central molecule of the BCR signaling, efficiently induced apoptosis *in vitro* but its effectiveness *in vivo* is still unclear. In CLL cells, LYN is found to be aberrantly expressed and highly activated. Not only the classical BCR molecules downstream of LYN such as SYK or PI3K, but also several other substrates such as HS1, procaspase-8, cortactin or PP2A have been indicated to be deregulated in CLL. These downstream molecules could support the aggressiveness and apoptosis resistance of the malignant cells. Besides, the expression levels of LYN mRNA was associated with treatment-free survival of CLL patients, providing further perspectives for LYN as a valuable therapy target. However, studies about the role of LYN in CLL have only been conducted from isolated cells *in vitro*, and modulations of Lyn activity in CLL were only performed using inhibitors with insufficient specificity. Thus, the contribution of LYN in CLL pathogenesis is far from being fully understood. We have demonstrated that Lyn kinase is essential for the development of CLL *in vivo* (Nguyen *et al.*, in revision). Interestingly, loss of Lyn in leukemic B cells results in higher proliferation rate, early malignant transformation and apoptosis resistance, and can potently induce CLL in immune competent syngeneic recipients. In stark contrast, our results suggest a striking role of Lyn for the formation of a tumor microenvironment that supports CLL expansion.
Aims: So far studies about the role of LYN in CLL have only been conducted from isolated cells *in vitro*, and modulations of Lyn activity in CLL were only performed using inhibitors with insufficient specificity. Thus, the contribution of LYN in CLL pathogenesis is far from being fully understood. Using the well-established mouse model for CLL, the Eμ-TCL1 mouse model, we investigated the functional contribution of LYN in the pathogenesis of CLL in on the *in vivo* level.
Methods: To better understand the physiological consequences of overexpressing LYN observed in human CLL cells, we generated a novel mouse model with constitutively active LYN mutation. The conditional knockin mice have a point mutation at the inhibitory phosphorylation tyrosine of the Lyn gene (Y508F), resulting in its constitutively active conformation that expresses specifically in B cells due to Cre-induced recombination under the CD19 promoter ($LYN^{up\text{-}B}$). This novel mouse model was intercrossed with the Eμ-TCL1 model ($TCL1^{+}LYN^{up\text{-}B}$) and monitored for CLL development.
Results: B cells isolated from $TCL1^{+}LYN^{up\text{-}B}$ mice exhibited remarkably enhanced activation of BCR-associated kinases such as SRC family kinases, SYK, AKT and ERK1/2 compared to control $TCL1^{+}LYN^{wt}$ B cells, particularly upon IgM stimulation. Our analysis also revealed a higher malignant transformation rate of $TCL1^{+}LYN^{up\text{-}B}$ B cells starting at six months of age, and a more progressive CLL burden in the peripheral blood of $TCL1^{+}LYN^{up\text{-}B}$ mice, particularly when the CLL clones had been well established at eight months of age. However, moribund mice from both cohorts showed similar infiltration of CLL cells in the lymphoid organs, and the slight increased CLL burden in peripheral blood did not affect overall survival of $TCL1^{+}LYN^{up\text{-}B}$ mice compared to $TCL1^{+}LYN^{wt}$ counterparts.
Summary/Conclusions: Our results suggest that constitutively active LYN and the hyperactive BCR signaling in leukemic cells contribute moderately to the progression of CLL in the Eμ-TCL1 mouse model.

P199

MICROENVIRONMENTAL STROMAL CELLS TRIGGER HYPOXIASIGNALLING IN CLL CELLS – A MECHANISM THAT PROVIDES PROTECTION FROM APOPTOSIS AND CAN BE TARGETED THERAPEUTICALLY
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Background: Microenvironmental support protects malignant cells from apoptosis and cancer therapy, in part by induction of hypoxia-regulated pathways. Hypoxia-regulated pathways are conceivable as therapeutic targets in solid cancers, but not so much in leukemias.
Aims: Here we show that in chronic lymphocytic leukemia (CLL) cells hypoxia-regulated pathways are the most important mediators of support by bone marrow derived stromal cells (BMSC). These pathways can be targeted therapeutically.
Methods: We compared the transcriptomes of primary CLL cells protected by coculture with BMSCs to the transcriptomes of non-protected CLL cells that undergo apoptosis. To identify compounds that are active against the microenvironmental support, we determined the differential transcriptomic signature of CLL cells between *in vitro* co-culture and unsupported culture and compared it to a database of transcriptomes induced by bioactive compounds in cell lines ("connectivity map"). We tested emetine for efficacy in a syngeneic transplantation model of murine CLL, using three different treatment schedules - a short high dose intensity and two continuous lower dose intensity scheme.

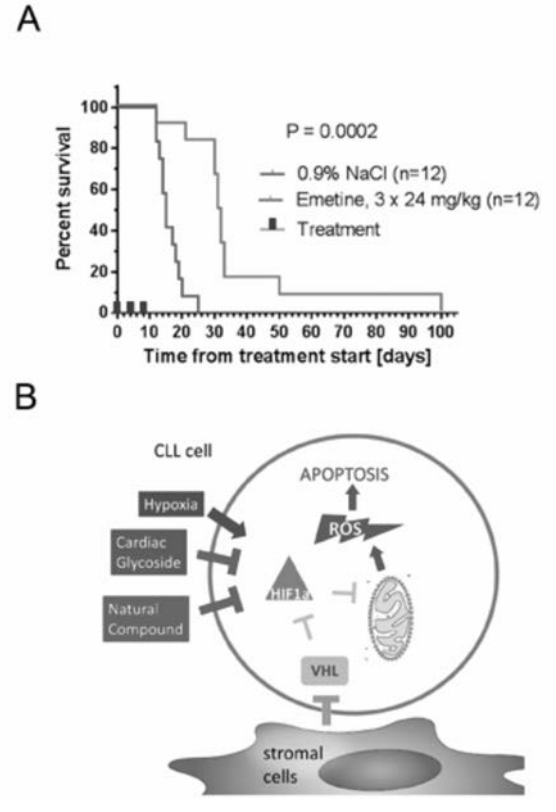

Figure 1.

Results: The cellular pathways most differentially regulated in primary CLL cells protected by coculture compared to unprotected CLL cells were involved in oxidative phosphorylation and mitochondrial function. Transcriptional differences were already evident at 6-8 h, directly before induction of apoptosis in unprotected cells. In line with this finding, the support of primary CLL cells by the BMSCs could be mimicked *in vitro* by hypoxia. By the comparison of the dysregulated CLL transcriptomic signature to the connectivity map database, we identified the cardiac glycoside ouabain and the ipecac alkaloid emetine as inducing the most correlated transcriptomic signatures. *In vitro*, these substances were highly active on primary CLL cells protected by coculture (IC50s 287 nM, 190 nM and 35 nM, respectively; the IC50 of fludarabine was 5 μM). *In vitro* characterization of the substances revealed suppression of HIF-1 alpha activity. This suppression coincided with a stronger oxidation of the mitochondrial and cytoplasmic redox homeostatic system compared to 20 redox-active compounds. We tested the efficacy of emetine *in vivo* using three different treatment schedules - a short one of high dose intensity (24 mg/kg every 4th day for 3 applications) and two continuous ones of lower dose intensity (3 or 8 mg/kg every 4th day). We transplanted murine CLL tumors induced by Eμ-driven overexpression of TCL1A into microenvironmentally intact wildtype syngeneic mice. Continuous low dose treatment did not lead to weight reduction in mice, whereas treatment with three high doses of emetine resulted in a significant loss of weight. However, while continuously administered low doses of emetine were not beneficial for survival of transplanted mice (Figure 1a), three high doses of emetine doubled median survival of diseased mice (31.5 days *vs* 15 days, p=0.0002, Figure 1b). This enhanced survival coincided with decreased numbers of CLL cells in peripheral blood, spleen and most pronounced in bone marrow (BM), which led to recovery of hematological parameters.

Summary/Conclusions: CLL cells are supported by bone-marrow derived stromal cells first of all through modulation of hypoxia-regulated pathways. This microenvironmental support can be therapeutically targeted by cardiac glycosides and ipecac alkaloids, both of which increase the intracellular oxidation of CLL cells even when they are protected by coculture. In a mouse model of CLL, this is reflected by doubled median survival of mice treated with emetine.

P200

IBRUTINIB INHIBITS CD20 UP-REGULATION ON CLL B CELLS MEDIATED BY THE CXCR4/SDF-1 AXIS

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Background: It was shown that BCR inhibitors such as ibrutinib interrupt microenvironmental interactions and mobilize CLL cells from lymph node niches and bone marrow into the blood stream. Therefore, it has been suggested that a combinatorial therapy of BCR-inhibitors with anti-CD20 or other antibodies might be an effective therapeutic combination.
Aims: The aim of this study was to test for the ibrutinib effect on the expression of selected CLL cell-surface molecules that could be potentially targeted by available monoclonal antibodies.
Methods: Peripheral blood samples were obtained from untreated CLL patients or patients treated with ibrutinib as a single agent (420 mg once daily). The study was approved by the institutional review board and samples were obtained with written informed consent.
Results: We performed gene expression profiling in samples obtained from CLL patients treated with ibrutinib as a single agent (pre-ibrutinib *vs* day 15 and/or week 5/12) and analyzed changes in >20 cell-surface molecules that could be potentially targeted by different available therapeutic antibodies. Surprisingly, we observed that CD20 mRNA had the most significantly changed expression (down-modulation of 3.4-fold; P<0.0001). This suggested that CD20 expression might be regulated by a yet unknown mechanism in the context of microenvironmental interactions impaired by ibrutinib. The co-culture of primary CLL cells with stromal cell line HS-5 induced higher CD20 surface levels on CLL cells, and ibrutinib inhibited this CD20 up-regulation. Then we assessed the CD20 expression on CLL cell populations defined according to CXCR4 and CD5 levels. CLL cells that have recently exited the lymph node microenvironment to the peripheral blood express lower levels of chemokine receptor CXCR4 and higher levels of activation marker CD5 ($CXCR4^{dim}CD5^{bright}$ cells) than those cells circulating in the blood stream for a long time ($CXCR4^{bright}CD5^{dim}$ cells). The $CXCR4^{dim}CD5^{bright}$ cells had 2-times higher CD20 surface as well as mRNA expression (P<0.01) suggesting that changes of CD20 levels within immune niches reflect the changes in its transcription. Moreover, CD20 expression gradually decreased with CLL cells' transition from $CXCR4^{dim}CD5^{bright}$ to $CXCR4^{bright}CD5^{dim}$ (P<0.01). This led us to hypothesize that CXCR4/SDF-1 is directly implicated in CD20 regulation. Indeed, *in vitro* treatment of CLL cells with SDF-1α (ligand for CXCR4 produced by stromal cells) up-regulated CD20 expression (P<0.01). The application of plerixafor (CXCR4 inhibitor) or ibrutinib abolished the SDF-1 mediated CD20 up-regulation (P<0.01). Altogether, this represents the first known mechanism of CD20 regulation in CLL cells. Notably, it has been shown that addition of rituximab to ibrutinib *in vivo* is able to eliminate CLL cells despite lower levels of CD20 suggesting that there must be other mechanism of ibrutinib action that allows for the effect of rituximab. The screening of anti-apoptotic molecules revealed that Mcl1 levels were significantly down-modulated after ibrutinib treatment *in vivo* (P=0.002), and ibrutinib also inhibited Mcl1 induction in CLL cells co-cultured with HS-5. As Mcl1 was described to directly protect CLL cells from rituximab-induced apoptosis and CDC, we suggest that ibrutinib-mediated Mcl1 reduction is a mechanism that facilitates rituximab efficacy.
Summary/Conclusions: We have described the first known mechanism of CD20 regulation in CLL cells. The CXCR4/SDF-1 axis up-regulates CD20 expression in CLL B cells, this is inhibited by ibrutinib. Supported by the Ministry of Education, Youth and Sports of the Czech Rep, project CEITEC 2020 (LQ1601); Ministry of Health of the Czech Rep, grant nr. 16-29622A; GACR (GA16-13334Y); TACR (TEO2000058/2014-2019); MUNI/A/1028/2015; MSMT COST CZ (LD15144); Horizon 2020 (No. 692298); and G.P. is a city of Ostrava scholarship holder.

P201

FUNCTIONAL IMPACT OF BONE MARROW MESENCHYMAL STROMAL CELL-DERIVED EXTRACELLULAR VESICLES ON CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS: APOPTOSIS, MIGRATION, CHEMORESISTANCE AND GENE EXPRESSION MODIFICATION

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Background: Chronic Lymphocytic Leukemia (CLL) is the most common leukemia in Western countries and is characterized by the accumulation of CD5/CD19+ monoclonal B-cells in peripheral blood and secondary lymphoid tissues. The interactions between CLL cells and the bone marrow (BM) microenvironment (notably composed by mesenchymal stromal cells - MSC) play an important role in promoting the increased survival of leukemic B-cells. Extracellular vesicles (EVs) produced by leukemic cells and the microenvironment may be implicated in this cross-talk. EVs, including microparticles and exosomes, are small plasma membrane fragments with sizes ranging from 0.01 to 1µm, and contain products specific to the original cell, such as microRNA, mRNA and proteins.
Aims: Our objective is to assess the role of EVs in the cross-talk between malignant cells and their microenvironment by functional studies and microarray analyzes.
Methods: Ultracentrifugation (150000g) was applied to isolate EVs from supernatant of BM-MSC. Different concentrations of EVs were added to CLL B-cells to evaluate their impact on cell survival, migration and chemoresistance. The gene expression change induced by EVs in CLL cells was also defined by microarray analysis (Affymetrix) after their incubation (24h) with BM-MSC-EVs. Some genes identified as differentially expressed were validated by real-time PCR.
Results: We demonstrated that BM-MSC EVs are able to enter in CLL-B-cells (PKH67 labeling): after 24h, >95% of CLL cells had integrated EVs. Apoptosis was assessed by flow cytometry: addition of increasing concentrations of EVs showed a protective effect on CLL-B-cells from spontaneous cell death (n=21/p-value<0.0001). CLL-B-cells pre-incubated with EVs (4h) displayed an increased spontaneous migration index and also in response to SDF1α (n=10/p-value=0.002). We also observed that EVs rescue CLL-B-cells from drug induced apoptosis. Microarray analysis reveals a significant effect on the expression of 987 genes after the integration of EVs in CLL-B-cells, notably implicated in apoptosis, epigenetic, adhesion, migration, cell activation, actin cytoskeleton regulation and immune pathways.
Summary/Conclusions: Here we show for the first time that BM-MSC-EVs protect CLL cells from spontaneous apoptosis and influence their migration and chemoresistance capacities. The implication of EVs in several cell functions was observed by microarray analyzes. This study demonstrates the existence of a powerful cross talk between BM-MSC and CLL-B-cells mediated by EVs. It provides new insight in the biology of CLL.

P202

THE T-CELL/CLL/MACROPHAGE TRIAD SHAPES A SUPPORTIVE TUMOR MICROENVIRONMENT IN CLL

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Background: Survival of chronic lymphocytic leukemia (CLL) cells critically depends on signals from bystanders cells, such as T-cells and macrophages, within micro-environmental niches such as lymph nodes. The mechanisms of this intricate interplay are however unknown. Moreover, whether CLL cells actively participate in shaping their pro-survival niche is poorly understood.
Aims: 1) whether T-cell stimulation initiates active recruitment of monocytes by CLL cells, 2) whether CLL cells are able to differentiate these monocytes towards a supporting phenotype and 3) by which mechanism macrophages induce CLL survival.
Methods: migration assays with inhibitors, microarray, Luminex, flow cytometry, western blot, immunohistochemistry, survival assays, RNAi, polysome gradient fractionation
Results: 1) In *in vitro* migration assays we found that CLL cells can actively recruit monocytes after T-cell or CD40L stimulation. T-cell stimulation of CLL cells resulted in secretion of several chemokines, of which CCL2 was responsible for the enhanced monocyte migration. 2) We next examined and compared polarization patterns of monocytes after differentiation with serum derived from CLL patients (N=25) or pooled healthy donor serum and found that CLL serum was able to differentiate macrophages towards a tumor supporting M2 phenotype. This finding was confirmed *ex vivo* by IHC, as M2 marker CD206 co-localizes with CD68 cells in CLL LNs, while the majority of macrophages in non-CLL derived LNs are CD80+ (M1 type). 3) Lastly, we found that macrophages induce CLL cell survival and that this survival resulted from an AKT-dependent upregulation of pro-survival protein MCL-1, which was translationally stabilized. Interestingly, the mechanism of MCL-1 stabilization was independent of NF-κB signaling.
Summary/Conclusions: These studies shed light on reciprocal cellular interactions in the CLL LN that shape pro-tumor differentiation of supporting cells, that in turn cause survival by changing the apoptotic balance. These interactions can be targeted at different levels, creating new treatment venues for this still incurable disease.

P203

METABOLIC ALTERATIONS INDUCED BY IBRUTINIB IN CLL CELLS AS A BIOCHEMICAL BASIS FOR MECHANISM-BASED DRUG COMBINATION TO ENHANCE THERAPEUTIC ACTIVITY

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Background: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western countries and is currently still incurable. Ibrutinib, a small molecule compound designed to target Bruton's tyrosine kinase (BTK) essential for CLL cell survival, exhibits impressive therapeutic activity in CLL, and significantly improves the clinical outcome of CLL patients. However, drug resistance and disease relapse may occur in a small percent of patients.
Aims: The main objectives of this study were to investigate the potential effect of Ibrutinib on CLL metabolism, and to develop novel strategies to enhance the therapeutic activity of Ibrutinib and reduce drug resistance.
Methods: Primary CLL cells were isolated from the peripheral blood samples of CLL patients for *in vitro* study. Metabolic alterations including changes in oxygen consummption (OCR) as an indicator of mitochondrial respiration and extracellular acidification rate (ECAR) as an indication of lactate production from glycolysis were measured by a Seahorse metabolic analyzer XF^{e}24. Cellular reactive oxygen species (ROS) were detected using a redoxsensitive fluorescence probe DCFH-DA.
Results: Since active generation of reactive oxygen species (ROS) is a metabolic feature of CLL cells, we first used DCFH-DA to test the potential effect of Ibrutinib on ROS production in primary CLL cells isolated from patients. We found that Ibrutinib induced a substantial increase of ROS as early as 90 min and lasted for up to 24 h. Mechanistic study suggests that the mitochondrial respiratory chain might be the major source of this abnormal ROS generation, since inhibition of the mitochondrial respiratory complex I by rotenone or suppression of flavoproteins (components of mitochondrial respiratory chain or NAD(P)H oxidase) by diphenyleneiodonium largely abrogated Ibrutinib-induced ROS generation. To further investigate the potential effect of Ibrutinib on mitochondria, we used the extracellular flux analyzer (XF^{e}24). In a CLL cells-stromal cells co-culture system (to maintain long-term viability of CLL cells), Ibrutinib induced little changes in OCR and ECAR during the first 48-72 h. However, prolonged drug exposure (up to 7 days) caused a consistent and significant decrease in OCR compared to the untreated CLL cells co-cultured with stromal cells for the same period. Importantly, CLL cells isolated from patients under Ibrutinib treatment for 7 days also exhibited a significant decrease in OCR compared to the pre-treatment samples from the same patients, suggesting that the impact of Ibrutinib on mitochondria occurred *in vivo*. Western blot analysis revealed that Ibrutinib induced a decreased expression of certain respiratory chain components. Functional analysis showed that Ibrutinib caused CLL cells to take up less glutamine but did not affect glucose uptake, suggesting that Ibrutinib might preferentially impact glutamine metabolism. Based on these new findings, we further tested the possibility that the metabolic alterations and mitochondrial dysfunction induced by Ibrutinib might render CLL cells vulnerable to compounds that impact mitochondria or inhibit glutamine metabolism. Indeed, our further study showed that combination of Ibrutinib with metformin (a drug that inhibits mitochondrial OXPHOS) or with C968 (a glutaminase inhibitor) had synergistic activity against CLL cells and induced massive leukemia cell death.
Summary/Conclusions: Overall, our study suggests that ibrutinib could induce metabolic alterations characterized by decreased mitochondrial respiration and attenuated glutamine metabolism. Such metabolic alterations provide a biochemical basis for mechanism-based drug combination to enhance the therapeutic activity of Ibrutinib. Since metformin is a diabetes drug with low toxicity, it is feasible to test its combination with Ibrutinib for treatment of CLL to potentially enhance therapeutic activity.

P204

BLOCKING ENDOTHELIN-1 SIGNALING DISRUPTS LEUKEMIC CELL HOMING AND B-CELL RECEPTOR PATHWAY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Endothelin 1 (ET-1) is a 21-aa peptide that mediates its action by activating two G-protein-coupled receptor (GPCR) subtypes, ET_A and ET_B receptors. CLL cells secrete ET-1 and express ET_A and ET_B receptors on the cellular surface. ET-1 signaling improves CLL survival and proliferation and

reduces CLL sensitivity to phosphoinositide-3-kinase δ inhibitor idelalisib and fludarabine.
Aims: We asked whether ET-1 pathway may be involved in CLL homing and B-cell receptor (BCR) signaling pathway. Macitentan, a novel double inhibitor of ET_A and ET_B receptors, was tested in CLL in combination with the Btk inhibitor ibrutinib.
Methods: CD19+ CLL cells isolated from patients were treated *in vitro* with 100nM ET-1 with or without the addition of BQ-123, specific ET_AR inhibitor, BQ-788, specific ET_BR inhibitor or macitentan, a double ET_AR/ET_BR inhibitor. In some instances, CLL cells were pre-incubated with ibrutinib 0.5μM. We evaluated CLL migration, adhesion to stromal cells, calcium efflux and BCR signaling pathway. Moreover, the expression of ET-1, ET_AR and ET_BR in CD49d+ CLL *vs* CD49d- CLL as well as in $CXCR4^{high}$ CLL *vs* $CXCR4^{low}$ CLL was measured in peripheral blood of CLL patients by flow cytometry. We also evaluated big ET-1 level by ELISA in plasma samples from MBL, CLL patients at diagnosis and at first progression, in fludarabine resistant CLL and in CLL after ibrutinib treatment.
Results: First, we found that circulating $CXCR4^{high}$ CLL cells express higher levels of ET-1 and ET1 receptors than $CXCR4^{low}$ fraction ($p<0.05$). *In vitro*, ET-1 acts as a chemotactic factor for CLL cells. The blockage of ET receptors impairs CLL migration to ET-1 and also to other stimuli as CXCL12. In addition, ET-1 signaling is involved in CLL adhesion, as inferred on the basis of the following observations: (i) the blockage of ET receptors leads to CLL detachment from stromal cell supports; (ii) ET-1 stimulation promotes the phosphorylation of FAK, GSK3β, AKT and increases ILK expression in CLL cells and (iii) higher levels of ET-1 and ET receptors characterize CD49d+ CLL subset. Furthermore, we found that ET receptor triggering promotes calcium release in CLL. Blockage of both ET receptors by macitentan disrupts BCR signaling pathway and BCR-mediated calcium release in CLL cells in combination with ibrutinib. In plasma samples, big ET-1, the precursor of ET-1, was detectable in variable levels ranging from 0.6 to 67 pg/mL. Mean big ET-1 levels were equal to 2 pg/mL in MBL patients, 3.5 pg/mL in CLL at diagnosis, 7.9 pg/mL at first progression, 8.2 pg/mL at relapse, and 28.8 pg/ml in fludarabine-resistant CLL patients. In 4 CLL cases with plasma samples available before and after ibrutinib treatment, we found a significant decrease of big ET-1 in plasma from 10.8 to 3.9 pg/mL ($p<0.05$).
Summary/Conclusions: Collectively, our data describe for the first time an involvement of ET-1 signaling in CLL homing and BCR pathway. Moreover, ET-1 seems to accumulate in CLL patients with worse clinical features. Our results also envision the possibility to interfere with ET receptors activity using macitentan as a possible novel therapeutic strategy for CLL patients.

P205

GENE EXPRESSION PROFILES OF PERIPHERAL BLOOD T AND NK-CELLS REVEALED DIFFERENT PATTERNS OF IMMUNE DYSFUNCTION IN CLL-LIKE MBL AND EARLY STAGE CLL

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Background: Immune evasion is important for the expansion of the tumoral clone in chronic lymphocytic leukemia (CLL). Impaired NK-cell activity and dysfunctional CD4+ and CD8+ T-cells with abnormal cytoskeletal dynamics and immune synapses have been reported in CLL. T and NK-cell dysregulation may be relevant in CLL-like monoclonal B-cell lymphocytosis (MBL) and initial stages of CLL, although it has not been studied in depth.
Aims: To compare the gene expression patterns of CD4+ and CD8+ T-cells and NK-cells from the peripheral blood of healthy controls, MBL subjects and CLL-A(0) patients. To assess the T and NK-cell dysfunction in these entities.
Methods: Samples from thirty-eight individuals (17M/21F, median age=72yrs) were collected: 9 healthy controls, 15 MBLs and 14 CLL-A(0) with an absolute lymphocyte count $<12x10^9$/L. T-cells (CD4+ or CD8+) and NK-cells (CD56+ CD3-) from all subjects were isolated by immunomagnetic methods (Miltenyi Biotec). Good quality extracted RNA (RIN>7) was hybridized to 113 Human Gene 2.0 ST arrays (Affymetrix). Differential gene expression between groups was evaluated employing linear models for microarrays in R. Genes with a P-value<0.05 and |logFC|>0.58 were considered as potentially relevant. Functional analysis was performed using Ingenuity Pathway Analysis (IPA). For the CD4+ T-cell fraction, expression results were validated by qPCR in the same and in an independent cohort composed by 10 MBL and 5 CLL-A(0) individuals.
Results: Unsupervised hierarchical clustering analysis clearly discriminated healthy controls from both MBL and CLL for CD4+ and NK-cells, where the major differences in the gene expression were found. Regarding CD4+, gene upregulation was notably predominant in MBL and CLL-A(0), mainly in terms of cytotoxicity (*GZMB, PRF1, GNLY, SERPING1, CLU, NKG7* and *FCRL6*) and signaling, cytoskeleton and membrane dynamics leading to an increased inflammatory response (*RAB31, VNN1, TNFSF4, ITGAM, TLR2*). Strikingly, MBL showed more upregulated networks and expressed genes with higher |logFC| values than CLL-A(0). Furthermore, some of the activated genes (*CCL5, MAP3K8, IL18RAP, NKG7, GZMH* and *PRF1*) have been previously described in Th1-cells, which points to a bias towards this T-cell subset in the Th1-Th2 axis. Additional qPCR analysis validated the expression results in this cell fraction. In contrast to previously described dysfunction of CD8+ T-cells in CLL, functional analysis did not reveal differences involving cellular migration, adhesion or cytotoxicity neither in MBL nor CLL-A(0). Regarding NK-cells, functional analyses showed a decrease of the NK function in both MBL and CLL-A(0), the latter showing higher number of differentially expressed genes and |logFC| values. The most relevant downregulated pathways were related to chemotaxis, adhesion and cell-to-cell binding (downregulation of *AMICA1, CCR4, CD4, FPR2, FPR3, KIT* and *XCL1*), as well as activation, cytotoxicity and cytoskeleton organization (down-regulation of *CD28, CD86, FCAR, FPR2, FPR3, ICOS* and up-regulation of *FCGR2B*).
Summary/Conclusions: 1. CD4+ T-cells show an unusual activation of cytotoxicity and inflammation that are higher in MBL than in CLL-A(0) patients. A Th1 expression pattern is also observed in these entities. 2. CD8+ T-cells dysfunction do not occur in our cohort of MBL and initial CLL. 3. NK-cell dysregulation in cytotoxicity and migration is already detectable in MBL, and increases progressively with the evolution of the disease. 4. These are preliminary results, validation and functional studies are ongoing.
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P206

CD49D EXPRESSION IMPACTS ON THE IBRUTINIB-INDUCED LYMPHOCYTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA: A ROLE OF BCR-MEDIATED INSIDE-OUT VLA-4 INTEGRIN ACTIVATION

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Background: Treatment with the B-cell receptor (BCR) signaling inhibitor ibrutinib (IB) has demonstrated high response rates and improved survival in chronic lymphocytic leukemia (CLL). IB treatment is associated with a highly variable degree of transient lymphocytosis, due to a reduction of CLL cell adhesion and efflux of CLL cells from tissue sites. CD49d, a strong negative prognosticator in CLL, is the alpha-chain of the integrin heterodimer CD49d/CD29 (VLA-4), a key molecule in CLL microenvironmental interactions. Inside-out activation of VLA-4 by BCR signals increases its adhesive properties, although the role of the BCR as a VLA-4 activator in CLL is unknown.
Aims: To correlate the kinetics of transient lymphocytosis with the expression and levels of activation of VLA-4.
Methods: Two cohorts of IB treated patients (IB-CLL) with data of absolute lymphocyte count (ALC) at pre-treatment and at different time points of IB treatment were available: 1) 28 refractory/relapsed CLL patients (15 CD49d+) treated with IB on a compassionate basis (IT cohort); 2) 26 CLL patients (14 CD49d+) enrolled in the investigator-initiated phase II study of ibrutinib NCT01500733 (US cohort).VLA-4 activation was assessed by flow cytometry using conformation sensitive anti-CD29 mAb HUTS-21 in conjunction with LDV-containing VLA-4 specific ligand. The levels of activated VLA-4 was measured as VLA-4 receptor occupancy (RO) in values ranging from 0.0 (no RO) to 1.0 (100% RO) as in Chigaev *et al.* (J Biol Chem, 2009): higher RO indicates a larger fraction of activated (*i.e.* with a higher affinity) VLA-4. BCR engagement was performed using goat F(ab')2 anti–human IgM. CD49d, CD29, IgM expression, and Ca^{++} release were cytometrically analysed. Adhesion assays were performed on VCAM-1-coated slides.
Results: Correlation between CD49d expression and IB-induced lymphocytosis was performed in both cohorts. Median ALC at baseline were $23x10^6$/mL and $29x10^6$/mL (IT cohort) and $67x10^6$/mL and $95x10^6$/mL (US cohort) for CD49d- and CD49d+ CLL, respectively. Despite these different baseline ALC, and although ALC data were available at slightly different timepoints, CD49d- CLL showed a more pronounced increase in ALC than CD49d+ cases in both cohorts (Figure 1). To explain the observed differences in ALC kinetics in CD49d+ and CD49d- CLL, VLA-4 inside-out activation upon BCR stimulation

was evaluated in CD49d+ CLL cells that were exposed in-vivo to IB. Cells collected from IB-CLL on t30 (n=7), showed slightly decreased (79-86%) CD49d, CD29, and IgM MFI levels compared to pre-treatment cells. Despite an overall impairment of BCR-dependent Ca^{++} signaling (mean Ca^{++} release: 12.4% at pre-treatment, 3.6% at t30), IgM stimulation increased both VLA-4 RO (mean 0.53-range 0.40-0.73-, compared to 0.36-range 0.22-0.52-, at pre-treatment, p=0.004; 0.53-range 0.41-0.84-, compared to 0.27-range 0.01-0.45-, at t30, p=0.010) and CLL cell adhesion (mean values of adherent cells/control= 4.6 *vs* 3.7 at pre-treatment and 4.5 *vs* 1.6 at day 30). Similarly, cells from IB-CLL on day 60-90 of treatment (n=3) kept showing an increased VLA-4 RO upon IgM stimulation (from 0.14, range 0.10-0.17 to 0.34, range 0.20-0.56), although Ca^{++} release was relevantly decreased.

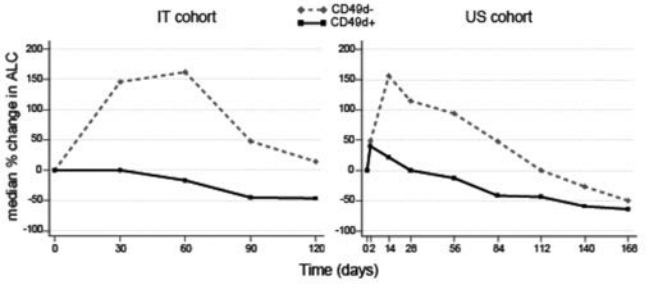

Figure 1.

Summary/Conclusions: BCR triggering in CLL cells activates VLA-4 via an inside-out pathway at least in part independent from IB binding to BTK. CD49d+ cells retain VLA-4 activation after IB treatment, with implications for CLL cell adhesion, and treatment-induced lymphocytosis. These observations should be considered in the design of IB therapies.

CLL - Efficacy and safety of new treatments 1

P207

LONG-TERM FOLLOW-UP OF THE NEXT GENERATION PI3K-DELTA INHIBITOR TGR-1202 DEMONSTRATES SAFETY AND HIGH RESPONSE RATES IN CLL: INTEGRATED-ANALYSIS OF TGR-1202 MONOTHERAPY AND COMBINED WITH UBLITUXIMAB

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Background: TGR-1202 is a novel, once-daily PI3Kδ inhibitor with a differentiated safety profile from other PI3Kδ inhibitors, and proven activity in patients (pts) with advanced hematologic malignancies. TGR-1202 is in clinical development as monotherapy and in combination with the glycoengineered CD20 mAb, ublituximab (UTX).

Aims: An integrated-analysis was conducted of pts dosed with TGR-1202 monotherapy or combined with ublituximab with a focus on outcomes in patients with CLL/SLL.

Methods: In both studies, there were no limits on prior therapies (Tx) and TGR-1202 was administered once-daily and escalated in 3 + 3 design with expansion cohorts explored. In the combo study, UTX was administered at a fixed dose of 900 mg per infusion. The primary endpoint was safety and efficacy was a secondary endpoint.

Results: Across both studies, a total of 152 pts (81 monotherapy/71 combined with UTX), including 40 CLL/SLL and 112 NHL patients were exposed to at least one dose of TGR-1202. Median age 65 yrs (22-86); 100 M/52 F; median # prior Tx=3 (1-14); 53% refractory to immediate prior Tx. Most frequent reported AE's (all grades; Gr 3/4): nausea (44%; 1%), diarrhea (42%; 2%), fatigue (36%; 3%), vomiting (23%; 0%) and neutropenia (19%; 16%). AST/ALT increase was 6% (3% Gr 3/4), pneumonia 6% (5% Gr 3/4) and pneumonitis 1% (<1% Gr 3/4). 64 pts received TGR-1202 for ≥6 mos, 33 for ≥1 year, with longest on >30 mos. Discontinuations due to AE's occurred in 8% of pts. Across both studies, 27 CLL/SLL pts received the therapeutic targeted dose of ≥800 mg (17 single agent, 10 combo with UTX) and were evaluable for efficacy. 48% of CLL patients exhibited high-risk cytogenetics (17p and/or 11q del). The combined ORR was 89% (4% CR) of which 22% had persistent lymphocytosis (all PR-L pts were on monotherapy). The median PFS in CLL was 24 mo (95% CI: 7.4, NR) as a single agent, and has not been reached for the TGR+UTX combo.

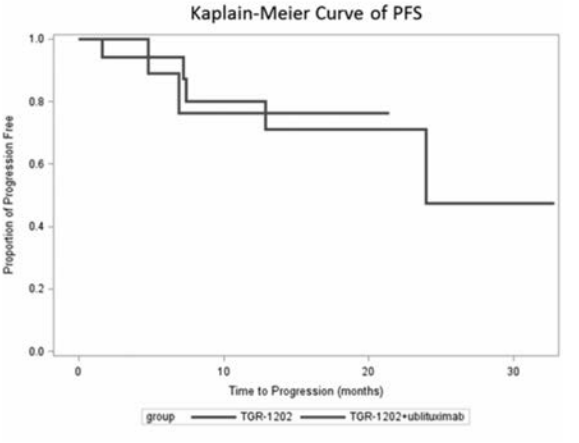

Figure 1.

Summary/Conclusions: TGR-1202 has exhibited a markedly differentiated safety profile from other PI3Kδ inhibitors to date with few discontinuations due to AE's and limited G 3/4 events. In particular we highlight the minimal rates of transaminitis / pneumonitis / colitis events across a large CLL/NHL population. Robust activity was observed in CLL, leading to a global Phase 3 trial evaluating

TGR-1202 in combination with UTX in treatment naïve and relapsed CLL. Based on the safety profile observed to date, combination Phase 1 & 2 trials exploring TGR-1202+ibrutinib, and the triple combinations of TGR-1202+ UTX+ibrutinib, and TGR-1202+UTX+pembrolizumab are ongoing. TGR-1202 is also being evaluated in a Phase 2 study in CLL patients intolerant to a BTK or PI3K delta inhibitor.

P208

REAL-WORLD EXPERIENCE OF IBRUTINIB IN >2900 CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS: DATA FROM A GLOBAL NAMED PATIENT PROGRAM

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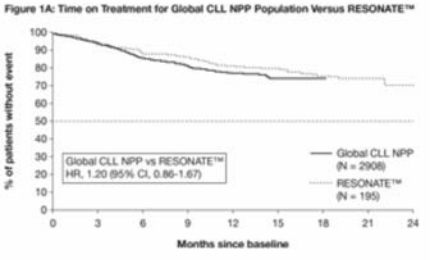
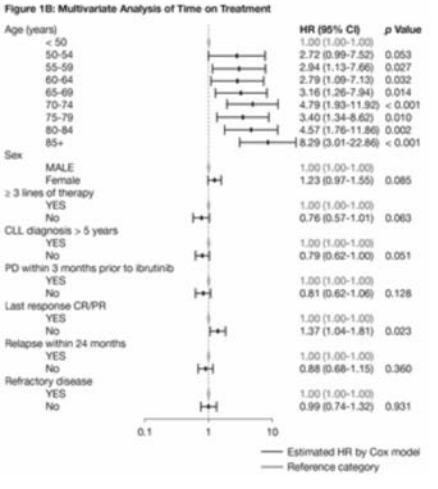
Background: A global named patient program (NPP) was opened in numerous countries worldwide in order to allow access to ibrutinib for eligible patients with relapsed/refractory chronic lymphocytic leukemia (CLL) prior to approval in those countries. This program provides real-world data on estimated outcomes with ibrutinib across a large, global CLL population.
Aims: To use real-world data from the ibrutinib CLL NPP to investigate whether treatment benefits reported in randomized clinical trials are reflected in outcomes observed in clinical practice.
Methods: In an analysis of ibrutinib ordering/reordering, we estimated patient time on treatment in order to provide a conservative approximation of progression-free survival (PFS) using Kaplan-Meier analysis and Cox proportional hazards regression. Reordering data were censored at the date of last ibrutinib supply or resupply (ibrutinib was resupplied every 1-3 months depending on stage of the NPP). Patients transferring to commercial ibrutinib after approval were censored at the time of NPP closure in their country.





Figures.

Results: Overall, 2908 patients with CLL from 30 countries participating in the global NPP were included in this analysis; median age was 69 years, and 66.3% were male. After 12 months, 77.3% (95% CI, 74.7-79.6%) of the global population remained on treatment. This estimate is similar to the 12-month time on treatment (81.5% [95% CI, 75.3-86.3%]) and PFS (83.8% [95% CI, 77.8-88.3%]) rates observed in the phase 3 RESONATE™ study of ibrutinib for relapsed/refractory CLL (inclusion criteria were similar for the CLL NPP and the RESONATE™ trial). Moreover, Kaplan-Meier curves for time on treatment for the global CLL NPP population and the RESONATE™ study were not statistically different (Figure 1A; CLL NPP *versus* RESONATE™: HR, 1.20 [95% CI, 0.86-1.67]). Limited baseline demographic information collected at NPP enrollment allowed an exploration of time on treatment via multivariate analysis (Figure 1B). Younger patients or those achieving complete/partial response to prior therapy had significantly longer time on treatment. Strong trends indicating longer time on treatment were observed for patients who had received fewer prior lines of therapy, as well as for male patients, those who were diagnosed with CLL within the last 5 years, and those with a progression-free interval of ≥3 months prior to ibrutinib treatment. Neither refractory disease (defined as no response to prior therapy [ie, stable disease or progression]) nor duration of response to prior therapy additionally impacted time on treatment. In total, 332 patients (11.4%) discontinued treatment during the observation period, the most common reasons being death (4.2%), disease progression (1.9%), and adverse events (1.7%).
Summary/Conclusions: Although NPP data are based on physician declarations and are unmonitored, this analysis provides a real-world estimate of time on treatment, which can be considered a conservative proxy for PFS. The estimates, determined from a large, global CLL population, were similar to those reported for RESONATE™, suggesting that results observed in ibrutinib clinical trials are reproducible in clinical practice.

P209

IMPACT OF ADDING RITUXIMAB TO VENETOCLAX ON THE RATE, QUALITY, AND DURATION OF RESPONSE IN PATIENTS WITH RELAPSED/ REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA: A CROSS-STUDY MULTIVARIABLE ANALYSIS

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Background: Venetoclax (VEN) is a selective, potent, orally bioavailable BCL-2 inhibitor. High overall response rates (ORR, 79%) and complete response rates (CR, 20%) were attained in a first-in-human dose-escalation study (M12-175) of VEN monotherapy in patients (pts) with relapsed/refractory (R/R) chronic lymphocytic leukaemia (CLL) or small lymphocytic lymphoma (SLL). The addition of monthly rituximab (R; months 1-6) in combination with VEN is being evaluated in an ongoing phase 1b dose-escalation study (M13-365) and as of 28Oct2015, the observed ORR is 86% and the CR rate is 47%. Entry criteria for the two studies were similar, but distribution of pt demographic and disease characteristics differed.
Aims: To assess the effect of adding R to VEN on response rate as well as on the quality and duration of the response, relative to VEN monotherapy using post hoc exploratory multivariable analyses.
Methods: Analyses used a cutoff of 25 Aug 2015 for M12-175 and 28 Oct 2015 for M13-365. The median follow-up was 17 and 21 months for M12-175 and M13-365, respectively. Demographic and disease characteristics were analyzed if their distribution differed between the studies and/or they had been identified as VEN response-modifiers in prior exploratory analyses (Roberts et al, NEJM 2016). Included variables were: age, number of prior therapies received (<3 *vs* ≥3), maximum lymph node diameter (≤5 *vs* >5cm), 17p status by FISH (not deleted *vs* deleted), and VEN dose (evaluated using assigned dose cohort in each study: 400 *vs* <400mg [150, 200, 300mg] or >400 mg [500, 600, 800mg]). Pt inclusion required available data for all variables analyzed. Forward stepwise selection was applied for the model, starting with therapy (VEN plus R *vs* VEN monotherapy); a significance threshold of $p<0.2$ was used to add or remove variables to/from the model. Logistic regression analyses were performed for ORR (responder *vs* non-responder) and CR (CR/CRi *vs* all other pts). A Cox proportional hazards model was applied to evaluate differences in duration of response (DoR).

Table 1.

	ORR, Odds Ratio (95% CI); p-value	CR, Odds Ratio (95% CI); p-value	DoR, Hazard Ratio for Progression (95% CI); p-value
VEN plus R vs. VEN	1.142 (.433 – 3.015); p=.79	3.292 (1.527 – 7.097); p=.002	.235 (.078 – .703); p=.01
Prior therapies <3 vs ≥3	4.579 (1.624 – 12.908); p=.004	NS	NS
Lymph node size ≤5 cm vs >5 cm	NS	3.921 (1.807 – 8.506); p=.0005	.406 (.163 – 1.009); p=.052
17p status not deleted vs deleted	NS	NS	.452 (.193 – 1.058); p=.067
Age continuous variable	NS	NS	NS
Dose of VEN <400 mg vs 400 mg	NS	NS	3.129 (1.025 – 9.551); p=.045
>400 mg vs 400 mg	NS	NS	1.365 (.447 – 4.171); p=.59

NS, not included in the final model.

Results: Final models from stepwise regression analyses are summarized in the table. Following selection of the final model, 157 pts were evaluated for ORR (84, M12-175; 45, M13-365), 156 for CR (83, M12-175; 45, M13-365), and 105 for DoR (66, M12-175; 39, M13-365). Combination therapy did not influence ORR relative to VEN monotherapy (odds ratio: 1.142 [95% CI: 0.433-3.015]; *p*=0.79). Responses of CR/CRi were more likely with combination therapy (odds ratio: 3.292 [95% CI: 1.527-7.097]; *p*=0.002) and they were more durable, with longer DoR with combination *versus* VEN monotherapy (hazard ratio for progression: 0.235 [95% CI: 0.078-0.703]; *p*=0.01). When analyzed across both studies, pts who received <3 prior therapies were more likely to achieve a response. Confirming prior exploratory analyses, pts with maximum nodal diameter ≤5 cm were more likely to achieve CR. Pts receiving <400 mg VEN (400 mg is the phase 2 dose of VEN combined with R or monotherapy) had shorter DoR. The effects of other variables were not significant.
Summary/Conclusions: Results of these exploratory, cross-study, post hoc analyses support the choice of 400 mg VEN dosing and indicate higher CR rates and longer DoR with the combination of VEN plus R after adjusting for pt demographics and disease characteristics. VEN combined with R is currently being evaluated in the ongoing, phase 3 MURANO study randomized against bendamustine with R.

P210

PRELIMINARY RESULTS OF A PHASE II STUDY OF IBRUTINIB IN COMBINATION WITH FCR (IFCR) IN PREVIOUSLY UNTREATED, YOUNGER PATIENTS WITH CLL

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Background: FCR is a standard initial therapy for younger patients (pts) with CLL; however, only about 20% of pts will achieve complete response (CR) with bone marrow minimal residual disease negativity (BM MRD-neg) (Boettcher *et al.*, 2012), which is likely a prerequisite for cure. Ibrutinib is an oral BTK inhibitor with an excellent efficacy and safety profile in CLL. We report on the preliminary results of an investigator-initiated, phase II study of ibrutinib plus FCR (iFCR) as upfront treatment for young, fit CLL pts (NCT02251548).
Aims: The primary aim of this open-label, single arm, multicenter phase II study is to determine the rate of CR with BM MRD-neg in younger CLL pts treated upfront with iFCR. Secondary aims are to assess response rates and safety/tolerability.
Methods: Ibrutinib 420 mg daily monotherapy is given for 7 days, and FCR per standard dosing is added on day 8. Up to 6 cycles of FCR are given concurrently with daily ibrutinib; growth factor support and antimicrobial prophylaxis are mandatory. Responders continue on ibrutinib maintenance until time of progression or unacceptable toxicity. Eligibility criteria include: age ≤65, requiring initial treatment by IW-CLL criteria, ECOG PS ≤1, and adequate organ function. CTCAE v4 and IW-CLL criteria are used to evaluate toxicity and efficacy, with response evaluations after 3 cycles, 2 mos. after final FCR, and q6 mos. thereafter. MRD is assessed by 4-color flow cytometry (10^{-4} sensitivity).
Results: As of 22 February 2016, 27 pts were enrolled. The median age at enrollment was 55 yrs (range 43-65). 7/25 (28%) had del (11q) and 1/25 (4%) had del (17p) with *TP53* mutation. Unmutated *IGHV* was present in 11/17 (65%), ZAP-70 was positive in 17/23 (74%), and 1/16 (6%) patient had *NOTCH1* mutation. In a 10 patient safety lead-in, there was no unexpected toxicity. Of the 27 total pts, hematologic toxicity includes neutropenia (26%; 15% gr 3-4), thrombocytopenia (56%; 19% gr 3-4), and anemia (19%, 4% gr 3-4). All grade non-hematologic toxicities occurring in ≥15% of pts include gr 1/2 nausea (63%), fatigue (33%), bruising (33%), rash (19%), and gr 1 diarrhea (15%). The only bleeding event was gr 1 epistaxis in 1 pt. SAEs include gr 4 febrile neutropenia, gr 3 atrial fibrillation, and gr 3 transaminitis in 1 patient each. In the 17 pts who have undergone re-staging after completing the iFCR combination, the ORR is 100%, including 8/17 (47%) with CR or CRi, all of whom are BM MRD-neg, and 9/17 (53%) with PR. The rate of MRD-neg in the BM is 13/17 (76%), including 5 patients in PR. 8/9 pts with PR have residual lymph nodes <2.5 cm in long axis by CT imaging. The median duration of therapy is 7.7 months (range 0.5-16.2), and 25 of the 27 pts remain on treatment. A pt who completed 6 cycles of iFCR and achieved CR with BM MRD-neg declined ibrutinib maintenance and remains in CR on observation, and the pt with del(17p) achieved PR and elected to pursue allogeneic stem cell transplant.
Summary/Conclusions: iFCR is a highly active combination for young, fit pts with CLL, with toxicities similar to FCR and ibrutinib given as individual therapies. Although the ORR and CR rates are similar to FCR historical controls, the 47% rate of CR with BM MRD-neg compares favorably to the 20% rate observed on the FCR arm of the CLL8 study. Additionally, several pts who achieved PR are BM MRD-neg, have small residual lymph nodes, and will be monitored for conversion to CR while on ibrutinib maintenance in this ongoing trial.

P211

PRESENCE OF MULTIPLE UNMUTATED IGHV REARRANGEMENTS IN CLL PATIENTS IS ASSOCIATED WITH A VERY SHORT TREATMENT-FREE SURVIVAL: RESULTS FROM 2 INDEPENDENT COHORTS

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Background: The immunoglobulin heavy-chain gene (IgHV) mutational status is considered the gold standard of prognostication in CLL and is currently determined by Sanger Sequencing (SSeq) that only allows for analysis of the major clone. Next-generation sequencing (NGS), in the other hand, is able to highlight multiple rearrangements within a patient. However, the prognostic significance of multiple subclones in CLL is still unknown.
Aims: The aim of the present work was to investigate the prognostic impact of multiple IgHV rearrangements on treatment-free (TFS) and/or overall (OS survival).
Methods: Using NGS, we sequenced the IgHV gene from 2 independent cohorts: (I) 270 unselected CLL patient samples obtained at diagnosis with a median follow-up of 83 months (range, 1-397) and (II) 227 patients from the UK Arctic-Admire FCR-based clinical trial with a median follow-up of 24 months (range, 0.1-215). The data were after correlated with TFS and/or OS.
Results: The presence of multiple rearrangements was confirmed by fluorescence electrophoresis of DNA fragments. We also showed that some IgHV biclonal patients had multiple clones by flow cytometry using a CD5/CD19/Kappa/Lambda staining. Accuracy of sequences obtained by NGS was validated by SSeq. In order to control for potential PCR/sequencing errors, only the most abundant sequences with a median depth of 32631 reads (range, 639-354763) were used to calculate the IgHV mutational status allowing a perfect concordance between SSeq and NGS. Reproducibility of results was confirmed by independent duplicate experiments. Based on IgHV-NGS subclonal profiles, we were able to define 5 different categories: patients with (a) multiple M clones, (b) 1M clone, (c) a mix of M-UM clones, (d) 1UM clone, (e) multiple UM clones. In the population obtained at diagnosis (composed of treated and untreated patients), IgHV-NGS classification stratified patients into 5 different subgroups with median TFS of >280 (a), 131 (b), 61 (c), 29 (d), 18 (e) months (P<0.0001) and a median OS of >397 (a), 292 (b), 196 (c), 137 (d), 106 (e) months (P<0.0001). The subgroups were associated with well-known prognostic factors such as ZAP70, CD38, LPL, lymphocyte doubling time or cytogenetic abnormalities. In the clinical trial population (composed only of treated patients), IgHV-NGS classification stratified patients into 5 different subgroups with median TFS of >38 (a), 37 (b), 17 (c), 19 (d) and 4 (e) months (P=0.0017). The very favorable prognosis of patients with multiple mutated rearrangements was not confirmed in the clinical trial population: indeed, this subgroup is composed of 85% of untreated patients in population (I) who were excluded in trial population (II) which by definition only contains treated patients. On the contrary, the very short median TFS of patient with multiple unmutated rearrangements was confirmed in both populations: patients with multiple unmutated IgHV have a significantly shorter TFS than patients with only one unmutated rearrangement (I. P=0.0190 and II. P=0.0359). In addition, their chance of requiring a treatment within the 4 first years after diagnosis is associated with a positive predictive value of 94% (I) and 90% (II).

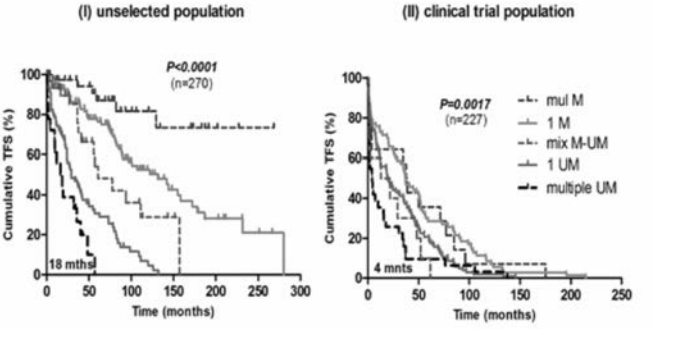

Figure 1.

Summary/Conclusions: Here, we showed for the first time that patients with multiple unmutated IgHV rearrangements determined by deep NGS have very aggressive disease with a very short TFS compared to patient with only one unmutated rearrangement. These finding will help identify patients at diagnosis who will require early treatment and consequently closer follow-up.

P212

NON-CODING NOTCH1 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKAEMIA; THEIR CLINICAL IMPACT IN THE CLL4 CLINICAL TRIAL

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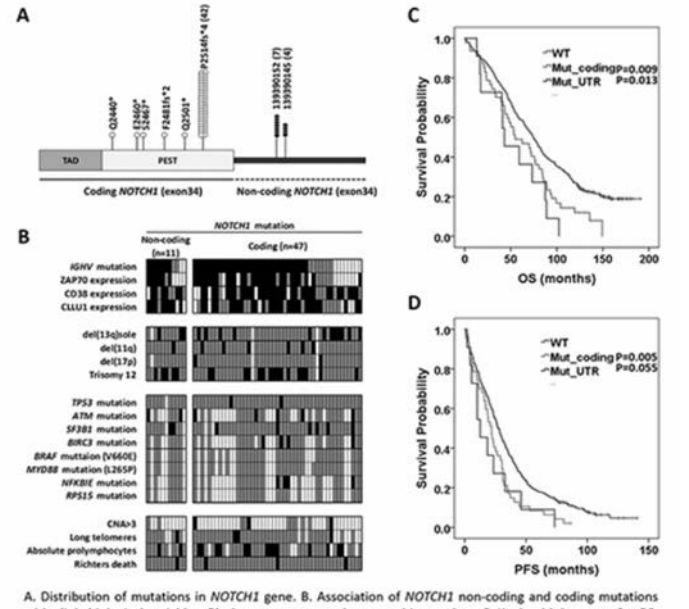
pital, Bournemouth, [3]Haemato-oncology Research Unit, Division of Pathology, The Institute for Cancer Research, Sutton, [4]Cancer Sciences Unit, University of Southampton, Southampton, United Kingdom

Background: *NOTCH1* is recurrently mutated in approximately 10% of patients with Chronic Lymphocytic Leukaemia (CLL) and associates with reduced survival and Richters transformation. Whilst mutations were initially localized to exon 34 (termed 'coding'), recent whole genome sequencing analysis (Nature 2015, 526:519) showed additional mutations in the 3' UTR of *NOTCH1* (termed 'non-coding') resulting in in the same constitutive activation of Notch1. However, the clinical impact of these mutations on patients requiring therapy, enrolled into a clinical trial has not been ascertained.
Aims: To assess the incidence of *NOTCH1* non-coding mutations in the UK CLL4 trial and identify any associations with clinico-biological characteristics, progression free- (PFS) and overall survival (OS).
Methods: 489 CLL patients with available material were included in this study. High-resolution melt analysis, Sanger and high-throughput sequencing were employed to identify *NOTCH1* mutations.
Results: We identified 47/489 (9.6%) and 11/489 (2.2%) coding and non-coding mutations, respectively, with the latter being the those previously reported [139390152 A>G (n=7) and 139390145 A>G (n=4), Nature 2015, 526:519] (**Figure 1A**). The non-coding variants constituted 19% of the total *NOTCH1* mutations, with 11.8% of CLL4 patients carrying either *NOTCH1* mutation. *NOTCH1* non-coding mutations were mutually exclusive of *TP53, MYD88, RPS15* and *NFKBIE* mutations (**Figure 1B**). When considering all 58 mutations together, mutant *NOTCH1* was associated with unmutated *IGHV* genes (OR:2.93, 95%CI:1.38-6.23, P=0.005), CD38 (OR:4.48, 95%CI:2.30-8.72, P<0.001) and ZAP70 positive expression (OR:3.11, 95%CI:1.52-6.37, P=0.002) and the presence of trisomy 12 (OR:4.00, 95%CI:2.16-7.38, P<0.001). For non-coding mutations in isolation, only the association with trisomy 12 remained significant (OR:5.55, 95%CI:1.64-18.83, P=0.006). We analysed the impact of the two types of *NOTCH1* mutations on survival. Considered together, coding and non-coding mutations were associated with a significant reduction in OS and PFS with medians of 53.4 months *versus* 74.7 months (HR:1.63, 95%CI:1.22-2.17, P=0.001) and 19.3 *versus* 27.7 (HR:1.59, 95%CI:1.20-2.11, P=0.001), respectively, compared to wild-type patients. Considered separately, *NOTCH1* non-coding and coding mutations presented a significantly shorter OS than wild-type patients (median 43.2, 54.8 and 74.6 months to death, respectively, P=0.012 and P=0.013, respectively) (**Figure 1C**). Only coding *NOTCH1* mutations were significantly associated with reduced PFS compare to wild-type (median 22.0 *versus* 27.7 months, respectively, P=0.005) (**Figure 1D**). However, whilst not statistically significant, UTR mutations did exhibit reduced PFS (median 12.72, P=0.055) compared to wild-type patients. Cases with non-coding *NOTCH1* mutations were 80% (HR: 1.80, 95%CI: 0.99-3.28, P=0.055) more likely to progress and two-fold more likely to die (HR: 2.14, 95%CI: 1.17-3.92, P=0.013) than wild-type patients at last follow-up. When non-coding and coding mutations were combined, two additional features were noted: 1) combined *NOTCH1* status captured 11 more of both PFS and OS events compared to coding mutations alone, and 2) a trend was observed between the presence of *NOTCH1* mutations and death from Richters transformation (P=0.062).



A. Distribution of mutations in *NOTCH1* gene. B. Association of *NOTCH1* non-coding and coding mutations with clinic-biological variables. Black:presence, grey: absence, white:no data. C. Kaplan-Meier curve for OS. D. Kaplan-Meier curve for PFS.

Figure 1.

Summary/Conclusions: *NOTCH1* non-coding mutations constitute 19% of the total *NOTCH1* truncating mutational burden in patients requiring treatment in the UK CLL4 trial. Importantly, these non-coding mutations have a comparable impact on OS after first-line treatment with chemotherapy. This work supports the detection of non-coding *NOTCH1* mutations in patients requiring therapy.

P213

UPDATED RESULTS OF A PHASE 3 RANDOMIZED, CONTROLLED STUDY OF IDELALISIB IN COMBINATION WITH OFATUMUMAB FOR PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Idelalisib (IDELA) is an oral PI3Kδ inhibitor approved within the EU for use with rituximab in pts with relapsed CLL and as first-line treatment of CLL with either del(17p) or *TP53* mutation in patients unsuitable for chemoimmunotherapy. This open-label study (NCT01659021) compared IDELA + ofatumumab (OFA) *vs* OFA in pts with relapsed CLL. Results of the primary endpoint analysis were previously reported (Robak et al, EHA 2015) and are updated here with an additional 8.5 mos of follow-up. In the primary analysis, the combination yielded superior PFS and the KM estimated median OS was 20.9 and 19.4 mos in the IDELA + OFA and OFA arms, respectively.
Aims: To evaluate the safety and efficacy of IDELA + OFA *vs* OFA alone in patients with relapsed CLL.
Methods: Pts with CLL progressing ≤24 mo from last therapy, who had received ≥2 cycles of a purine analogue or bendamustine, were randomized 2:1 to either Arm A (IDELA 150 mg BID continuously plus OFA, 300 mg IV wk 1, then 1 g IV q wk x 7 and q 4 wk x 4) or Arm B (OFA, same as Arm A except 2 g was substituted for 1 g dosing) with stratification factors relapsed *vs* refractory, del(17p) and/or *TP53* mutation, and *IGHV* mutation. The primary endpoint was PFS as determined by an Independent Review Committee using modified IWCLL 2008 criteria.
Results: Pt characteristics were balanced in the 2 arms: med age 67; Rai II/III/IV 18/13/51%, med no. prior regimens 3, refractory 49%, del(17)p/*TP53*mut 40%, *IGHV* unmut 78%. Exposure, disposition, and efficacy are shown in the table. Results were consistent across risk groups. Gr ≥3 AEs in Arm A included diarrhea/colitis (23.1%), pneumonia (19.7%), and pneumonitis (4.6%).

Table 1.

	Arm A (IDELA/OFA)	Arm B (OFA)	HR / OR[2]
Pts randomized/dosed	174/173	87/86	-
Months on study (range)	16.1 (1.1 - 28.5)[1]	5.8 (0 - 25.4)	-
Reason for study D/C, n (%)			
PD[3]	52 (29.9)	44 (50.6)	-
Death	26 (14.9)	6 (6.9)	-
AE/Physician decision	29 (16.7)	19 (21.8)	-
Withdrew consent/other	15 (8.6)	17 (19.5)	-
Med PFS[4], mo	16.4	8.0	HR = 0.27, p < 0.0001
ORR, %	75.3	18.4	OR = 15.9, p < 0.0001
Q1[5] of OS[4] (95% CI), mo	18.2 (12.3, 22.7)	12.7 (6.0, 19.3)	-
Med OS[4] (95% CI), mo	NR (25.8, NR)	NR (21.7, NR)	HR = 0.75, p = 0.27
Del(17p)/*TP53*mut: Med OS[4] (95% CI), mo	25.8 (22.7, NR)	19.3 (10.7, NR)	HR = 0.52, p = 0.03

[1]IDELA med exposure 12.3 mo (0.2-23.9); [2]odds ratio; [3]per physician; [4]KM estimation; [5]1st quartile

Summary/Conclusions: With >8 mos longer follow-up, IDELA + OFA *vs* OFA continues to show superior PFS and ORR, and now demonstrates superior OS in pts with del(17p)/*TP53*mut and a trend of improvement of OS in the ITT population.

P214

AN EVALUATION OF THE CLL-IPI SCORE AND COMPREHENSIVE PROGNOSTIC FACTOR ANALYSIS IN PATIENTS WITH R/R CLL IN IDELALISIB PHASE 3 RANDOMIZED STUDIES

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Background: The International Prognostic Index for patients (pts) with chronic lymphocytic leukemia (CLL-IPI) is a scoring system with prognostic value for overall survival (OS) in untreated CLL (Bahlo J *et al. Haematologica* 2015; 100: 313), but has not been studied in relapsed/refractory (R/R) CLL or in the context of novel agents. The CLL-IPI is a risk-weighted model consisting of 5 risk factors: age (relative weight, 1), stage (1), β2-microglobulin (2), IGHV mutation status (2), and del(17p)/TP53 mutation (17p-/TP53M) (4). Since IDELA is active in CLL with 17p-/TP53M, which is a major contributor to the CLL-IPI, we hypothesized that IDELA would overcome the negative impact of a high CLL-IPI.
Aims: 1) To assess the prognostic utility of CLL-IPI in R/R CLL in IDELA phase 3 randomized trials; 2) To determine if IDELA overcomes the negative impact of a high CLL-IPI; 3) To identify independent risk factors for OS in pts with R/R CLL and in pts treated with IDELA
Methods: The CLL-IPI was analyzed among 460 pts with R/R CLL requiring treatment who received IDELA+rituximab (R) *vs* placebo+R (NCT01539512), or IDELA+ofatumumab (O) *vs* O (NCT01659021). Subgroup analyses were conducted in 274 pts treated with IDELA+R or IDELA+O (IDELA cohort) and 186 pts treated with R or O (Control). Median OS was estimated using the Kaplan-Meier method for the 4 CLL-IPI risk groups: low (score 0-1), intermediate (2-3), high (4-6), and very high (7-10). The log-rank test was used to compare survival distributions across CLL-IPI risk groups. Multivariate analyses of prognostic factors for OS were performed. Factors with a p-value of <0.1 in the univariate analysis (log-rank test) were included in the multivariate analyses (Cox proportion hazards model).
Results: Most pts with R/R CLL have very high (40.9%) and high risk CLL-IPI (49.6%), and intermediate (8.3%) and low risk CLL-IPI (1.3%) are rare. Notably, only 0.6% pts with R/R CLL were Rai 0/Binet A. At a median follow-up of 14.7 months, the CLL-IPI score was validated in pts with R/R CLL with significant differences in OS across CLL-IPI risk groups (p=0.0001). In subgroup analyses, the CLL-IPI was prognostic for OS in the Control (p=0.0007) but not in the IDELA cohort (p=0.086). In the multivariate analyses: 17p-/TP53M, B2M, LDH and Karnofsky performance status (KPS) were independently prognostic for OS in pts with R/R CLL. While B2M, KPS and 17p-/TP53M were independently prognostic for OS in the Control cohort, only LDH and age were independently prognostic for OS in the IDELA cohort.

Table 1.

Multivariate analyses:	R/R CLL		IDELA Cohort		Control Cohort	
Overall Survival	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
B2M ≥3.5	2.29 (1.09, 4.8)	0.0283	1.69 (0.64, 4.45)	0.2912	3.29 (1.01, 10.69)	0.0482
LDH elevated	1.65 (1.15, 2.36)	0.0064	1.73 (1.03, 2.89)	0.0376	1.63 (0.97, 2.74)	0.0665
Age ≥65	1.44 (0.97, 2.15)	0.074	2.01 (1.11, 3.62)	0.0202	0.94 (0.54, 1.65)	0.8333
KPS ≤70	1.93 (1.33, 2.8)	0.0005	1.47 (0.86, 2.52)	0.161	2.91 (1.71, 4.93)	0.0001
17p-/TP53M	1.63 (1.15, 2.32)	0.0066	1.41 (0.86, 2.3)	0.1732	1.88 (1.12, 3.15)	0.0161
IGHV unmutated	1.45 (0.88, 2.37)	0.1448	1.48 (0.76, 2.86)	0.2475	1.32 (0.61, 2.85)	0.4764

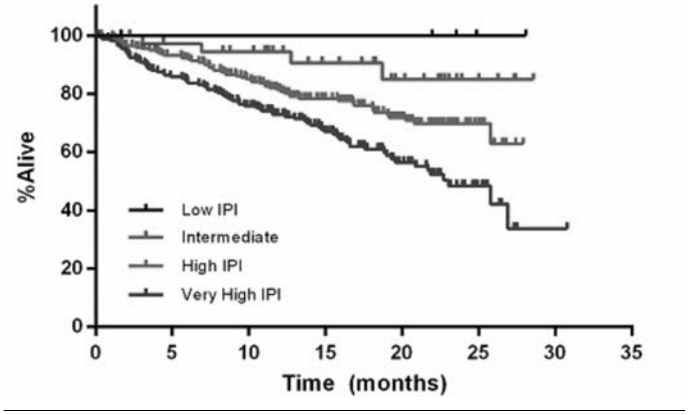

Figure 1.

Summary/Conclusions: Although low/intermediate-risk CLL-IPI is uncommon in R/R CLL, the CLL-IPI may be prognostic for OS in this population. These data suggest that IDELA overcomes the negative impact of high-risk CLL-IPI. While 17p-/TP53M, B2M, LDH and KPS were prognostic for OS in pts with R/R CLL, the current analysis indicates that only age and LDH may be prognostic for OS in pts treated with IDELA in combination with an anti-CD20 monoclonal antibody. Additional work is needed to develop adjusted risk models for pts with R/R CLL and for pts treated with targeted agents.

P215

DISCONTINUATION OF IDELALISIB TREATMENT DUE TO DISEASE PROGRESSION IN PATIENTS WITH RELAPSED AND REFRACTORY CLL: AN EVALUATION OF OUTCOMES

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Background: There have been reports of rapid decline leading to death in patients with CLL following discontinuation of B-cell receptor pathway-targeted therapy (Jain, *et al. Blood*. 2015;125(13):2062-7). Idelalisib (IDELA) is a first in class PI3Kδ inhibitor which inhibits multiple signaling pathways, including those downstream of the B-cell receptor, CXCR4, and CXCR5. It is administered orally until disease progression or intolerance. Outcomes of patients with CLL who discontinued IDELA treatment either due to progressive disease (PD) or adverse events (AEs) have not been described comprehensively.
Aims: The objective of this post hoc analysis was to examine the outcomes of patients with R/R CLL who discontinued IDELA.
Methods: Data were pooled for patients with R/R CLL who were randomized to treatment with either IDELA+ofatumumab (n=173) in Study 119 or IDELA+rituximab (n=110) in Study 116 (including long-term follow up in extension Study 117). Time-dependent endpoints were calculated from the dates of IDELA discontinuation, including: time to next therapy (TTNT), and time to death (TTD). Kaplan-Meier analysis was used to estimate overall survival (OS) from the date of randomization. Subgroup OS analyses were performed using the integrated safety data set.
Results: Of 283 patients in the safety population, 124 (44%) remained on IDELA, 28 (10%) discontinued due to PD (22 CLL progressions and 6 Richter's transformations (RT), and 131 (46%) discontinued for reasons other than PD. Presence of -17p/TP53 mutation at baseline was reported for 54% of patients who discontinued due to PD, 41% of patients who discontinued due to AEs, and 37% of patients who remained on IDELA treatment. The clinical outcomes for those patients who discontinued IDELA treatment due to PD are tabulated.

Table 1.

	IDELA D/C due to PD (n=28)
OS, mos	
Median (95% CI)	18.8 (15.5, NR)
OS at 24 months, %	44.4%
TTNT, mos	
n	7
Median (95% CI)	0.9 (0.2, 3.7)
TTD, mos	
n	9
Median (95% CI)	2.4 (0.1, 9.9)

Summary/Conclusions: In contrast to reports for other B-cell receptor pathway inhibitors, discontinuation of IDELA due to progression of CLL does not appear to be associated with shortened survival.

P216

ASSOCIATION BETWEEN KIR/HLA GENOTYPE AND OUTCOME IN THE CLL11 STUDY OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS RECEIVING CHLORAMBUCIL ALONE OR IN COMBINATION WITH OBINUTUZUMAB OR RITUXIMAB

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Background: Interaction between killer cell immunoglobulin-like receptors (KIRs) and HLA class I molecules results in negative regulation of natural killer (NK) cells. Depending on an individual's KIR/HLA genotype, the number of possible inhibitory KIR/HLA interactions can range from 1-3. Previous analyses suggest a negative correlation between response to monoclonal antibody therapy and number of KIR/HLA interactions, with a lower number resulting in increased NK cell activation and a stronger response to treatment. Findings from in-vitro studies suggest that inhibitory signaling through KIRs observed with the conventional anti-CD20 antibody rituximab (R) may be overridden by the enhanced NK-cell activation associated with the glycoengineered anti-CD20 antibody obinutuzumab (GA101; GAZYVA/GAZYVARO; G).
Aims: To analyze the association between KIR/HLA genotype and treatment outcome using data from the CLL11 study (NCT01010061) comparing chlo-

rambucil (Clb), R plus Clb (R-Clb), and G plus Clb (G-Clb) as first-line treatment in patients (pts) with CLL.
Methods: Pts with available DNA who provided informed consent for genomic analysis were included. Genotyping was performed for the inhibitory KIR genes *2DL1*, *2DL2/3* and *3DL1* and their respective HLA ligands *HLA-C2*, *HLA-C1* and *HLA-Bw4*. Multivariate Cox regression analysis was performed to determine the association between number of KIR/HLA interactions (based on the presence of a receptor-ligand pair) and progression-free survival (PFS). Overall response rate (ORR) and minimal residual disease (MRD) negativity at end of treatment by number of KIR/HLA interactions was also evaluated. In the main analysis, pts carrying genes for 1 or 2 interactions (1-2i) were compared with pts carrying genes for all 3 interactions (3i). As this was an exploratory analysis, p-values were not adjusted for multiple testing.
Results: In total, 489 pts were included in the analysis (Clb, n=74; R-Clb, n=203; G-Clb, n=212). The number of KIR/HLA interactions was: 1 in 104 pts (21%); 2 in 221 pts (45%); and 3 in 164 pts (34%). In the Clb arm, KIR/HLA genotype did not significantly influence PFS (median PFS: 1-2i, 11.1 months [mo]; 3i, 10.6 mo; adjusted HR 0.75, 95% CI 0.42-1.34, p=0.33). The impact of KIR/HLA genotype on PFS tended to be stronger in pts treated with R-Clb (median PFS: 1-2i, 18.0 mo; 3i, 14.7 mo; HR 0.79; 95% CI 0.56-1.10; p=0.16) or G-Clb (median PFS: 1-2i, 29.6 mo; 3i, 22.9 mo; HR 0.69, 95% CI 0.47-1.00, p=0.05). A similar effect was seen for ORR, although differences were not statistically significant (Clb: 1-2i, 31% *vs* 3i, 39%; R-Clb: 1-2i, 70% *vs* 3i, 59%; G-Clb: 1-2i, 79% *vs* 3i, 71%). MRD negativity appeared higher for 1-2i pts in the G-Clb arm (39% *vs* 3i, 26%), but not in the R-Clb arm (3% *vs* 3i, 2%); p>0.05 for both arms. No pt achieved MRD negativity in the Clb arm. Analysis of pts with 1i *vs* 2i *vs* 3i suggested a trend of association between the KIR/HLA interaction number and PFS in G-Clb pts (median PFS: 33.5 mo *vs* 29.1 mo *vs* 22.9 mo, respectively; HR 1i *vs* 3i 0.60, 95% CI 0.36-1.00, p=0.05; HR 2i *vs* 3i 0.73, 95% CI 0.49-1.10, p=0.13).
Summary/Conclusions: Pts with a lower number of KIR/HLA interactions appeared to have a consistently better outcome across a number of efficacy parameters when treated with anti-CD20 antibodies; the strongest effect was observed in the G-Clb arm. These findings warrant investigation of combined KIR-blockade with anti-CD20 antibody treatment in pts with CLL to decrease the impact of these inhibitory interactions on antibody-dependent cell-mediated cytotoxicity.

CLL - Efficacy and safety of new treatments 2

P217

IMPROVEMENT IN QUALITY OF LIFE AND WELL-BEING IN OLDER PATIENTS WITH TREATMENT-NAÏVE (TN) CLL: RESULTS FROM THE RANDOMIZED PHASE 3 STUDY OF IBRUTINIB (IBR) *VERSUS* CHLORAMBUCIL (CLB) (RESONATE-2(TM))

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Background: CLL primarily affects older pts who often have comorbidities and disease-related immunosuppression. Improvement of health-related quality of life (QOL) is an important therapy endpoint. In pts with CLL, QOL per EORTC QLQ-C30 is significantly compromised (Holtzer-Goor Qual Life Res 2015). Ibr is the first-in-class, once-daily, oral inhibitor of Bruton's tyrosine kinase approved for CLL pts with ≥1 prior therapy. Ibr was associated with improvement in QOL in R/R CLL pts on RESONATE study (Barrientos ASH 2014). The recent phase 3 PCYC-1115 trial (RESONATE-2) showed superior PFS, OS, response, and hematologic improvement with ibr *vs* clb in older TN pts with CLL, with a favorable safety profile (Burger NEJM 2015).
Aims: To evaluate outcomes associated with pt well-being from RESONATE-2 in 1st-line CLL.
Methods: After informed consent, TN pts ≥65 years (yrs) received 420 mg ibr once daily until progression or clb for up to 12 cycles. Patient-reported QOL was measured by change from baseline to each assessment time for FACIT-Fatigue (F) and QLQ-C30 in the ITT population.

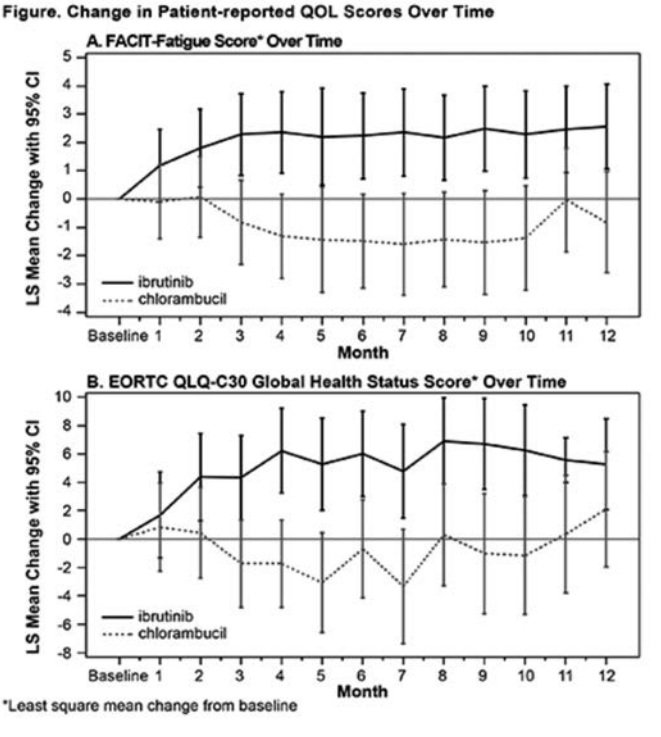

Figure 1.

Results: In 269 randomized pts, median age was 73 yrs (≥70 yrs in 70%) and 69% had comorbidities (CIRS score >6, reduced creatinine clearance, or ECOG status 2). Median follow-up was 18.4 mo. Ibr resulted in greater improvements over time compared to clb in FACIT-F (P=0.0004) and QLQ-C30 global health (P=0.0002) by repeated measures (Figure 1). Baseline hemoglobin (Hb) correlated with FACIT-F; Hb improvement was associated with meaningful improvement in fatigue. Sustained improvement in hematologic function occurred in 84% of ibr pts with baseline anemia and 77% with baseline thrombocytopenia (vs 45% and 43% for clb), both occurring earlier with ibr; median time to first sustained improvement in Hb was 4 mo (vs 12 mo with clb; P=0.0003) and in platelets was 3 mo with ibr (vs 11 mo with clb; P=0.0269). Lymph node (LN) and spleen reductions ≥50% occurred in 95% and 95% of ibr pts *vs* 40% and 52% with clb, with complete resolution in lymphadenopathy and splenomegaly in 42% and 56% *vs* 7% and 22%, respectively. At first assessment (4 mo), median reductions of 73% in LN and 72% in spleen occurred with ibr *vs* 17% and 17% with clb. Disease symptoms including fatigue, night sweats, and weight loss improved more frequently with ibr *vs* clb. No decrement in median IgG or IgM levels was observed for ibr, with relative increase in IgA. Median exposure for ibr was 17.4 mo *vs* 7.1 mo for clb. Most frequent ibr adverse events (AEs) were largely mild (grade 1) including diarrhea, fatigue, cough and nausea. Atrial fibrillation occurred in 8 pts on ibr (6 within the first 6 mo), which was largely grade 1-2 with 2 grade 3 events. Anticoagulants and antiplatelet agents were frequently used (53% of pts), most commonly aspirin. Major hemorrhage was observed in 6 ibr pts including 2 within first 6 mo, 3 during mo 6-12, and 1 during mo 12-18. Clb arm had 2 major hemorrhages, 1 each in first 6 mo and 6-12 mo. The exposure-adjusted infection rate for pts treated with ibr *vs* clb was 7.5 *versus* 10.1 per 100 patient-mo. Grade ≥3 infections decreased with time for ibr. AEs leading to ibr discontinuation were infrequent with most occurring during first 6 mo. 87% of pts continued ibr.

Summary/Conclusions: In TN CLL pts, ibr compared with clb was associated with greater improvements in QOL and hematologic function, as well as reduction in disease burden. The safety profile was favorable allowing for 87% of this elderly population with frequent comorbidity to continue therapy after median 1.5 yrs of follow-up.

P218

ULTRA DEEP TARGETED NEXT GENERATION SEQUENCING FOR THE DETECTION OF SUBCLONAL TP53 ABERRATIONS IN CLL

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Background: *TP53* aberrations (del(17p) and/or *TP53* mutations) are well-characterized as a prognostic marker in chronic lymphocytic leukemia (CLL). However, subclonal *TP53* aberrations as low as 0.3% allelic burden has been demonstrated to result in as poor survival as for patients (pts) with clonal *TP53* aberrations (Rossi *et al. Blood.* 2014, Nadeau *et al. Blood*. 2016). As low allelic burden *TP53* mutations are expected to influence choice of treatment for pts with CLL, it is of the outmost importance to develop a robust, reproducible, and sensitive assay. We here describe the development of an assay refining the detection of subclonal levels of *TP53* as low as 0.1% based on algorithms combined with a dilution step.

Aims: To establish a clinical tool to evaluate very small subclonal *TP53* aberrations.

Methods: Purified DNA from four CLL pts with known clonal levels of *TP53* mutations (detected by Sanger) were diluted with wild type (wt)-TP53 DNA to 10%, 1%, and 0.1%. wt-TP53 DNA was used as reference. Twenty-one amplicons of approximately 180 base pairs (bp) lengths covering *TP53* exons 2-10 were generated and in order to minimize the error rate of nucleotide incorporation a proofreading DNA polymerase was used. Amplicons were purified, adaptor ligated, and sequenced using targeted next generation sequencing (tNGS). All sequences were trimmed 5' and 3' to remove primer DNA *in silico*. To diminish background noise, variants were only called if a minimum of 10 reads of the aberration were detected, and only if variants were also present in a dilution at the expected frequency. The same method was applied with four breast cancer pts with known clonal levels of *TP53* insertions or deletions (INDELS), to test performance of our algorithm with INDELS. These samples were first diluted with wt-TP53 DNA into subclonal levels (5% patient DNA) and then further diluted in a 1:3 and 1:10 ratio. We further optimized our test by reducing the number of 180-220 bp amplicons needed to cover *TP53* exons 2-10.

Results: With a median coverage of 30,587x (range: 0-94,696x, with 85% >13,331x) and 41,465x (range: 134-113,468x, with 85% >14,116x) for the pts with CLL and breast cancer respectively, we were able to detect the known mutations in all eight samples (3 substitutions, 3 deletions, and 2 insertions). One CLL sample also contained a previously unknown subclonal substitution. All background noise could be clearly eliminated *in silico* with the inclusion of the dilution step. Including a 1:3 or a 1:10 dilution step showed same results. The current detection level for the assay is approximately 0.1% allelic burden (Figure 1). However, mutations were still detectable as low as 0.02% allelic burden. One single variant call within the tested eight samples was above 0.3% allelic burden but discarded as background noise (163 reads at 51,000x) based on our novel dilution algorithm. This false positive aberration would have been called as a true mutation with the cut-off based on statistical algorithms used by other groups in previous publications.

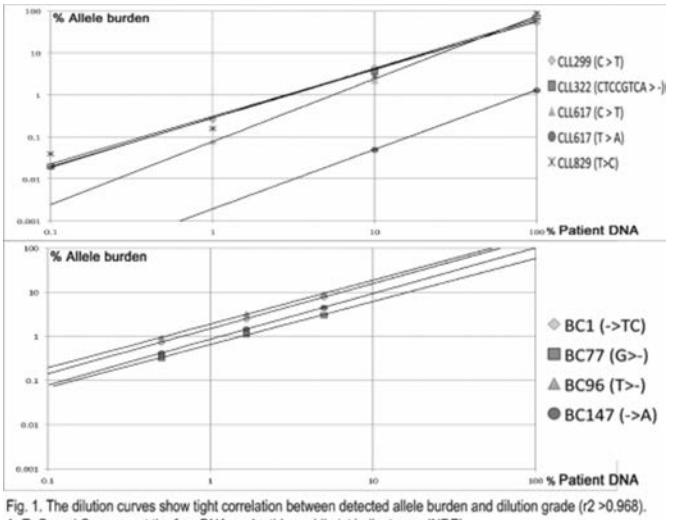

Fig. 1. The dilution curves show tight correlation between detected allele burden and dilution grade (r2 >0.968). A, T, G, and C represent the four DNA nucleotides, while '–' indicates an INDEL.
A) All four CLL TP53 mutations diluted in wt-TP53 DNA to 10%, 1%, and 0.1% were detected. Sample CLL617 show excessive dilution with an allelic burden of less than 0.1% at 1% dilution, thus not detectable at the highest dilution. Sample CLL617 also harbored a subclonal T>A substitution at chr17:7579394 (hg19), which was detected for the first time by our assay.
B) All four breast cancer TP53 INDELS diluted to 5%, 1.67%, and 0.5% in wt-TP53 DNA were detected. There were no differences between including a 1:3 or a 1:10 dilution step, when calling the variants, indicating a lower level of detection of only 0.06% allele burden with the inclusion of a 1:3 dilution step.

Figure 1.

Summary/Conclusions: We here describe for the first time the development of a more robust tNGS assay enabling the detection of very low allelic burden *TP53* mutations. At the same time, false positive variant calls can be discarded based on the inclusion of a dilution step, while the sensitivity for both substitutions and INDELS is as low as 0.1%. The assay is undergoing further validation in a retrospective cohort of pts with CLL. The assay will subsequently be implemented as part of the diagnostic tools available at Copenhagen University Hospital, Rigshospitalet.

P219

COMPARABLE OUTCOMES IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH REDUCED DOSE IBRUTINIB: RESULTS FROM A MULTI-CENTER STUDY

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Background: Ibrutinib (Ibr) is a first-in-class oral covalent inhibitor of Bruton's tyrosine kinase (BTK). Ibr is dosed according to guidelines for chronic lymphocytic leukemia (CLL) patients at 420mg daily to achieve >90% BTK occupancy (≥2.5mg/kg/day). Although fewer patients achieve >90% occupancy at <420mg daily, it is unknown whether lower Ibr doses translate into inferior outcomes. To address this question, we conducted a multi-center study to capture experiences of CLL patients treated with Ibr below suggested starting doses.

Aims: This study evaluated survival outcomes for CLL patients treated with Ibr at reduced doses.

Methods: We conducted a multi-center, IRB approved, retrospective study of all Ibr-treated CLL patients between 1/2010 and 10/2015. Demographics, Ibr dose, clinical responses, and outcomes were recorded. Reduced Ibr dose was defined as sustained (≥2 months) dosing at <420mg/day, either at treatment initiation or within 3 months of initiation. The primary study endpoints were progression-free survival (PFS) and overall survival (OS), estimated by the Kaplan Meier method and stratified by Ibr dose (standard dose *versus* reduced dose). For comparison, log rank tests and Cox regressions were performed.

Results: 197 Ibr-treated CLL patients were identified (83% with relapsed or refractory disease). 37 patients (19%) received reduced dose Ibr. Median follow-up time was 13.5 months. For reduced dose patients, median dose was 4.3mg/kg/day (4% <2.5mg/kg/day). The most common reasons for reducing

Ibr dose were GI toxicity, bleeding, rash, cardiotoxicity, and pre-existing renal insufficiency. 5% of patients in the reduced dose group were taking a mild-moderate CYP3A4 inhibitor. The best overall response rate (CR+PR+PR-L) for the entire cohort was 85%, *versus* 84% in the reduced dose group. Median PFS for standard dose and reduced dose cohorts were 37.4 months and not reached, respectively (p=0.6, LR test). Median OS for standard dose and reduced dose cohorts were both not reached (p=0.5, LR test). Ibr dose did not impact PFS (1.2, 95% CI 0.5-2.6) or OS (0.6, 95% CI 0.14-2.7).

Figure 1.

Summary/Conclusions: We describe the largest real-world experience of reduced dose Ibr in CLL patients. Reduced Ibr doses do not appear to affect clinical outcomes. Most patients treated with doses <420mg/day achieve a dose of >2.5mg/kg/day, and therefore adequate BTK occupancy. Weight-based dosing should be considered in future studies and pharmacoeconomic analyses. Comparative analyses of toxicity profiles stratified by Ibr dose are underway.

P220

Abstract withdrawn.

P221

PATIENTS WITH COMPLEX KARYOTYPE (CK) LACKING TP53 DELETIONS SHOW AN EQUIVALENT DISMAL CLINICAL OUTCOME AS THOSE WITH TP53 DELETIONS AND NON-CK IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Cytogenetic abnormalities detected by FISH define prognostic groups in chronic lymphocytic leukemia (CLL). Complex karyotypes (CK) confer poor prognosis in patients treated with chemoimmunotherapy and novel agents. However, only 28-50% of patients with CK carry *TP53* deletions [delTP53]. The relationship between delTP53 and CK, and its clinical impact, remain to be elucidated.
Aims: 1. To describe the clinico-biological profile of CLL and monoclonal B-cell lymphocytosis (MBL) patients regarding the karyotype complexity by chromosome banding analysis (CBA) 2. To compare the clinical outcome of patients with CK lacking high-risk FISH deletions (HR-FISH), *TP53* and/or *ATM,* and those with HR-FISH and non-CK.
Methods: Our database comprising 2756 MBL and CLL patients from Grupo Cooperativo Español de Citogenética Hematológica and Grupo Español de Leucemia Linfática Crónica was screened for patients with CBA results available at diagnosis or prior to treatment. The clinico-biological data and outcome of patients with CK (≥3 abnormalities) were compared with those with normal or <3 abnormalities [non-CK]. Time to first treatment (TFT) and overall survival (OS) were assessed according to both karyotype complexity and HR-FISH deletions.
Results: CBA results were available for 1149 patients (703M/446F, median age 67), 199 MBL and 1050 CLL. Most karyotypes (75%) were assessed on peripheral blood TPA-based cultures. 438/1149 (38.1%) patients displayed abnormal karyotypes, 82 showing a CK. CK cases showed features of more advanced and aggressive disease than non-CK: more Binet B/C stages (23 *vs* 12%; P=0.008), higher LDH (379 *vs* 304 IU/L; P<0.001) and β2-microglobulin (2.8 *vs* 2 mg/L; P<0.001), higher positivity rates for CD38 (38% *vs* 21%; P<0.001) and ZAP70 (50% *vs* 33%; P<0.001) and increased +12 (32% *vs* 18%; P<0.001), delATM (19% *vs* 7%; P=0.001) and delTP53 (42% *vs* 6%; P<0.001) by FISH. Indeed, patients with CK showed a shorter median TFT (119m *vs* 43m, P<0.001) and OS (163m *vs* 93m; P<0.001). Although the CK group was significantly enriched in delTP53, only 29/78 (37.2%) patients with delTP53 showed CK. Of note, CK negatively impacted on OS even among patients with delTP53 (132m *vs* 38m; P<0.001). Categorization of patients regarding HR-FISH and CK allowed stratification in terms of TFT (Figure). Nonetheless, while patients with both HR-FISH and CK showed the worst OS (39m) being clearly distinct from those non-CK and non-HR-FISH (not reached), survival analysis did not show significant differences between patients with only one high-risk cytogenetic marker (CK or HR-FISH) (Figure). Similarly, no differences in OS for CK patients lacking delTP53 and those with delTP53 and non-CK were observed (209m *vs* 133m; P=0.795). In the multivariate analysis for TFT and OS, both CK and del*ATM* or del*TP53* remained statistically significant (OS HR: 2.6, 2.1 and 2, respectively; P<0.001).

Table 1.

Table. Adverse events of any grade occurring in >25% and grade ≥3 adverse events occurring in ≥10% of patients

AE,n (%)	5/14 Schedule OPZ150 mg/d (n= 4)		2/7 Schedule OPZ 210 mg/d (n= 17)		2/7 Schedule OPZ240 mg/d (n= 10)	
	Any grade	Grade ≥3	Any grade	Grade ≥3	Any grade	Grade ≥3
Hematologic						
Anemia	2 (50)	2 (50)	10 (59)	8 (47)	3 (30)	2 (20)
Neutropenia	1 (25)	1(25)	6 (35)	6 (35)	2 (20)	0
Thrombocytopenia	1 (25)	0	6 (35)	4 (24)	1 (10)	0
Decreased platelet count	0	0	0	0	3 (30)	2 (20)
Decreased neutrophil count	0	0	4 (24)	3 (18)	2 (20)	1 (10)
Febrile neutropenia	0	0	3 (18)	3 (18)	0	0
Clinical interest						
Nausea	2 (50)	0	10 (59)	0	10 (100)	1 (10)
Diarrhea	2 (50)	0	14 (82)	2 (12)	9 (90)	4 (40)
Vomiting	3 (75)	0	7 (41)	0	8 (80)	1 (10)
Constipation	2 (50)	0	6 (35)	0	6 (60)	0
Peripheral neuropathy	0	0	1 (6)	0	4 (40)	1 (10)
Acute kidney injury	0	0	1 (6)	0	1 (10)	1 (10)
Gastric hemorrhage	0	0	1 (6)	1 (6)	0	0

AE, adverse event; OPZ, oprozomib

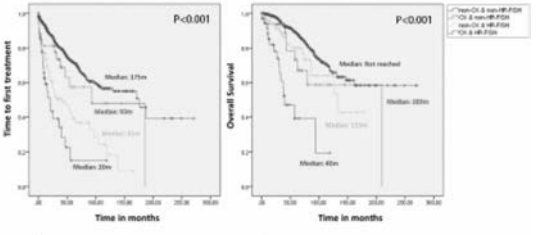

Figure. Kaplan Meier plots for TFT and OS and presence of CK and/or HR FISH

Figure 1.

Summary/Conclusions: 1. CK is associated to advanced disease and poor prognostic markers; however, only 42% of them carry delTP53. 2. Patients with CK lacking delTP53 show an equivalent impaired clinical evolution as those with delTP53 and non-CK. 3. Other mechanisms than *TP53/ATM* dysfunction could be responsible for the outcome of CK. A better characterization of CK patients is needed to elucidate if they could benefit from new therapeutic strategies.
Acknowledgements: PI11/01621;PI15/00437;RD12/0036/0044,FEDER;2014/SGR585.

P222

LARGE SCALE, REAL-WORLD RESULTS ON IBRUTINIB FOR 428 FRENCH PATIENTS WITH 17P DELETION OR RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA INCLUDED IN A TEMPORARY AUTHORIZATION FOR USE (ATU) PROGRAM

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Background: Ibrutinib is an oral once-daily first-in-class covalent Bruton's tyrosine kinase inhibitor approved in Europe for adult patients with CLL who have received at least one prior therapy, or in first line in the presence of 17p deletion or TP53 mutation in patients unsuitable for chemo-immunotherapy. We report here the largest series of real-world results in 428 patients receiving ibrutinib through a temporary authorization for use (ATU) granted by the French National Agency for Medicines and Health Product Safety (ANSM).

Aims: Herein we describe key baseline characteristics and initial outcomes for all patients with CLL/SLL submitting an access-to-ibrutinib form throughout the period of the ATU (December 2013-November 2014).

Methods: Data reported herein represent the final report. As a real-world analysis, all data are declarative in nature (by physicians in charge of the patients), and medical files were not monitored.

Results: Among 428 CLL/SLL patients, most were male (67.1%) with a median age of 70 years (range 33-93, 35%>75y). Median time from diagnosis was 8.1 years. 50.4% of patients had lymph nodes ≥5 cm. Binet stage was A for 7,1%, B for 36.2% and C for 56.7%. Cytogenetic data were reported for 381 patients, among which 45.1% had del17p and/or TP53 mutation, and 24.6% had del11q. A median of 3 prior therapies was reported (range 0-10), including fludarabine-cyclophosphamide-rituximab (63.6%), bendamustine-rituximab (59.8%), and alemtuzumab (27%). Thirty seven patients had undergone prior stem cell transplantation (22 allo, 15 autoSCT). At least one concomitant disease was reported for 232 patients (54.2%), including 24.5% with vascular disorder, 16.6% with metabolism/nutrition disorder, and 11.2% with cardiac disorder. At least one other co-medication was reported in 66.1% of cases (including antithrombotic agents (14%)). The risk of grade 3-4 haemorrhages were 1.6% *vs* 2.6%, respectively for taking these drugs. (no or yes). After a median follow-up of 3 months (range 1-279 days), investigator-assessed ORR was 88.5% including 44.1% PR with lymphocytosis and 1.5% had progressive disease (out of 340 patients with assessable disease). The median PFS, OS and DOR have not been reached. Definitive discontinuation of ibrutinib was reported in 14.5% of 372 evaluable patients, with main reasons including adverse drug reaction (n=20), death (n=19), disease progression (n=8), contra-indication to pursuit of ibrutinib (n=3), and allogeneic transplantation (n=4). In the analysis for safety, 73.4% had at least one AE, 33.2% at least one SAE (among 150 patients above 75y: 34%). Of note, frequency of SAE either in total (34% *vs* 33.2%) or of special interest, infections (11.2% *vs* 10.7%), gastrointestinal disorders including diarrhoea (1.9% *vs* 2%), and cardiac disorders including atrial fibrillation (6.3% *vs* 9.3%), were not statistically increased in elderly patients.

Summary/Conclusions: This is the largest real-world analysis on ibrutinib given for 17p deletion or relapsed/refractory CLL, covering a vast territory (France), both in public/private practice. Efficacy and adverse events profiles were similar to those reported from published clinical trials, especially the RESONATE trial (with the acknowledged limitations inherent to such programs, declarative nature of the data and lack of patient monitoring).

P223

DURABLE TREATMENT-FREE REMISSION AND EFFECTIVE RETREATMENT IN PATIENTS WITH RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA WHO ACHIEVED A DEEP RESPONSE WITH VENETOCLAX COMBINED WITH RITUXIMAB

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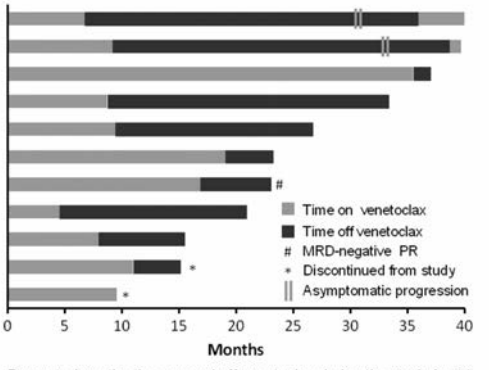
Background: Venetoclax (VEN) is a selective, potent, orally bioavailable BCL-2 inhibitor. VEN monotherapy induces rapid, deep, and durable responses, with a 79% ORR (20% CR) for patients (pts) with relapsed/refractory (R/R) chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL). Based on preclinical evidence of synergy, combination therapy of VEN plus rituximab (R) is being assessed in an ongoing Phase 1b study. In this study, pts with CR/CRi or MRD-negative (neg) PR may stop VEN, remain on study, and be retreated upon disease progression by iwCLL criteria.

Aims: To assess outcomes in pts with R/R CLL who had CR/CRi or MRD-neg PR and stopped therapy per study protocol.

Methods: Pts who achieved CR/CRi or MRD-neg PR could stop therapy and remain on study with clinical monitoring and peripheral blood (PB) counts every 3 months. Pts who then progressed could re-initiate VEN once daily with weekly ramp-up at 20, 50, 100, 200 to 400 mg. R could be added at month 4 or earlier per investigator's discretion. Responses and progression were assessed by iwCLL criteria with CT scan and bone marrow (BM) biopsy. MRD was assessed in blood and BM aspirates using ≥4 color flow cytometry (min sensitivity: 0.01%).

Results: 49 pts (48 CLL/1 SLL) were enrolled. Median number of prior regimens was 2 (range: 1-5); 45 (90%) received prior R (14 [29%] R-refractory) and 28 (57%) received fludarabine (9 [18%] fludarabine-refractory). 15/45 (33%) pts had del(17p) and/or *TP53* mutation. Common adverse events (AEs) were mild diarrhea, neutropenia, upper respiratory tract infection, and nausea. As of 28Oct2015, ORR was 86% (47% CR/CRi), with 55% MRD-neg (17 CR/CRi; 10 PR). While on therapy, disease progression was observed in 7 pts, with 4 occurring after PR, 1 after SD, and 2 had primary PD. 11 pts elected to stop therapy after achieving MRD-neg CR/CRi (8), MRD-pos CR/CRi (2), or MRD-neg PR (1); 9 remain in active follow-up and 2 discontinued from the study with no evidence of progression. Demographics and disease characteristics of these 11 pts were similar to the total study population and median time on VEN was 9 (range: 5–36) months. As of 28Oct2015, 7/9 pts have not progressed (all MRD-neg) and remain in follow-up for a median of 8 (range: 2-25) months off VEN. 2 pts with MRD-pos CR/CRi as best response had subsequent asymptomatic progression off VEN. The first pt stopped VEN plus R at month 7 and had asymptomatic progression (lymphocytosis, BM infiltrate, and splenomegaly) 24 months later. This pt was retreated with VEN monotherapy, achieved PR after 4 months of VEN, and remains in response with ongoing treatment. AEs reported during retreatment were all Grade 1/2 and similar to those seen during first treatment. The second pt stopped VEN at 9 months and had asymptomatic progression (lymphocytosis) 24 months later (PB MRD levels increased after 14 months off VEN). This pt recently reinitiated VEN monotherapy and has not yet had disease reassessment.



Presented are the time on and off venetoclax during the study for 11 patients who stopped venetoclax treatment after achieving CR/CRi or MRD-negative PR, with rows representing each patient. Two patients with MRD-pos CR/CRi as best response had asymptomatic progression after approximately 24 months off therapy. Both patients have reinitated venetoclax treatment.

Figure 1.

Summary/Conclusions: The combination of VEN plus R induced deep responses, with 47% pts achieving CR/CRi and 55% reaching MRD negativity in BM. The rate of disease progression was low for pts continuing VEN with current follow-up. Pts achieving deep responses who stop VEN per study protocol do not relapse rapidly, and treatment-free remissions can be durable, as 7 MRD-neg pts still off VEN maintain remission to date. Progression has been observed after 24 months off VEN in two pts whose best response was MRD-pos; preliminary data suggest that ongoing retreatment is well tolerated and effective.

P224

ATRIAL FIBRILLATION IN CLL PATIENTS TREATED WITH IBRUTINIB. AN INTERNATIONAL RETROSPECTIVE STUDY

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Background: Supraventricular tachyarrhythmias, particularly atrial fibrillation (AF), are an adverse effect of ibrutinib. In 3 phase 3 CLL studies, AF incidence was 5-7.7%, significantly higher than seen in control arms (0.5-2.4%). AF is

associated with deleterious effects on cardiac function and with risk of arterial thromboembolism, particularly stroke; patients receive prophylactic anti-platelet or anticoagulant medication according to individual thromboembolic risk (eg. CHADS2-VASC score). The clinical consequences of tachyarrhythmias and their optimal management in CLL patients treated with ibrutinib are unclear; in particular, the anti-platelet effect of ibrutinib may increase bleeding risk from thromboembolism prophylaxis.

Aims: We characterized clinical course and management of 59 CLL patients who received ibrutinib and developed supraventricular arrhythmia (AF/flutter 56, SVT 3).

Methods: We retrospectively reviewed data from 59 patients treated from 2010-2015 with ibrutinib 420mg/d (n=53) or ibrutinib plus rituximab (n=6): 31 patients were treated at FILO centers (France/Belgium), 22 at MD Anderson Cancer Center and 6 at Peter MacCallum Cancer Centre, Australia.

Results: Median age was 70. Median time to onset was 3.8 months. Baseline risk for AF was not increased: mean HATCH score (a risk index for AF) was 1.39, similar to an age-matched population. Hypertension requiring treatment was present in 63% cases, similar to an age-matched population; mild-moderate mitral regurgitation or aortic stenosis was present in 9/36 (25%) of cases with available echocardiogram results; LVEF was <50% in 10/36 (28%) and <35% in 5/36 (14%), with clinical congestive heart failure in 7/59 of all patients (12%) and angina in 7 cases (12%). Recent infection (<1 month) was present 10 cases (17%). Fifteen of 56 (27%) patients with AF/flutter had a previous history of AF/flutter; all were in sinus rhythm when ibrutinib treatment was started. 90% of the patients received an antiarrhythmic medication and/or procedure. Pharmacological antiarrhythmic therapy was: amiodarone in 19 cases (32%), flecainide in 5 (8%), beta-blockers in 40 (68%) and calcium channel blockers in 4 cases (6%). Seven patients underwent cardioversion; sustained conversion to sinus rhythm was achieved in 2/7. Tachyarrhythmias were persistent in 35/59 (59%) cases despite treatment, whether Ibrutinib was discontinued or not. 71% of patients with atrial fibrillation/flutter had a CHADS2-VASC score ≥2, considered an indication for anticoagulation: 48% of patients with AF/flutter received anticoagulation, 34% received anti-platelet therapy and 27% received neither therapy. Median CHADS2/VASC score was 2 in patients who received or did not receive thromboembolism prophylaxis. Clinical consequences of tachyarrhythmias were: 3 episodes of severe cardiac failure (one fatal); 1 stroke; 8 non-thrombocytopenic patients (13.5%) experienced grade 3-4 bleeding, including 2 subdural hemorrhages and 2 cases of hemorrhagic cardiac tamponade. Figure 1 outlines the management of ibrutinib dosing in patients with tachyarrhythmias. Altogether, Ibrutinib was permanently discontinued due to arrhythmia in 23/59 cases (39%), limiting effective antineoplastic therapy.

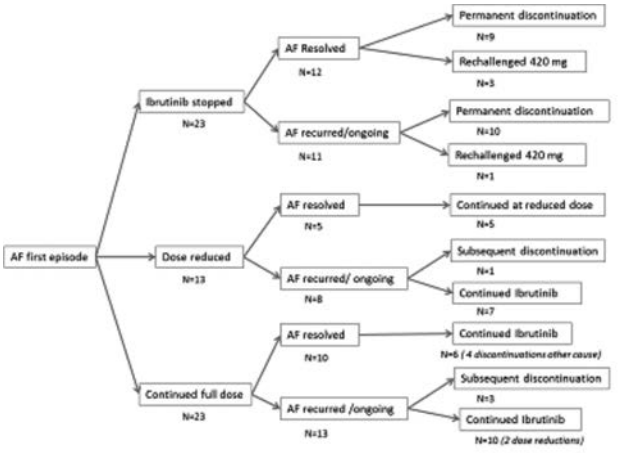

Figure 1.

Summary/Conclusions: AF in ibrutinib-treated patients with CLL is frequent and associated with significant consequences. Management, even in academic centers, was variable. Guidelines for prevention and management of AF in patients receiving ibrutinib are urgently required, particularly as ibrutinib use increases in community practice.

P225

INTEGRATED SAFETY ANALYSIS OF VENETOCLAX MONOTHERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Venetoclax is a potent, selective, oral BCL-2 inhibitor that has activity in patients (pts) with relapsed/refractory (R/R) CLL, with an objective response rate of >70%.

Aims: Here we report the aggregated venetoclax monotherapy safety analysis from 3 pooled trials in R/R CLL.

Methods: An integrated safety analysis was conducted for pts with R/R CLL who received venetoclax monotherapy in a phase 1 dose escalation and two phase 2 trials (N=328). Pts who received the recommended phase 2 dose of 400mg once daily (QD) were analyzed for incidence and timing of adverse events (AEs) and are reported here.

Results: Pts receiving 400 mg QD (n=279) included 181 pts with del 17p and 75 pts with previous B cell receptor inhibitor failure. As of Aug 25, 2015, the median duration on treatment was 10.3 months and 65.6% of pts were still on study. Discontinuation occurred in 96 pts, with 24 pts discontinuing due to adverse events (AEs). The most common (>20% pts) AEs of any grade (Gr) were neutropenia (38%), diarrhea (38%), nausea (36%), anemia (26%), fatigue (24%), and upper respiratory tract infection (21%). The most common (>10% pts) Gr 3/4 AEs were neutropenia (36%), anemia (15%), and thrombocytopenia (13%). Cytopenias occurred early and rates decreased over time. Serious AEs (>2% pts) were pneumonia (5%), febrile neutropenia (5%), pyrexia (3%), and autoimmune hemolytic anemia (3%). There were 41 deaths, 11 due to AEs not associated with disease progression. Under the most recent tumor lysis syndrome (TLS) mitigation guidelines, which align prophylactic measures with the pt's relative risk, 4/105 pts (4%) experienced laboratory TLS, all Gr 3. All TLS events were observed within the initial 5-wk dose ramp-up and were managed with hydration, electrolyte correction, and dose interruption. All pts were able to resume dosing without clinical sequelae.

Summary/Conclusions: Venetoclax 400mg QD has an established and manageable safety profile for the treatment of R/R CLL pts, including those who may not be appropriate candidates for other approved treatments.

P226

MANAGEMENT OF TRANSAMINASE ELEVATIONS ASSOCIATED WITH IDELALISIB

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Background: Idelalisib (IDELA), a first-in-class, selective oral PI3Kδ inhibitor, has been associated with increased alanine transaminase (ALT) and aspartate transaminase (AST) elevations in clinical trials. These transaminase elevations typically occur early during treatment (median onset of 8 weeks) and are generally asymptomatic. Currently recommended management of grade ≥3 (>5-20X ULN AST/ALT) elevation involves dose interruption with re-initiation of therapy possible following resolution to grade ≤1.

Aims: This post hoc analysis evaluated outcomes following treatment-emergent grade ≥3 ALT/AST in IDELA clinical trials.

Table 1.

n (%)	IDELA Alone (n=485)	IDELA Combination (N=588)	Total (N=1073)
Patients with any grade ALT/AST elevation	237/485 (49)	306/588 (52)	543/1073 (51)
Grade 1 (3 to < 5 × ULN)	136/485 (28)	193/588 (33)	329/1073 (31)
Grade 2 (5 to < 10 ULN)	31/485 (7)	38/588 (6)	69/1073 (6)
Grade 3 (10 to < 20 ULN)	49/485 (10)	63/588 (11)	112/1073 (10)
Grade 4 (≥ 20 × ULN)	19/485 (4)	14/588 (2)	33/1073 (3)
Patients with any grade ≥3 ALT/AST elevation	68/485 (14)	77/588 (13)	145/1073 (14)
Resolved to grade ≤1	64/68 (94)	69/77 (90)	133/145 (92)
Rechallenged	47/68 (70)	63/77 (82)	110/145 (76)
Recurrence of grade ≥3 ALT/AST	15/47 (32)	19/63 (30)	34/110 (31)
Recurrence resolved to grade ≤1	14/15 (93)	18/19 (95)	32/34 (94)

ULN=upper limit of normal

*pts were rechallenged at the discretion of the treating physician

Methods: An integrated analysis of safety was conducted for 1073 patients across 9 studies: 538 with previously treated chronic lymphocytic leukemia (CLL), 105 with untreated CLL or small lymphocytic lymphoma (SLL), and 430 with other relapsed/refractory B-cell malignancies (indolent non-Hodgkin lymphomas, mantle cell lymphoma, diffuse large B-cell lymphoma, Hodgkin lymphoma, acute myeloid leukemia, and multiple myeloma) who received IDELA alone (doses=50 mg BID to 350 mg BID) or as part of a combination regimen (IDELA doses=100 or 150 mg BID). Clinical laboratory findings were evaluated for highest grade of ALT/AST elevation and summarized according to MedDRA

and Common Terminology Criteria for Adverse Events (CTCAE) liver-related laboratory tests and categories. Those with grade ≥3 ALT/AST elevation were evaluated for rate of resolution to grade ≤1 ALT/AST, and recurrence of the event at any time on study. Patients were grouped by use of IDELA alone or as part of a combination regimen.
Results: Among patients with any disease type or regimen (N=1073), the incidence of any grade ALT/AST elevation was 51%, and the incidence of grade ≥3 ALT/AST elevation was 14% (Table). Of patients with transaminase elevation, 73% (398/543) experienced grade 1 or grade 2 events. The incidences of grade ≥3 ALT/AST elevations, and outcomes for patients with grade ≥3 ALT/AST elevation (Table) were comparable whether IDELA was used alone or in combination. 92% of patients with grade ≥3 ALT/AST elevation were managed with dose interruption and achieved resolution to grade ≤1. The majority (69%) of patients with subsequent IDELA rechallenge experienced no recurrence of the event; of events that did recur, the vast majority (94%) resolved in the analysis time period.
Summary/Conclusions: These data support the management of ALT/AST elevation with IDELA using dose interruption at grade 3 with reintroduction after resolution at the discretion of the treating physician. Studies Included in this Analysis: 101-02: NCT00710528; 101-07: NCT01088048; 101-08: NCT01203930; 101-09: NCT01282424; 101-10: NCT01306643; 101-11: NCT01393106; 101-99 (extension): NCT01090414; GS-US-312-0116: NCT01539512; GS-US-312-0117 (extension): NCT01539291; GS-US-312-0119: NCT01659021

Chronic myeloid leukemia - Clinical 1

P227

DE-ESCALATION OF TYROSINE KINASE INHIBITOR THERAPY IS SAFE IN CHRONIC MYELOID LEUKAEMIA IN DURABLE MOLECULAR RESPONSE (≥ MR3 FOR ≥12 MONTHS): INITIAL RESULTS IN THE BRITISH DESTINY STUDY

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Background: Some chronic myeloid leukaemia (CML) patients with prolonged and deep molecular remissions on tyrosine kinase inhibitor (TKI) therapy can discontinue treatment. However, study of this has been restricted to patients in stable MR4 (BCR-ABL1<0.01%), and it is not known whether some patients in stable major molecular response (MR3; BCR-ABL<0.1%) but not MR4 can discontinue treatment. In addition, it is plausible that while some patients might not be able to completely discontinue TKI therapy, they may be able to maintain good molecular remissions on TKI doses that are less than standard, with concurrent improvement in any TKI-related adverse events; this has not been previously investigated.
Aims: The British DESTINY study is examining the safety and efficacy of initially de-escalating therapy to half the standard TKI dose (*i.e.* imatinib 200mg daily, nilotinib 200mg twice daily, or dasatinib 50mg daily) for 12 months, then stopping altogether.
Methods: Entrants must be in stable MR3 or better, on all PCR results (minimum of 3) in the preceding 12 months, and must have received TKI for at least 3 years, and not switched TKI (except that a single switch was permitted if solely due to intolerance). The primary endpoint is loss of MR3; monthly molecular monitoring is carried out centrally.
Results: During 16 months recruitment to April 2015, 174 patients (male 98; female 76) were recruited after giving informed consent by 20 sites. At entry, 125 (72%) were in MR4 and 49 (28%) in MR3 but not MR4; 148 were receiving imatinib, 16 on nilotinib and 10 on dasatinib. During 12 months of de-escalation in the first 100 patients, no deaths, disease progressions or losses of cytogenetic response have been seen, though 7 serious adverse events have occurred (6 Grade 3+), all unrelated to TKI. Thirteen patients reported 14 new events of musculoskeletal disorders since decreasing TKI therapy (all Grade 1), comprising pain, stiffness or cramps in the joints, legs, chest, neck and/or back (13) with one additional event of arthritis. Individual side effects (lethargy, diarrhoea, rash, nausea, periorbital oedema, hair thinning) all improve in the first 1-2 months of de-escalation but not significantly thereafter, though the FACT-BRM or EQ-5D Quality of Life data are already optimal at trial entry, suggesting that TKI side effects in entrants do not impact Quality of Life. During the de-escalation phase in the first 100 patients there have been 7 molecular relapses (defined as loss of MR3 on 2 consecutive samples), occurring during the second (1 case), third (2), seventh (1) and eighth (3) month of de-escalation. Four of these were in the 31 patients in MR3 but not MR4 at trial entry, giving a relapse rate of 12.9%; similarly 3 of 69 patients (4.3%) relapsed in the 'MR4 at entry' group. No relapses have occurred in the quartile with the shortest duration of prior TKI treatment (less than 4.8 years), while 3 of the 7 relapses have occurred in the quartile with the longest pre-entry TKI treatment (>10.2 years). All 7 patients have regained at least MR3 within 4 months of resumption of standard dose TKI. Overall, 26 (84%) 'MR3 but not MR4' and 64 (93%) 'MR4 at entry' patients have proceeded to the stopping phase.
Summary/Conclusions: In this first DESTINY trial report focussed on de-escalation, halving the standard TKI dose for 12 months in CML patients in at least MR3 appears safe, does not compromise disease control, and is associated with a rapid improvement in side effects. Mild musculoskeletal symptoms may occur in about 13% of patients.

P228

LONG-TERM EFFICACY AND SAFETY OF PONATINIB IN HEAVILY PRETREATED LEUKEMIA PATIENTS: 4-YEAR RESULTS FROM THE PIVOTAL PHASE 2 PACE TRIAL

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Background: The approved tyrosine kinase inhibitor (TKI) ponatinib is potently active against native and resistant BCR-ABL, including T315I.
Aims: The pivotal phase 2 PACE trial (NCT01207440) evaluated the efficacy and safety of ponatinib.
Methods: Patients with chronic myeloid leukemia (CML) or Philadelphia-positive acute lymphoblastic leukemia (Ph+ALL) refractory to dasatinib or nilotinib, or with T315I, were enrolled in PACE (starting dose 45 mg once daily). All patients gave informed consent. Dose reductions were recommended in October 2013 due to observed arterial occlusive events (AOEs). Efficacy and safety at 4 years, as well as by year for chronic phase (CP) CML patients are reported (data as of 3 August, 2015). Exposure-adjusted incidence rates of new AOEs are reported as the number of events/100 patient-years.
Results: Of 449 patients, 59% received ≥3 prior TKIs. At analysis, 30% (133/449) of patients (median follow-up 37.3, range 0.1–58.5 months) and 41% (110/270) of CP-CML pts (48.2, 0.1–58.5 months) remained on study. Primary reasons for discontinuation were disease progression (22.5% overall, 10.4% CP-CML) and adverse events (AEs) (16.0% overall, 18.5% CP-CML). Responses continued to deepen over time (Table 1**,** CP-CML patients) despite dose reductions. Among CP-CML patients, estimated 4-year rates for PFS, OS, and maintenance of major cytogenetic response (MCyR) and major molecular response (MMR) were 56%, 77%, 82% and 61%, respectively. For accelerated phase patients, the estimated 4-year OS was 51%; median OS for blast phase/Ph+ALL patients was 6.9 months (95% CI, 5.0-9.2). Common (in ≥30% of patients) treatment-emergent AEs were thrombocytopenia 44%, abdominal pain 43%, rash 42%, constipation 37%, headache 37%, dry skin 36%, fatigue and hypertension 30%. AOE rate/serious AOE rate was 23%/19%, including cardio- 13%/9%, cerebro- 9%/7%, and peripheral-vascular 9%/7%. Of patients with AOEs (n=104), 38% remained on study. Exposure-adjusted incidence rates of new AOEs fell after the first 2 years: 15.5 Year 1, 15.7 Year 2, 10.4 Year 3, and 9.6 Year 4. Nearly 2 years after recommended dose reductions, 87% (114/131) and 74% (70/95) of CP-CML pts were estimated to maintain MCyR and MMR, respectively, and 8% (6/75) of all dose-reduced pts without a prior AOE on trial had an AOE.

Table 1.

Percentage cumulative response rates with ponatinib in CP-CML pts (n=267, efficacy evaluable)

	MCyR	CCyR	MMR / MR4.5
Year 1	55	51	30 / 9
Year 2	58	53	36 / 16
Year 3	59	53	39 / 22
Year 4	59	54	39 / 23

Summary/Conclusions: After 4 years, heavily pretreated patients continue to show deep and lasting responses on ponatinib, and approximately 2 years post recommended dose reductions, maintenance of response is high, and the incidence of newly occurring AOEs has decreased.

P229

SUCCESSFUL TREATMENT FREE REMISSION IN CML AFTER 2 YEAR CONSOLIDATION WITH NILOTINIB OF AN MR4.5 RESPONSE LEVEL ACHIEVED ORIGINALLY WITH IMATINIB TREATMENT: FIRST REPORT FROM STAT2 TRIAL IN JAPAN

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Background: Nilotinib is a second-generation tyrosine kinase inhibitor (TKI) that exhibits significant efficacy as first- or second-line treatment in patients with chronic myeloid leukemia (CML). Superior rates of deeper molecular responses (DMR) were achieved with nilotinib *vs* imatinib in patients newly diagnosed with CML in chronic phase (CML-CP) in the ENESTnd trial. Recently, treatment free remission (TFR) is one of the goals in CML treatment. Indeed, prospective trials suggest that imatinib therapy may be safely and successfully discontinued in 40% of CML patients with DMR (Mahon et al, Lancet Oncol 2010, Ross et al, Blood 2013).
Aims: The purpose of this study is to evaluate the efficacy of two-year consolidation by nilotinib for successful TFR in patients with CML-CP who had achieved $MR^{4.5}$ with imatinib.
Methods: Patients with CML-CP who had achieved $MR^{4.5}$ (BCR-ABL1 ≤0.0032% on the International Scale [IS]) by real- time quantitative polymerase chain reaction (RQ-PCR) on imatinib were eligible. At study entry, patients treatment was switched from imatinib to nilotinib and nilotinib was taken twice daily (600mg/day) for 2 years as a consolidation phase. Patients who had sustained $MR^{4.5}$ for 2 years after initiation of the study were able to stop nilotinib in TFR phase. Thirty-three institutions in STAT study group participated. The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was written by all patients according to institutional guidelines. The study was approved by all institutional review boards and registered with ClinicalTrials.gov (number UMIN000005904). The primary objective of this multicenter phase II, single-treatment arm, open-label clinical study was to identify the $MR^{4.5}$ rate at 12 months after discontinuation of nilotinib treatment in patients who have sustained $MR^{4.5}$ for 2 years after initiation of the study. The molecular response was evaluated using IS-PCR (Molecular MD One-Step qRT-PCR BCR-ABL kit) at a central laboratory (BML, Inc., Kawagoe, Japan) upon study entry, every 3 months during the consolidation phase, every month the first year in TFR phase, every 2 months the second year in TFR phase, and every 3 months the third year in TFR phase thereafter. Molecular recurrence of CML was defined as confirmed loss of $MR^{4.5}$ (2 consecutive BCR-ABL1 IS >0.0032%) according to STIM criteria (Mahon Lancet Oncol 2010).
Results: Between July, 2011 and December, 2012, 96 Japanese patients were enrolled, and 75 patients who had sustained $MR^{4.5}$ during the consolidation phase were eligible to discontinue nilotinib. The median age was 56 years. The ratio of men to women was 60:36 and the risk groups according to Sokal Score were low: 56, intermediate: 20, high: 18, respectively. Sixteen patients were treated with interferon prior to imatinib therapy. All patients showed $MR^{4.5}$ at the time of entry into the study and the median time to DMR on imatinib therapy was 50.8 months. Median follow up duration in TFR phase of STAT2 trial was 16 month (range: 3 - 30 months). Among the 75 patients who entered the TFR phase, 52 patients (69.4%, 95%CI: [58.9% to 79.8%]) remained without molecular recurrence the first 12 months (Figure 1). TFR rate at 12M was 69.4%, which was superior to the previous reports, although we have to carefully validate that the population baseline characteristics are similar to those from other trials. Furthermore, the 23 patients with molecular recurrence who were treated with nilotinib again achieved $MR^{4.5}$ within 6 months.

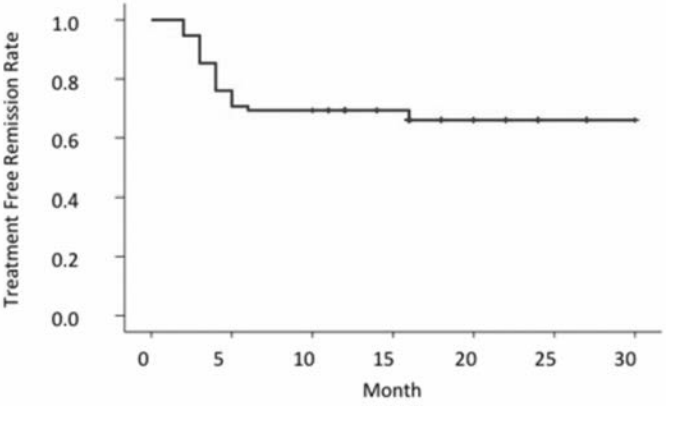

Figure 1.

Summary/Conclusions: 2-year consolidation by nilotinib is associated with successful TFR in CML patients with $MR^{4.5}$ on imatinib.

P230

IMPACT OF SECOND DECLINE RATE OF BCR-ABL1 TRANSCRIPT BETWEEN M3 AND M6 ON CLINICAL OUTCOME FOR CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH IMATINIB: PRACTICAL ASPECT FOR THE TRUE LIFE

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Background: Early molecular response (*BCR-ABL1* level at 3 months) has been associated with clinical outcome in chronic phase chronic myeloid leukemia (CML) patients treated with tyrosine kinase inhibitors (TKI). However, a single measurement of *BCR-ABL1* transcript levels does not seem enough to define failure and the European Leukemia Net (ELN) recommendations define first line-failure only at 6 months if *BCR-ABL1* transcript level is above 10%. The transcript rate decline from baseline has been demonstrated to be more predictive than a single *BCR-ABL1* level at 3 months however it cannot be used routinely because *ABL1*, as an internal gene control reliable for *BCR-ABL1* quantification is above 10%.
Aims: This study aimed to compare clinical outcome and molecular response of CML patients, depending on the percentage of decrease of *BCR-ABL1* transcript from M3 to M6 using *ABL1* as an internal control gene, used by most laboratories that provide results on the International Scale.
Methods: The first CML patient cohort (University Hospitals of Bordeaux n=116) was used to develop the model and the second one (University Hospitals of Lyon n=107) was taken to confirm and validate the predictive model. All patients were in chronic phase from September 2000 to October 2014, treated by frontline imatinib 400 mg, and for whom M3 and M6 molecular data were available. Event-free (EFS), failure-free (FFS), progression-free (PFS) and overall survivals (OS) defined according to ELN, were measured from M6 to the date of event or death. Survival differences estimated by Kaplan-Meier analysis were assessed using a log-rank test. The analysis of the area under the time-dependent ROC curve (AUC) using the inverse probability of censoring weighting (IPCW) approach was used to define the best cut-off for the percentage of decline. The cut-off was obtained by the closest "top left" method. Associations with MR^4 or $MR^{4.5}$ and multivariate analysis including Sokal, age and *BCR-ABL1* level ≤ or >10% at 3 months were assessed by Cox regression. Significant results obtained in the first cohort were validated in the second cohort.
Results: The percentage of *BCR-ABL1* decline from M3 to M6 was significantly linked to the EFS and the PFS (p=0.002). A common cut-off of 72% of decline at 24, 36 and 60 months after M6, predicted the better risk of having an event with respectively a sensibility of 0.69, 0.72, 0.56 and a specificity of 0.74, 0.80, 0.82 for each time point. Patients with a percentage of decrease below 72% have worse EFS as compared to those having a higher decrease (p<0.001 cohort 1; p=0.028 cohort 2; figure1). The estimated 5-years EFS are 60.75% and 51.76% for patients with less than 72% of decline *vs* 84.78% and 71.63% for patients with a higher decline, for cohort 1 and 2 respectively. The impact was maintained in the first cohort if we remove from all events treatment cessation for toxicity (p=0.017). However, only a trend is observed in the second cohort (p=0.073). The impact is independent of the Sokal score, age and *BCR-ABL1* level ≤ or >10% at 3 months.

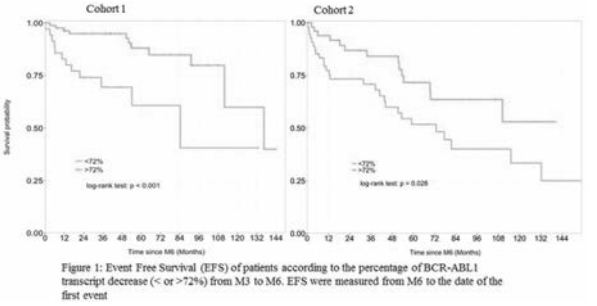

Figure 1: Event Free Survival (EFS) of patients according to the percentage of BCR-ABL1 transcript decrease (< or >72%) from M3 to M6. EFS were measured from M6 to the date of the first event

Figure 1.

Summary/Conclusions: Since the slope between diagnosis and 3 months cannot be reliable using *ABL1* as an internal gene control, the second decline rate of *BCR-ABL1* transcript between M3 and M6 could efficiently identify patients at higher risk of presenting an event and could help physicians to manage CML patients treated with imatinib or generic imatinib and to decide to switch to another TKI in the true life.

P231

EFFICACY AND SAFETY OF FIRST-LINE TREATMENT WITH NILOTINIB IN ELDERLY CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: In chronic phase (CP) chronic myeloid leukemia (CML) nilotinib has shown better efficacy compared to imatinib, but it has been associated to a higher incidence of arterial thrombotic events (ATEs). Considering that aging is linked to a higher cardiovascular risk, elderly patients treated with nilotinib may experience more ATEs, which ultimately may impact on their outcome.
Aims: To investigate the efficacy and safety, particularly the cardiovascular safety, of nilotinib in elderly chronic phase chronic myeloid leukemia patients.
Methods: We analyzed 345 patients with CP CML enrolled in clinical trials of the GIMEMA CML WP investigating nilotinib as first-line treatment. Patients were treated with: nilotinib 400 mg BID (n=73); rotation of nilotinib 400 mg BID / imatinib 400 mg OD (3-month periods for each drug)(n=123); nilotinib 300 mg BID (n=149). The median follow-up was 58 months. The median age was 53 years. We analyzed in detail the response rates, events and outcome of 89 patients ≥65 years of age; these patients were also compared to the 245 patients <65 years. Definitions: Major molecular response (MMR): BCR-ABL≤0.1% (IS), with >10.000 ABL copies; MR4: BCR-ABL≤0.01% (IS), with >10.000 ABL copies. Events: permanent discontinuation of nilotinib for adverse events, progression to accelerated/blast phase (AP/BP), or death for any reason. ATEs: peripheral arterial obstructive disease (PAOD), acute coronary syndrome, chronic ischemic heart disease, significant carotid stenosis and ischemic stroke.
Results: In elderly patients, the Sokal risk distribution was: low, 9%; intermediate, 69%; high, 22%. The median age was 71 years. Males were 49%. Molecular response rates were as follows: BCR-ABL/ABL <10% at 3 months, 89%; MMR at 12 months, 54%; MR4 at 12 months, 25%; cumulative incidence of MMR by 5 years, 93%; cumulative incidence of MR4 by 5 years, 75%. These response rates were similar to those observed in patients <65 years (all p >0.05). Overall, events leading to permanent discontinuation of nilotinib were observed in 38% of elderly patients, a proportion significantly higher than that of patients <65 years (23%; p=0.005). ATEs occurred in 15 (16.9%) elderly patients, and were the reason of permanent nilotinib discontinuation in 12 (13.4%) of them. As expected, the rate of ATEs in younger patients was significantly lower (5.8%; p=0.0035). Nine (10%) elderly patients died during the follow-up: a patient after progression to AP/BP (the only progression observed in patients ≥65 years), while the others died in CP for concomitant diseases / age-related conditions (2 patients had a previous ATE). The 6-year progression-free survival and overall survival in elderly patients were 85%.
Summary/Conclusions: Nilotinib as first-line treatment of newly diagnosed CP CML patients showed high efficacy in patients ≥65 years, with molecular response rates comparable to those observed in younger patients. However, a significantly higher proportion of elderly patients discontinued nilotinib, partially because of the occurrence of ATEs. Even though a relevant morbidity was associated to some ATEs, they did not significantly impacted on the long-term outcome of elderly patients.

P232

LATE RELAPSES, UP TO 45 MONTHS AFTER IMATINIB DISCONTINUATION: RESULTS FROM THE ISAV STUDY

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Background: As already demonstrated in different trials, it is possible to safely discontinue imatinib treatment in patients (pts) affected by Chronic Myeloid Leukemia (CML) who achieve a durable complete negativity by Q-RT-PCR. However a variable fraction of them become PCR positive but never loose Major Molecular Response (MMR). Here we report an update of the Imatinib Suspension And Validation (ISAV) study at 52 months (mts) of follow-up (FUP).
Aims: The ISAV study is aimed at validating the capability of digital PCR (dPCR) to predict relapses after imatinib discontinuation in CML pts with negative Q-RT-PCR results and to evaluate relapse rate, time of recurrence, survival and the impact of imatinib treatment on Quality of Life (QoL).
Methods: This study involves 15 sites worldwide. CML pts (Chronic or Accelerated Phase) under imatinib therapy since more than 2 years and in complete molecular remission (CMR) were eligible. Patients had to be in CMR for at least 18 mts, with a minimum of 3 Q-RT-PCR performed at their own sites. After discontinuation of imatinib therapy, Q-RT-PCR was performed monthly (mts 1-6), bimonthly for 36 mts and then every 6 months for additional 2 years, to assess the maintenance of the molecular remission. The loss of molecular remission was defined as two consecutive positive Q-RT-PCR tests with at least one BCR-ABL/ABL value above 0.1%. Patients losing molecular remission resumed imatinib treatment at the same dosage used before interruption. Patients' QoL during imatinib discontinuation/resumption was evaluated through the EORTC QLQ–C30 questionnaire.
Results: The ISAV study enrolled 112 pts with a median FUP time of 35.5 mts [95% CI: 33.5-36.0]. The cumulative probability of survival at 3 years is 94.7% [95% CI: 81.4-98.6]. dPCR results showed that 23.1% of pts were positive and 75.9% negative at the time of discontinuation, with a Negative Predictive Value ratio (dPCR/Q-RT-PCR) of 1.01 [95% CI: 1.22-0.99]. At 52 mts from imatinib discontinuation, 55 pts (50.9%, 95% CI: 41.1-60.7) of the 108 eligible ones relapsed and resumed imatinib; 70.9% of them relapsed in the first 9 mts. Of the 53 not-relapsed pts, 39 (36.1% of the total) regained Q-RT-PCR positivity without losing MMR. The median time to Q-RT-PCR positivity was 3.0 mts [95% CI: 2.1-3.1] in the relapsed pts and 4.8 mts [95% CI: 2.9-7.8] in pts who developed only PCR positivity. In this latter group 2 pts experienced late relapses, at 30.5 and 45.5 mts respectively (Fig. 1). No case of CML progression or resistance to imatinib was observed. No significant correlation between relapse and previous duration of imatinib treatment, use of interferon, time to CCyR, Sokal score or duration of CMR was identified, while an inverse relationship between pts age and risk of relapse was evident. The analysis of QoL evidenced a statistically significant improvement in the general well-being and symptoms scales at 1 month after imatinib discontinuation and in particular nausea, diarrhea and fatigue (p<0.01). An inverse and transient trend toward increased pain emerged at mts 1 and 3.

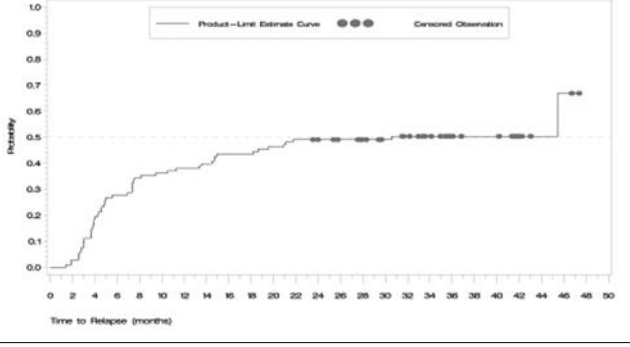

Figure 1. Time to Relapse (months) Kaplan-Meier curve evaluable patients

Summary/Conclusions: At 52 mts from the beginning of the study, with a median FUP of 35.5 mts, 50.9% of pts relapsed; the majority of relapses developed in the first 9 mts after imatinib discontinuation but late relapses also occurred, up to the 4th year. Therefore pts who discontinue imatinib should be monitored for a long period of time, especially if they showed positive PCR values after imatinib discontinuation.

P233

ASSESSMENT OF THERAPY RESPONSE IN CHRONIC MYELOID LEUKEMIA VIA 1 MONTH BCR-ABL1 TRANSCRIPT DECLINE

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Background: The majority of chronic myeloid leukemia (CML) patients achieve excellent therapy responses to tyrosine kinase inhibitors (TKIs). However, a proportion of patients still fail to respond to the assigned first-line TKI. Currently, treatment responses are usually assessed first time at the 3 months time-point. As the treatment responses to TKIs are in general very fast, we hypothesized that the prediction of overall treatment response could be done at an earlier time-point than 3 months especially with emerging of newer TKIs.
Aims: To evaluate the feasibility of using the reduction of *BCR-ABL1* levels after one month of TKI treatment to predict therapy response.
Methods: 52 first-line TKI treated chronic phase CML patients were included (imatinib n=26, dasatinib n=21, nilotinib n=5). *BCR-ABL1* transcript levels were measured at diagnosis, 1, 3, 6, 12, 18, 24, and 36 months in EUTOS standardized laboratories. The patients were divided into groups based on the decline of *BCR-ABL1* at 1 month compared to the diagnostic values. The patients with a fold change (FC) higher than 1 (*i.e.* no decrease in *BCR-ABL1* values during the first month [FC>1]) were named *non-responders*, while those lower than 1 (FC<1) were *responders*. The median FC for the *responders* was 0.31, and this group of patients was further divided into *intermediate responders* (FC 0.31-1) and *good responders* (FC<0.31)
Results: One fifth (n=11, 21% of total) of patients had no decrease in *BCR-ABL1* after 1 month and were identified as *non-responders*. All *non-responders* had used full doses of their assigned TKIs according to their drug accountability logs in the clinical trial records and therefore the lack of response was not due to non-compliance. Surprisingly, at the diagnosis, the *non-responders* had lower *BCR-ABL1* compared to the *responders* (31% *vs* 48%, p=0.0083). Further, the *non-responders* had significantly more often enlarged spleen (55% *vs* 15%; p<0.01), lower hemoglobin levels (median 105g/L, range 88-122g/L *vs* 128g/L, range 82-154g/L; p<0.001), and higher percentage of Ph+ CD34+CD38- cells in the bone marrow (91% *vs* 75%, p<0.05). These three factors might be a better reflection of the total tumor load in the body than the initial level of *BCR-ABL1*, and therefore explain the slower response to therapy. In addition, the eradication of leukemic stem cells was slower in the *non-responders* in comparison to the *responders*. The *non-responders* had a modest decrease in Ph+CD34+CD38- after 1 month (medians at dg 91.3% *vs* 56.2% at 1m; p<0.05), while the decrease was quite dramatic in *responders* (at dg 75.3% *vs* 11.1% at 1m; p<0.0001). Similarly for Ph+CD34+CD38+ progenitor cells, the *non-responders* still had high percentages at 1 month (median at dg 99% and at 1m 86.5%; p<0.05,) while they were rapidly decreased in the *responders* (at dg 94.7% and at 1m 32.0%, p<0.0001). Long-term follow-up of molecular responses showed that the major molecular response (MMR) rates were inferior in the *non-responders* when compared to the *responders* (at 12m 18% *vs* 64%, p<0.01; 18m 27% *vs* 75%, p<0.01, 24m 55% *vs* 87%, p<0.01). In the latest ELN recommendations the 3 month BCR-ABL<10% is a marker for good response. In our patients, we observed that *BCR-ABL1* was <10% at 3 months in all *good responder* cases (n=17), while 24% (5/21 cases) of the *intermediate group* and 36% (4/11 cases) of the *non-responder* group had *BCR-ABL1* >10% at 3 months (p<0.01).
Summary/Conclusions: The early 1 month treatment response analysis defines a biologically distinct patient subgroup with less satisfactory long-term response.

P234

DEVELOPMENT AND EVALUATION OF A MR1 – MR4.5 SECONDARY LYOPHILIZED CELL REFERENCE PANEL FOR BCR-ABL1 QUANTIFICATION ON THE INTERNATIONAL SCALE

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Background: Frequent molecular monitoring of chronic myeloid leukemia patients using high quality *BCR-ABL1* tests standardized to the International Scale (IS) is recommended for proper disease management, especially when

treatment cessation is considered. Currently, many laboratories standardize to the IS by exchanging patient samples with reference laboratories, an expensive and time-consuming process. A *BCR-ABL1* reference panel accredited by the World Health Organization (WHO) has been developed as a primary standard for IS calibration (MR^1-MR^4), but access to the material is restricted.
Aims: (1) To develop a secondary reference panel that is traceable to and faithfully replicates the WHO panel in manufacturing materials and methods, with an additional $MR^{4.5}$ level. (2) To assess the secondary panel in an international multi-center study.
Methods: As with the WHO panel, the secondary panel was manufactured by lyophilizing K-562 and HL-60 cell mixtures. Reverse-transcription droplet digital polymerase chain reaction (RT-ddPCR) was used to quality assess and calibrate the secondary panel to the WHO panel. The secondary panel was subsequently evaluated by 44 laboratories to determine the optimal sample input for each assay, and to assess assay performance including PCR efficiency, IS accuracy, sensitivity, linearity and precision.
Results: A secondary panel with *BCR-ABL1* levels of MR^1, MR^2, MR^3, MR^4 and $MR^{4.5}$ was successfully developed and IS calibrated to the WHO panel using RT-ddPCR against *ABL1*, *BCR* and *GUSB*. Quality control assessments indicated that the secondary panel had minimal residual moisture, excellent vial-to-vial homogeneity and >2.5 years real-time stability. The multi-center evaluation of the panel demonstrated compatibility with >44 *BCR-ABL1* assays of different configurations. Interestingly, in a standard curve experiment, we found that >40% of *BCR-ABL1* assays showed signs of inadequate optimization such as poor linearity and suboptimal PCR efficiency. When optimized sample inputs were used, 60% of tests achieved mean%BCR-ABL1 values within 2-fold of the panel's assigned values. Furthermore, 84% achieved good precision (≤0.25 log standard deviation) from MR^1 to MR^4, and 76% achieved a 100% detection rate at $MR^{4.5}$. Finally, 58% obtained IS conversion factors from the secondary panel equivalent to their current ones, most of which were obtained via sample exchange. Correlation analysis indicated that better PCR optimization was associated with better assay performance, and increased sample input improved detection rate and precision at $MR^{4.5}$. Different assay configurations were not found to be correlated with alterations in%BCR-ABL1 results.
Summary/Conclusions: We successfully developed the first secondary panel that is traceable to and fully replicates the WHO primary standards, with an additional $MR^{4.5}$ level. The panel was shown to be compatible with *BCR-ABL1* assays of different configurations in an international multi-center study. Importantly, once assay conditions were optimized, a high degree of precision and $MR^{4.5}$ sensitivity were achievable, yet IS accuracy was demonstrated in only 60% of cases. These findings indicate that there remains an unmet need for a simple and broadly available calibration mechanism, such as this secondary panel, to ensure IS accuracy is maintained in laboratories over time. The secondary panel and its derivatives can also be used as reference samples for assay analytical validation, optimization and quality assessment.

P235

TYROSINE KINASE INHIBITORS DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA: A RETROSPECTIVE ANALYSIS OF 208 ITALIAN PATIENTS

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Background: In the last 10 years different studies analyzed the outcome of patients (pts) with sustained complete molecular remission (CMR) who discontinued imatinib, reporting rates of treatment-free remission (TFR) ranging from 33 to 66% depending on the definition of molecular relapse. On these bases it is judged safe to discontinue treatment with tyrosine-kinase inhibitors (TKIs) in experimental contexts.
Aims: To evaluate TFR in the setting of clinical practice according to the Italian experience where most of the pts who discontinued TKIs were not included in prospective protocols.
Methods: We retrospectively analyzed the outcome of pts treated in 23 Divisions of Hematology in Italy, who discontinued TKIs in deep molecular response (MR). General and clinical information such as TKI at discontinuation, line of treatment, type of MR at discontinuation, reasons for discontinuation were collected. We estimated TFR with the Kaplan-Meier method. Prognostic factors for TFR were assessed by univariate Cox regression model analysis.
Results: We analyzed a total of 208 pts who discontinued TKIs from June 2003 to October 2015. Median age was 59 years (Interquartile Range, IQR, 46-72). 102 were male, 106 female; 52%, 35% and 13% were low, intermediate and high Sokal score respectively. 168 pts (81%) discontinued in first line; 38 pts in second line (63% for intolerance to prior TKIs) and 2 pts in third line. 153 pts (74%) were on treatment with imatinib (all frontline), 26% with either nilotinib or dasatinib. Median duration of treatment with the last TKI was 75.2 mos (IQR 50-114); median time to CMR (undetectable transcript or MR4/MR4.5/MR5) with the last TKI was 23.3 mos (IQR 11.1-45.2). Median duration of CMR was 23 mos (IQR 11-45) before stop. At 3 mos of last TKI 28% of pts were in MR3, 26% were in PCyR and/or had a transcript <10%, 45% were in CCyR and/or had a transcript <1%, and 1% had no response. 184 pts had a response defined according to molecular standardization: 8% were MR3, 30% were MR4, 36% were MR4.5, 26% were MR5. Reasons for discontinuation were: toxicity for 28% of pts, pregnancy for 10%, pt request for 56%, enrollment in ISAV protocol for 10 pts. After a median follow-up of 11 mos (IQR 1-149), estimated TFR was 71% (95%CI 64.6%>78.1%) (figure 1). 69 pts restarted treatment. Reasons for restarting were: loss of MR4 for 20% of pts, loss of MR3 for 55%, loss of CCyR for 12%, other reasons for 13%. Median time to restart treatment was 6 mos (IQR 4-11). We assessed age, sex, Sokal score, type of transcript, previous IFN therapy, duration of TKI therapy, response at 3 mos, time to CMR, CMR duration, line of therapy at stop, depth of MR, reasons for stop as potential prognostic factors for TFR, but no statistically significant association were found, with the exception of response MR5 at stop (MR5 *vs* MR4, HR: 0.43, 95%CI 0.21-0.87, p=0.02) and age [HR 0.62 (95%CI 0.42-0.93, p=0.02), *i.e.* a decreased risk in older *vs* younger pts (difference of age=26 years)]. Pts who had to restart therapy were treated with imatinib (57), nilotinib (10), dasatinib (2). All of them regained at least MR3. No pts progressed. All pts were alive at the last follow up with the exception of 7 who died for reasons unrelated to CML.

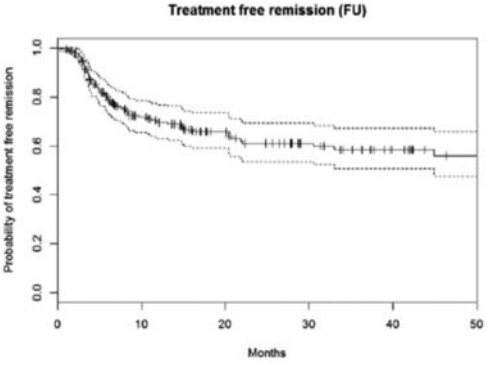

Figure 1. TFR of patients who discontinued TKIs in Italy.

Summary/Conclusions: Although our population is heterogeneous and worthy of specific sub-analysis, our experience confirms that discontinuation of imatinib, nilotinib and dasatinib is feasible and safe in the clinical practice. No progressions occurred, considering that 1/4 of our population had a follow-up longer than 12 years.

P236

MANAGING CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH HIGH-DOSE IMATINIB. THE ITALIAN EXPERIENCE

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Background: Background The European LeukemiaNet (ELN) has produced recommendations for monitoring and assessing the response to tyrosine kinase inhibitors (TKI) at particular time points. Response criteria hve been developed and revised by ELN over the years and have been validated in adults with chronic myeloid leukemia (CML). In 2014, de La Fuente et al (BJH,2014) proposed the modified ELN guidelines for the management of children and young adults with CML treated with imatinib (IM). To date, both the 2013 ELN guideline and the modified ELN recommendations have not been yet validated in children with CML.
Aims: The aims of this study were: a) to analyze treatment results in patients less than 18 years of age at initial diagnosis of chronic phase CP-CML, treated and monitored according to local guidelines designed to standardize disease management in pediatric patients; b) to evaluate the 2013 ELN recommendations and the modified ELN guidelines in this pediatric population.
Methods: CML patients in CP, diagnosed between March 2001 and March 2014 and followed for at least 18 months were included in this analysis. The local guidelines provided cytogenetics on bone marrow (BM) cells before and every 3 months during IM therapy as well as molecular analysis on peripheral blood (PB) monthly and on BM every 3 months. Allogeneic stem cell transplantation (SCT) was considered for children who failed IM. Quantitative RT-PCR (qPCR) was assessed according to the ELN recommendations. BCR-ABL1 kinase domain mutation analysis was planned in patients with IM failure. Cytogenetic and molecular monitoring at planned time points, as well as treatment response according to the 2013 ELN recommendations and modified ELN guidelines were evaluated in this cohort of patients managed according to the local guidelines.
Results: Fifty-one CP-CML patients, 19 females and 32 males, median age at diagnosis: 11.2 years (range: 3.1-17,9), were managed at 11 Italian pediatric centers according to the local guidelines. Assessments of response at 3, 6, 12 and 18 months were carried out in 92%, 91%, 91%, 95% and 92% of patients, respectively. Figure 1 shows the response rates according to the 2013 ELN and modified ELN guidelines (B). The response rates at 3, 6, and 12 months were higher according to the modified ELN guidelines compared to the 2013 ELN criteria, owing to the high number of not evaluable (NE) patients using the modified ELN guideline. IM was discontinued in 29.5% of patients, due to toxicity (8%) or failure (21.5%), that occurred after a median of 24 months. Patients who failed IM had an optimal (82%) or a suboptimal response (18%) at 3 months. BCR-ABL35INS and no mutations were found in 45.5% and 54.5% of patients who failed IM, respectively. Fifteen% of patients with optimal response at 3 months underwent a SCT, all before 2009. IM was successfully discontinued in 8% of patients showing a durable deep molecular response. All patients are alive at a median follow-up of 75 months (range: 21-151.3).

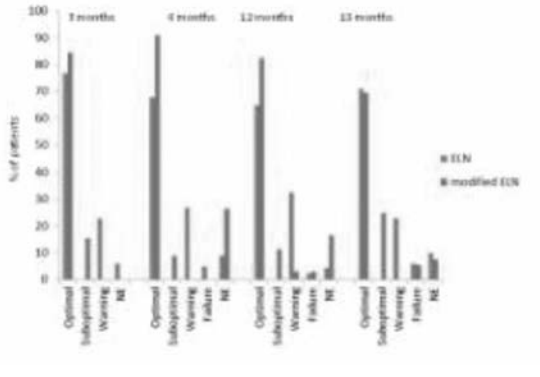

Figure 1.

Summary/Conclusions: Our experience confirms that collaborative studies have been useful to better manage children and adolescents with CML. Taking into account the rarity of the condition and the differences in the biology, clinical presentation, progression and response to treatment in adults compared tochildren, international collaborative studies and a shared clinical database for pediatric CML will be useful to address unanswered questions and issues.

LB237

RESULTS FROM ENESTOP: TREATMENT-FREE REMISSION (TFR) FOLLOWING SWITCH TO NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP)

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Background: Prior clinical studies of imatinib (IM) have demonstrated proof of concept for TFR; in the STIM trial, TFR (no loss of deep molecular response [MR]) was maintained 1 y after treatment discontinuation by ≈40% of pts with sustained deep MR on long-term frontline IM. In ENESTcmr, pts who did not achieve deep MR ($MR^{4.5}$; *BCR-ABL1* ≤0.0032% on the International Scale [IS]) with IM were more likely to achieve this response with a switch to NIL.
Aims: To present results from the single-arm phase 2 ENESTop study (NCT01698905) evaluating the ability to maintain TFR in pts who achieve and sustain $MR^{4.5}$ on NIL following a switch from IM to NIL treatment.
Methods: Eligible pts had CML-CP, received prior tyrosine kinase inhibitor (TKI) treatment for ≥3 y (including IM for >4 wk followed by NIL for ≥2 y), and achieved $MR^{4.5}$ with NIL. All pts provided informed consent. Upon enrollment, pts continued NIL for 1 y (consolidation phase; RQ-PCR assessments every 12 wk). Pts without confirmed loss of $MR^{4.5}$ (consecutive *BCR-ABL1*^{IS} >0.0032%) during the consolidation phase were eligible to stop NIL (TFR phase; RQ-PCR assessments every 4 wk for the first 48 wk). NIL was reinitiated (ReRx phase) upon confirmed loss of MR^4 (consecutive *BCR-ABL1*^{IS} >0.01%) or loss of MMR (*BCR-ABL1*^{IS} >0.1%). The primary endpoint was the proportion of pts with ongoing TFR 48 wk after stopping NIL. This analysis was conducted when all pts who entered the TFR phase had completed 48 wk of TFR, entered the ReRx phase, or discontinued from the study (data cutoff, 26 Nov 2015).
Results: A total of 163 pts were enrolled and entered the consolidation phase; 126 entered the TFR phase. Median age at baseline was 56 y; prior to entering the TFR phase, median durations of TKI and NIL treatments were 88 (range, 49-171) and 53 (range, 37-109) mo, respectively. Of 126 pts, 57.9% (95% CI, 48.8-66.7%) remained in TFR at wk 48 (primary endpoint); 53.2% (95% CI, 44.1-62.1%) of pts had $MR^{4.5}$ at wk 48. Of 53 pts who discontinued from the TFR phase, 51 entered the ReRx phase (loss of MMR, n=34; confirmed loss of MR^4, n=17), and 2 permanently discontinued from the study (1 with confirmed MR^4 loss did not enter ReRx phase due to investigator decision; 1 was found to have atypical transcripts). In the ReRx phase, 50 pts (98.0%) regained MMR or better by the data cutoff, and 48 (94.1%) and 47 (92.2%) regained MR^4 and $MR^{4.5}$, respectively; median times to regain MR^4 and $MR^{4.5}$ were 12.0 and 13.1 wk, respectively. No new safety signals were observed with NIL. Adverse event rates were 77.0% *vs* 73.8% in the consolidation *vs* the TFR phase (full analysis set). Events in the musculoskeletal pain grouping (defined as musculoskeletal pain, myalgia, pain in extremity, arthralgia, bone pain, and/or spinal pain) were reported in 42.1% (n=53) of pts (grade 3/4, 1.6%) in the first 48 wk of the TFR phase; most of these pts (37 of 53; 69.8%) had no history of musculoskeletal pain. In the TFR phase, the majority of events (46 of 54) in this grouping occurred during the first 24 wk; most events were still ongoing.
Summary/Conclusion: ENESTop provides the largest set of prospective TFR data to date in pts with CML-CP who achieved sustained deep MR after switching from IM to NIL. After stopping NIL, 58% of pts remained in TFR at 48 wk. The results of ENESTop, together with those from ENESTcmr showing that a higher proportion of pts switching to NIL reach deep MR, suggest that a higher proportion of pts switching to NIL will achieve TFR compared with pts continuing on IM.

Myelodysplastic syndromes - Biology

P237

IMPACT OF MUTATION-DERIVED ANTIGENS IN IMMUNE RECOGNITION OF HEMATOLOGICAL MALIGNANCIES, SPECIFICALLY MYELOID DYSPLASTIC SYNDROMES (MDS)

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Background: Mutation-derived neoepitopes have been suggested as a major component for immune recognition of solid tumors with a high mutational load, *e.g.* Melanoma and Non-Small-Cell Lung Cancer (NSCLC). Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms characterized by increasing bone marrow failure due to clonal expansion of immature dysplastic cells in the bone marrow. Compared to Melanoma and NSCLC, these dysplastic cells carry low numbers of point mutations, but high levels of frameshifts, indels, splice variations or epigenetic changes. All of which may contribute to the generation of tumor-specific neoepitopes.
Aims: Assess if immune recognition of tumor specific neoepitopes is evident in MDS. Such neoepitopes may serve a potential important novel targets for immune therapy in hematological malignancies.
Methods: We identified personalized tumor specific mutations based on whole exome sequencing of MDS blasts and corresponding germline DNA. HLA binding regions covering these mutation sites were characterized by prediction of peptide-HLA binding using a specially designed program (MuPeXI, the Mutant Peptide Extractor and Informer), including state-of-the-art prediction tools based on netMHC.
Results: We identified 60-250 peptides per patients, for 6 MDS patient. These neoepitopes covered point mutations (70%) and indels/frameshifts (30%). Peptide-MHC multimer reagents where generated for each of these peptides, matching the HLA expression of the patient. We screened bone-barrow-derived T cells for recognition of these large pMHC libraries using a novel multiplex technology based on DNA barcode labeled MHC multimers. This technique allows screening for T cell recognition of >1000 different pMHC complexes in a single sample.
Summary/Conclusions: Potential neoepitopes where identified from all 6 MDS patients included. Bone marrow-derived T cells where successfully analyzed both directly ex-vivo and following in-vitro expansion. Data analyses are currently ongoing. This study proves the feasibility for identification of neoepitopes in MDS and confirms the barcode-labeled MHC multimer complexes as a strong tool to discover the T cell recognition of mutation derived neoepitopes.

P238

VITAMIN C INCREASES VIRAL MIMICRY INDUCED BY 5-AZA-2'-DEOXYCYTIDINE

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Background: Cancer patients are often deficient in vitamin C, which is an essential cofactor for the ten-eleven translocation (TET) enzymes. Since TET enzymes initiate DNA demethylation by oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), restoring vitamin C to physiological levels might raise the levels of 5hmC in cancer cells. DNA methyltransferase inhibitors (DNMTi's), 5-aza-2'-deoxycytidine (5-aza-CdR) and 5-azacytidine (5-aza-CR), are drugs of choice in higher risk myelodysplastic syndromes (MDS) and have shown efficiency in the treatment of acute myeloid leukemia (AML). Our group recently showed that low dose DNMTi causes upregulation of endogenous retroviruses (ERVs) that induce the viral defense pathway to elicit an interferon response, which may play an important role in the efficiency of DNMTi's.
Aims: The aim of this study was to explore if vitamin C would enhance growth inhibition and apoptosis induced by DNMTi via; 1) improved activation of TET enzymes and the formation of 5hmC and 2) enhanced induction of ERVs and activation of the viral defense pathway.
Methods: We measured the blood levels of vitamin C in 24 patients with hematological malignancies using high performance liquid chromatography (HPLC). Next, we explored the synergy between physiological doses of vitamin C and low doses of 5-aza-CdR in five cell lines measuring growth inhibition by CellTiter-Glo luminescent cell viability assay and apoptosis by annexin V affinity assay using flow cytometry. Upregulation of ERVs and viral defense genes were determined by RT-qPCR, gene expression microarrays and RNA sequencing. Finally, to detect global 5hmC changes we performed dot blot analysis using a specific antibody against 5hmC as well as Illumina Infinium HM450 DNA methylation assay to detect global 5mC changes. Bisulfite sequencing and a Hydroxymethyl Collector kit were used for detection of 5mC and 5hmC at the long terminal repeat (LTR) regions of the ERVs.
Results: We first document that hematological cancer patients are indeed vitamin C deficient. By adding vitamin C at physiological concentrations to low doses of 5-aza-CdR *in vitro*, we showed a synergistic inhibition of cancer cell proliferation and enhanced apoptosis. These effects are associated with enhanced immune signals including increased expression of bi-directionally transcribed ERV transcripts, increased presence of cytosolic double stranded RNAs (dsRNA), and activation of an interferon induced cellular response to these transcripts. In addition, we show that the TET product, 5hmC, is enriched both globally and locally at the LTR regions, which initiates transcription of ERVs.
Summary/Conclusions: Our work shows a remarkable synergy between physiological doses of vitamin C and 5-Aza-CdR, and the combination enhances a "viral mimicry" response by upregulation of ERVs, including dsRNA production and induction of an innate immune response. This may be essential for the responses of patients to this class of drugs. Since patients with hematological cancers are markedly vitamin C deficient, the addition of vitamin C to treatment protocols may therefore be a straightforward way to increase the clinical efficacies of these drugs in MDS/AML patients. A.D.Ø., M.L., W.Z. and H.O. contributed equally to this work.

P239

GENETIC PREDISPOSITIONS TO SPORADIC MYELOID NEOPLASMS CAUSED BY GERMLINE DDX41 MUTATIONS IN ASIAN AND CAUCASIAN POPULATIONS.

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Background: Studies on germline mutations responsible for cancer predisposition provide an important clue to the pathobiology and potentially the management of relevant cancers. Within acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), only a handful of genes, including *RUNX1, CEBPA, GATA2, ETV6,* and *ANKRD26*, have been implicated in early onset familial cases, with germline mutations rarely seen in sporadic cases. Recently, we have identified novel germline variants of the *DDX41* gene that predispose to AML/MDS, using whole exome sequencing (WES) of familial cases with MDS/AML. In contrast to the variants of other MDS/AML-predisposing genes, germline *DDX41* variants are associated with a late onset disease typically diagnosed at the age of over 60, which could obscure their genetic predisposition, raising a possibility that germline *DDX41* variants might be found in apparently sporadic cases with MDS/AML.
Aims: This study was designed to assess the impact of germline DDX41 mutations on the AML/MDS development in Asian and Caucasian cohorts of sporadic AML/MDS.

Methods: We performed WES (N=55) or targeted-capture sequencing (N=1,035) in patients from the Asian cohort of sporadic AML/MDS (N=1,090), where the germline or somatic origin of the detected mutations was determined by using paired tumor and matched germline DNA if available. These results were compared to those from the Caucasian cohort of AML/MDS (N=1,034). The effect size of each variant for AML/MDS development was estimated by using the data from healthy populations in Asian countries (N=6,434) (BioBank Japan, Tohoku Medical Megabank Organization, and Exome Aggregation Consortium (ExAC) database) and in Caucasian countries (N=33,364)(ExAC database).
Results: In the Asian cohort of AML/MDS, germline *DDX41* variants were found in 42 patients (3.9%), of whom 15 (1.4%) also harbored a somatic *DDX41* mutation. In Caucasians, 8 patients (0.8%) had germline variants, and 2 (0.19%) for simultaneous germline and somatic mutations. Asian patients with germline variants had a median age of 56.6 (21-89), which was almost similar with that of the whole Asian cohort of AML/MDS {55 (6-93)}. In total, 8 and 1 recurrent germline variant alleles were observed in the Asian and Caucasian cohorts, respectively. Among them, the most predominant variants were p.A500fs (n=23; 55%) and p.Y259C (n=6; 14%) in the Asian cohort and p.D140fs (n=6; 75%) in Caucasians. Notably, no variants were shared by both populations, except for p.E256K, which are thought to be derived from a common ancestor. In contrast, a prominent hotspot mutation involving a highly conserved amino-acid within the helicase domain (p.R525H) was observed in both cohorts, accounting for 67% of second-hit *DDX41* somatic mutations. As a whole, these germline variants showed significant enrichment in AML/MDS cases compared to the respective control populations: odds ratio (OR)=18, {95% confidence interval (CI): 10-33} for Asian variants, and OR=10.8 (4.9-24) for Caucasian variants. As for individual variants, the enrichment in AML/MDS also showed various effect sizes: OR=19 (8.5-46) for p.A500fs, 8.9 (2.5-32) for p.Y259C in Asians, and 19.5 (7.1-54) for p.D140fs in Caucasians.
Summary/Conclusions: Germline *DDX41* variants are found in 0.8% to 3.9% in sporadic cases with MDS/AML depending of different ethnic populations. These germline *DDX41* alleles are present in the general population at low frequencies but account for the largest genetic predisposition to MDS/AML with high absolute risks. Second-hit mutations are common and show a prominent hotspot within the catalytic domain (p.R525). Given the high prevalence of *DDX41* germline variants and the late onset of associated MDS/AML, their detection may help clinicians to better manage patients with AML/MDS and to find other family members at risk, even when no family history is known.

P240

IDENTIFICATION OF ALTERED AND CRYPTIC-SPLICING IN SF3B1 MUTANT MYELODYSPLASTIC SYNDROMES AND ISOGENIC MODELS OF SF3B1-K700E MUTATION

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Background: In recent years, mutations in the SF3B1 gene have been discovered in a variety of cancers, especially myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL). The majority are a single A>G transition resulting in a K700E codon change within the HEAT repeats. Very recent data have demonstrated that this mutation drastically alters the branch point preference of the SF3B1 protein. The high prevalence of mutations in SF3B1 in MDS alludes to an important role in the disease. In terms of clinical utility, identifying therapies which target cells harbouring mutant SF3B1 would be of great benefit, as there is pressing need to improve available therapies for MDS, a disease of the elderly.
Aims: To identify the downstream effects of mutant SF3B1 in primary MDS and assess the sensitivity of SF3B1 mutant cells to standard and advanced molecular therapies; using isogenic pre-clinical models of SF3B1 mutant MDS and AML.
Methods: An RNA-Seq data set from $CD34^+$ cells from a panel of SF3B1 wild-type and mutant MDS together with normal donors was analysed. More specifically, the dSpliceType tool was used to identify differential and cryptic-splicing between both SF3B1 mutant and wildtype MDS samples or SF3B1 mutant MDS and normal donors. In addition to analysis of primary material, we used genome-editing via CRISPR/Cas9 nucleases to create isogenic models for *in vitro* comparisons. The generated SF3B1 mutant cell lines have been validated by mass spectrometry and profiled by numerous assays. In order to ascertain the effects of SF3B1 mutation on both splicing and the wider transcriptome, we are currently validating RNA-Seq from these isogenic clones.
Results: The analysis of primary MDS RNA-Seq characterised nearly 3,000 differentially-spliced transcripts. Perhaps unsurprisingly, there was an enrichment of genes with roles in RNA processing, suggesting a possible feedback or compensatory mechanism in SF3B1 mutant cells. Of the altered transcripts, only around 150 were related to alternative 3' splice site recognition, which is likely to be directly attributable to $SF3B1^{K700E}$. Interestingly, this set of genes was enriched for phosphoproteins, which may suggest an impact of $SF3B1^{K700E}$ on kinase cascades within the cell. The isogenic models were successfully created by introducing the A>G transition at the K700 codon in one allele of the endogenous SF3B1 gene. The expression of the mutant was confirmed both by sequencing and mass spectrometry. The mutant cells do show a slightly slower growth rate, but are generally similar to wildtype cells. Altered splicing in these clones was validated using qPCR, which targeted transcripts identified in the primary samples. Further studies comparing differentiation and clonogenic potential, together with response to a wide range of therapeutics, are starting to yield interesting differences.
Summary/Conclusions: This study has shown that SF3B1 mutation leads to an altered transcriptome in primary MDS samples and many atypical transcripts. Furthermore, it has identified a potential role for SF3B1 in modulating the splicing of phosphoproteins representing a potential source of clinical targets that could be employed in the clinic. In addition, the successful creation of an isogenic model that recapitulates the altered splicing seen in the primary disease provides an opportunity for the screening of therapeutics for MDS or secondary AML.

P241

IMPACT OF SOMATIC MUTATIONS ON OUTCOME IN PATIENTS WITH MDS AFTER STEM-CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative therapy for patients with myelodysplastic syndromes (MDS), but its benefit is often offset by high mortality and morbidity accompanied. During the past decade, substantial advances have been made in our understanding of the genetic basis of myelodysplastic syndromes (MDS), through which a full spectrum of major mutational targets was delineated and their impacts on disease phenotypes and clinical outcome have been intensively studied. However, few studies addressed the effects of these mutations and other genetic alterations in the setting of allo-HSCT in a large series.
Aims: We aimed to elucidate the effect of genetic alterations on the clinical outcome of allo-HSCT for MDS based on a large scale-genotyping.
We aimed to elucidate the effect of genetic alterations on the clinical outcome of allo-HSCT for MDS based on a large scale-genotyping.
Methods: We performed targeted deep sequencing of genomic DNA from 865 MDS patients who received unrelated bone marrow transplantation via Japan Marrow Donor Program between 1998 and 2013, through which somatic mutations in the major drivers in MDS (68 genes) and other copy number abnormalities(CNAs) and their association to clinical outcomes were interrogated.
Results: The median age at HSCT and observation period were 52 years (1-66) and 1121 days (48-5,747), respectively. At the diagnosis, 5%, 26%, 20%, and 8% of the patients were classified into low, intermediate-1, intermediate-2, and high risk categories based on the International Prognostic Scoring System, respectively (42% were not informative). At HSCT, 28%, 35%, 19%, 4%, and 15% were diagnosed with low-risk MDS, refractory anemia with excess blasts (RAEB), secondary acute myeloid leukemia (sAML), myelodysplastic/ myeloproliferative neoplasms (MDS/MPN), and MDS not specified, respectively. In total, 79% of the patients had either somatic mutations (75%) or CNAs (45%). *RUNX1*, *U2AF1*, *ASXL1*, and *TP53* were among the most frequently mutated genes and mutated in >10% of the cases. Complex karyotype, -7/del(7q), del(5q), and trisomy 8 were identified in 17.4, 16.3, 9.6, and 6.7%, respectively. In univariate analysis, mutations in *TP53* (hazard ratio (HR):2.76), *CBL* (2.15), *PHF6* (2.06), *NRAS* (1.85), *PTPN11* (1.83), *EZH2* (1.65), *FLT3* (1.63), and *RUNX1* (1.39) showed negative effect on overall survival. In multivariate analysis combining these mutations, CNAs and clinical parameters, mutations in *CBL* (HR:2.72), *KRAS* (2.60), *EZH2* (2.37), *PHF6* (2.29), and *TP53* (1.95) were independently associated with significantly poor survival, together with red blood cell transfusions before HSCT (2.99), multiple HSCTs (1.91), 3≥points of Hematopoietic Cell Transplantation-Comorbidity Index (1.72), male recipients (1.62), serological HLA mismatch (1.59), grade II-IV of acute graft *versus* host disease (GVHD) (1.50), and diagnosis of RAEB, sAML and MDS/MPN (HR:1.66, 1.76, and 2.08, respectively). We also analyzed the impact of mutations on relapse in cases who achieved complete response (CR) after HSCT (n=765; 88.4%). Multivariate analysis revealed mutations in *TP53* (HR:9.66), *ATRX* (7.12), *CUX1* (5.60), *ETV6* (5.15), *RUNX1* (2.71), del(11q) (11.7), loss of heterozygosity of 6p (5.03), disease status of relapse (7.89), refractory disease (2.69), CR (2.98), and partial response (2.69) at HSCT (compared to treatment naive cases), and diagnosis of sAML (2.85) significantly correlated with unfavorable outcomes, whereas *PTPN11* (0.04) mutations predicted a favorable prognosis.

Summary/Conclusions: This large study of MDS cases treated by unrelated HSCT demonstrated that somatic mutations of specific driver genes could predict clinical outcomes of MDS patients treated by HSCT and better guide therapy.

P242

17P DELETIONS AND TP53 MUTATIONS IN PATIENTS WITH MDS/AML AND COMPLEX ABERRANT KARYOTYPE

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Background: Alterations of the *TP53* gene, either by molecular mutations or by cytogenetic deletions of the 17p13 region (where *TP53* is localized), are frequent in patients (pts.) with myelodysplastic syndromes (MDS) with complex aberrant karyotypes (≥3 clonal cytogenetic aberrations (CA)). Both, molecular as well as cytogenetic alterations of *TP53* were shown to be prognostically adverse in pts. with MDS.
Aims: This study investigates the interrelations between mutational status (hetero- *vs* homozygous, cytogenetic deletion *vs* molecular mutation) of *TP53* with the extent of (cyto)genetic instability and with prognosis of MDS/AML pts. with complex aberrant karyotypes.
Methods: We included 123 pts. with MDS (N=91), sAML after MDS (N=15) and *de novo* AML (N=17) all with complex aberrant karyotypes (≥3 clonal cytogenetic aberrations). Pts. were comprehensively characterized by chromosomal banding analysis (CBA), interphase fluorescence *in situ* hybridization (FISH) including 17p13/*TP53*, multicolor FISH (mFISH), sequencing of *TP53* and molecular karyotyping (MK, SNP-array analysis). The extent of genetic imbalances was quantified by the number of CA, the number of fusions as shown by mFISH and the size of total genomic aberrations (TGA) measured by MK.
Results: We identified molecular *TP53* mutations in 62/123 (50.4%) pts. from our study cohort (all with complex aberrant karyotypes). FISH/CBA revealed deletions of *TP53* on 17p in 31/89 (34.8%) pts. Combining the different aberration types a total of 50/89 pts. (56.2%) showed molecular and/or cytogenetic changes of *TP53*. In detail, a total of 28/89 pts. (31.5%) showed any alteration (chromosomal or molecular) of one allele (corresponding to a heterozygous status of *TP53*), two *TP53* aberrations (corresponding to a homozygous state) were found in 22/89 pts. (24.7%). In 39/89 (43.8%) pts. we could not identify any *TP53* mutation. Pts. with a molecular *TP53* mutation showed a higher number of CA (median 7; range 1-41; *vs* 4; 3-23; p=0.014), a tendency for larger TGA size (median 362 Mb; range; 97-981; *vs* 163 Mb; 64-600 Mb; p=0.135) and for a higher number of fusions (median 2; range, 0-17; *vs* 3.5; 0-13; p=0.093) as compared to pts. without a molecular *TP53* mutation. Likewise the number of CA and fusions was significantly higher in pts. with a homozygous mutational status of *TP53* (CA: median 8; range 3-41; fusions: 5; 0-13) and those with a heterozygous *TP53* mutational status (CA: 8; 1-25; fusions: 3; 0-10) in comparison to pts. with no aberration of *TP53* (CA: 4; 3-23; p=0.001; fusions: 2; 0-17; p=0.012). Preliminary follow-up analysis suggests an additional impact of the number of CA on survival outcomes of complex aberrant pts. with molecular *TP53* mutations. Currently, we are collecting further survival data for additional validation of this observation.
Summary/Conclusions: We were able to confirm a high proportion of molecular and/or cytogenetic aberrations of *TP53* in 56.2% of pts. with cytogenetically complex aberrant MDS/AML. Complex aberrant pts. with evidence of molecular and/or cytogenetic *TP53* aberrations showed higher levels of genetic instability as measured by the number of CA, genetic fusions and TGA size as compared to those without any *TP53* aberration. The extent of cytogenetic complexity may be prognostically relevant for complex aberrant MDS/AML pts. with molecular *TP53* mutations. We aim to collect further data to prove this observation. Those 43.8% of MDS pts. with a complex aberrant karyotype but without any *TP53* aberration raise the question whether other genes of the *TP53* pathway might be involved causing a "TP53-like" phenotype.

P243

TP53 AND EZH2 MUTATIONS PREDICT POOR SURVIVAL IN PATIENTS TREATED WITH HYPOMETHYLATING AGENTS IN MDS

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Background: Azacitidine (AZA) is a hypomethylating drug, which obtains a 40 to 50% of overall responses in MDS. Blast<15%, normal karyotype and no previous treatment seem to be independent factors for better response. However, the potential value of the gene mutations has not been extensively explored.
Aims: To evaluate the impact of gene mutations detected by next generation sequencing (NGS) on treatment response to hypomethylating agents in MDS patients; and to define predictive factors of survival.
Methods: Eighty-four MDS patients were included. According to WHO classification the diagnosis was: RA (1%), RCMD (22%), RAEB1 (25%), RAEB2 (23%), MDS isolated del5q (2%), CMML (8%), MDS/AML 20-30% blasts (13%) and unclassified MDS (6%). Very low, low, intermediate, high and very high-risk categories according to IPSS-R were found in 2%, 18%, 24%, 27%, and 25% of patients, respectively. For NGS, *Ion Torrent Proton (Life Technologies, Palo Alto, CA)* system was used. We selected a panel of 34 genes related to myeloid pathologies. An optimal depth of coverage, median about 2000, was reached, contributing to a high sensitivity. Additionally, clinical characteristics were considered: sex, age, WHO classification, cytogenetic risk, IPSS-R, response to AZA (IWG response criteria) and outcome.
Results: The median age was 69 years (range: 49-99). The median follow-up time was 17 months (range: 1-93). Mean numbers of cycles received per patient were 9 (range 1-55). Overall response to AZA was 42%, included complete remission 17%, partial remission 5% and hematologic improvement 20%. A total of 181 somatic mutations were found in 78 out of 84 patients (93%). The more frequent mutations involved *TET2* (25%), *TP53* (20%), *RUNX1* (18%), *DNMT3A* (18%), *EZH2* (14%), *ASXL1* (13%), *U2AF1* (12%), *SF3B1* (11%), *ZRSR2* (10%), and the remainder had frequencies of <10%. Considering clinical characteristics and mutational status in the univariate analysis of the impact on response, the number of AZA cycles (*p=0.003*), and the number of mutations per patient (*p=0.032*) were associated with overall response. Taking into account genes related to DNA methylation pathway (*TET2, DNMT3A, IDH1* and *IDH2*), the presence of at least 1 mutation in any of these genes was associated with complete remission (*p=0.031*). In the multivariate analysis, these parameters remained significant for overall response after adjusting by IPSS-R and age: number of AZA cycles (*p=0.006*; OR=1.2; 95%CI 1.1-1.4), number of mutations per patient (*p=0.042*; OR= 0.6; 95%CI 0.3-0.9), and mutations in methylation pathway genes (*p=0.024*; OR=4.4; 95%CI 1.2-15.7). In univariate analysis of survival, platelet counts, cytogenetic risk, IPSS-R, WHO classification, response to AZA and *TP53*, *RUNX1*, and *EZH2* mutational status were associated with overall survival. By contrast, in multivariate analysis, considering these clinical and molecular variables significant, only *TP53* and *EZH2* mutations remained as independent adverse prognostic factors for overall survival (HR=3.9; 95% CI: 1.9-7.8; *p*<0.001 for *TP53*, and HR=2.5; 95% CI: 1.2-5.1; *p*=0.012 for *EZH2*), regardless of the response to AZA (figure 1).

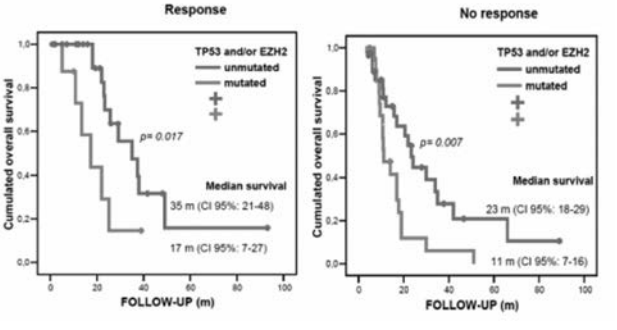

Figure 1.

Summary/Conclusions: In our series of patients treated with AZA, we found than more number of AZA cycles, less number of mutations per patient and the presence of at least 1 mutation in genes related to DNA methylation (*TET2, DNMT3A, IDH1, and IDH2*) were predictive factors for AZA response. However, a lower overall survival was associated with *TP53* and *EZH2* mutations regardless of this response.

P244

AZACYTIDINE RESTORES THE ABNORMAL STAT SIGNALING BIOSIGNATURE OF FOXP3+ T REGULATORY CELLS (TREGS) IN RESPONDING MDS PATIENTS

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Background: Tregs are expanded in late-stage MDS and may compromise tumor immunity. Signal transducer and activator of transcription (STAT) proteins regulate Treg differentiation, while aberrant immune signaling via STATs drives disease pathobiology in various malignancies. We have previously shown that, compared to RAEB-2 and AML/MDS patients, the CMML-2 ones display a distinct STAT signaling profile in Tregs which is also differentially modulated by azacytidine (AZA).
Aims: We have now updated our work by including more patients and normal donors and an extended panel of signaling nodes.
Methods: Peripheral blood mononuclear cells of 41 patients were obtained before, 15 days after AZA initiation and after the 6th cycle. According to WHO, 20 (48,7%) patients had RAEB-2, 12 (29,2%), 12 (29,2%) CMML-2, 7 (17%) AML/MDS and 2 (4,8%) RCMD. Based on the IWG criteria patients were divided into responders (CR and hematologic improvement, n=17) and non-responders (stable disease and failure, n=24). We applied phospho-specific flow cytometry to measure either basal or potentiated phospho-STAT1, STAT3 and STAT5 levels with simultaneous staining for FOXP3. The following potentiated, *i.e.* target/stimuli, signaling nodes (SN) were studied: IFNα/pSTAT1, IFNα/pSTAT5, IL-6/pSTAT1, IL-6/pSTAT3 and IL-2/pSTAT5. Clustering of SNs was performed with hierarchical cluster analysis and was correlated with response, disease subtype, transfusion burden, IPSS-R and cytogenetics by using χ^2 or Fisher Exact tests.
Results: Treg levels decreased in responders after cycle 6 (p=0.04), whereas non-responders showed a nonsignificant increase (p=0.17). Also, non-responders upregulated significantly the transcription factor Helios at 6 months (p=0.02), whereas responders retained stable levels. Unsupervised clustering of pretreatment SNs demonstrated that patients bear a different Treg STAT biosignature from age-matched normal donors (p=0.003), characterized by blunted response in cytokine stimulation and higher basal levels of STAT3. Normal donors still formed a separate cluster on day 15 (p=0.03), but clustering after cycle 6 separated two main clusters (figure 1). Cluster 1 encompassed all normal donors and was enriched in responders (p=0.04), whereas non-responders segregated in cluster 2 which was characterized by weak potentiated responses. Clustering of the fold-change of SNs in Tregs after the 6th cycle (MFI *cycle 6 – MFI pretreatment/MFI pretreatment*) discriminated responders from non-responders in 2 clusters (p=0.03). The first group upregulated both basal and potentiated nodes, while the second downregulated most SNs and upregulated pSTAT1 responses in IL-6 and IFNα.

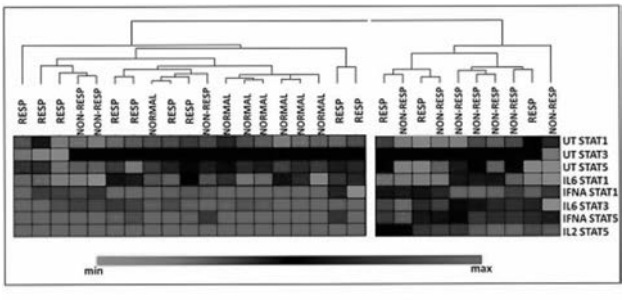

Figure 1. Hierarchical clustering of STAT signaling profiles in Tregs after 6 cycles of azacytidine. Resp=CR+HI; Non-Resp=Failure+stable disease

Figure 1.

Summary/Conclusions: Collectively, we demonstrate for the first time that the STAT signaling biosignature of Tregs in late-stage MDS patients is pathological, characterized by blunted response to cytokines. AZA induced a disparate effect in Tregs which was linked with clinical response; Responders decreased Treg numbers and normalized their STAT biosignature by restoring Treg responsiveness to cytokines, while non-responders tend to increase Tregs, displayed a marked increase in Helios expression and retained the abnormal STAT biosignature as they upregulated only pSTAT1 responses. Our results are suggestive of a clinically relevant immunomodulatory effect of AZA by modifying the cytokine/STAT signaling axis in Tregs. Further elucidation of the pathobiological significance of alterations in individual nodes will provide novel insights in the mechanisms of action and resistance to AZA.

P245

A CULTURE MODEL MIMICKING THE BM-NICHE ALLOWS FOR STUDYING THE DYSREGULATED ERYTHROPOIESIS OF SF3B1 MUTATED MYELODYSPLASTIC SYNDROME WITH RINGED SIDEROBLASTS

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Background: The bone marrow (BM) constitutes a complex microenvironment that maintains and regulates hematopoiesis, and promotes differentiation. Little is known about the microenvironment of myelodysplastic syndrome with ringed sideroblasts (MDS-RS), of which >80% of patients harbor splice factor *SF3B1* mutations. We recently showed that MDS-RS erythropoiesis fails in particular during its terminal phase, a process that normally requires the formation of erythroid islands (Conte *et al*, BrJH 2015). Currently available culture models remain inefficacious to simultaneously support the formation of erythroid islands, enucleation of erythroblasts, and ring sideroblast development in MDS-RS, hence we lack adequate systems to model and target terminal erythropoiesis in MDS.
Aims: Our aim was to develop an *in vitro* culture model to study normal and MDS-RS hematopoiesis under conditions simulating the human *in vivo* BM microenvironment, and for the first time to be able to generate an MDS-RS phenotype *in vitro*.
Methods: Mononuclear cells including autologous stromal cells (MNC+) and CD34+ cells were isolated from 8 MDS-RS patients and 9 healthy individuals, and seeded into scaffolds made out of collagen-coated polyurethane (3D culture), or into previously established suspension cultures. The *in vitro* conditions were optimized for erythropoiesis by testing different cytokines and media. Cells were extracted weekly to evaluate proliferation, cellular composition and enucleation by flow cytometry, and ringed sideroblast quantification using morphological assessments by Perl´s Prussian blue staining. Self-renewal potential of cultured cells was assessed by long-term culture initiating cell assays (LTC-ICs) and allelic burden of *SF3B1* mutations with pyrosequencing. Scaffolds were sectioned for morphological assessment.
Results: The optimal medium composition included an initial phase aiming for erythroid progenitor enrichment followed by a high concentration of iron loaded human transferrin and EPO to induce erythroid maturation. Both MNC+ cultures and CD34+ 3D cultures maintained proliferation for up to 4 weeks. Interestingly, self-renewal potential, as assessed by LTC-ICs, was also maintained following this culture period. By contrast, conventional CD34+ cultures lost proliferative capacity after 2 weeks and did not give rise to LTC-ICs. The allelic burden of *SF3B1* was maintained in the 3D cultures (with MNC+ and CD34+ cells), but decreased within 4 weeks in suspension cultures. The MNC+ cultures were able to retain different lineages of hematopoiesis (including myeloid, lymphoid and erythroid) although primarily facilitating erythroid cells, while the CD34+ cultures (3D and suspension) only generated cells of the erythroid lineage. We identified enucleated erythroid cells from all cultures except for CD34+ suspension cultures. Importantly, erythroid islands carrying central macrophages were formed during week 3 and 4 in the MNC+ 3D culture. Morphologic quantification confirmed that 9-50% of the erythroblasts extracted at the end of the MNC+ cultures (3D and suspension) and CD34+ in 3D cultures were ringed sideroblasts.
Summary/Conclusions: We report for the first time that a 3D culture model seeded with BM MNC including autologous stromal cells is able to mimic hematopoiesis of healthy and MDS-RS BM *in vitro*, and provide support for formation of terminal erythropoiesis both from normal and *SF3B1* mutated bone marrow cells. This novel *in vitro* system should enable studies of MDS associated erythroid maturation defects *in vitro*.

P246

THE INCIDENCE AND CLINICAL IMPLICATIONS OF CHROMOTHRIPSIS IN BONE MARROW CELLS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Background: The increasing resolution of modern molecular genetics technologies has recently enabled the discovery of a novel phenomenon of genome instability that plays a role in cancer genesis and its progression. This phenomenon, termed chromothripsis, affects single chromosomes and/or chromosome regions that are shattered into many small pieces in a single catastrophic event. Chromothripsis was first described in 2011 by Stephens and colleagues. Since then, the occurrence of chromothripsis was detected in a wide range of tumor entities including hematologic malignancies. According to the published data it can be seen in approximately 2%–3% of all human cancers and is strongly associated with complex karyotypes. However, the real frequency of chromothripsis is likely to be much higher than was originally suspected.
Aims: The aim of the study was to assess the incidence and clinical significance of chromothripsis in bone marrow cells of the previously untreated patients with myelodysplastic syndromes (MDS) and complex chromosomal aberrations (CCAs).

Methods: A detailed retrospective genome-wide analysis of fixed bone-marrow cells from adults with CCAs (³3 aberrations) identified with conventional G-banding at the diagnosis of MDS was performed. The CCAs were studied through FISH with Vysis DNA probes (Abbott, Des Plaines, IL) and mFISH/mBAND methods using the 24*X*Cyte and the *X*Cyte color kits (MetaSystems, Altlussheim, Germany). Genomic imbalances were identified with CytoChip Cancer SNP 180K (BlueGnome, Cambridge, UK) or with Illumina Human CytoSNP-12 arrays (Illumina, San Diego, CA).
Results: We examined a large group of 222 patients with MDS and CCAs (104 females, 118 males; median age 68 years). The chromothripsis signature was revealed in 66.7% cases, both in the main clones as well as in one or more subclones. On the cytogenetic level, chromothripsis was manifested by multiple deletions, insertions, ring chromosomes, amplification of individual genes or chromosomal regions, and/or by the formation of chaotically reassembled marker chromosomes. The fragmentation or shattering of chromosomes into many pieces was observed as well. The most frequently shattered were chromosomes 5, 7, 17, and 12. In cases with signs of chromothripsis a higher frequency of the loss of heterozygosity (LOH) of 17p and/or the copy number neutral LOH (cnLOH) of 17p was observed (p=0.05). It is usually associated with homozygous mutations of *TP53* gene. Patients with chromothripsis had significantly worse overall survival (median OS, 3 months).
Summary/Conclusions: The results of this study confirmed chromothripsis as a frequent finding in patients with MDS and CCAs. This phenomenon was associated with a higher frequency of LOH/cnLOH 17p, very poor prognosis and rapid disease progression. The poor outcome could be explained by the fact that the function of many important genes becomes affected. The early identification of chromothripsis in patients with MDS can help with prognostication. Moreover, a better understanding of the mechanistic basis of chromothripsis could lead to the development of new treatment strategies based on drugs targeting genes present in amplified or deleted regions, and/or the DNA damage response pathways.
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P247

MODEST EPIGENETIC EFFECT OF AZACITIDINE IN GENOME-WIDE MAPPING OF DNA METHYLATION, H3K9ME3, H3K18AC AND GENE EXPRESSION IN MDS PROGENITORS

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Background: Azacitidine (Aza) is first-line treatment for patients with higher risk MDS. Since Aza is an inhibitor of DNMT1, demethylation of tumor suppressor genes has been proposed as the principal mechanism of action. Demethylation of specific genes has however been difficult to link to increased gene expression and a more comprehensive mapping of epigenetic and transcriptional effects of Aza on stem / early progenitor cells is warranted.
Aims: To comprehensively map epigenetic and transcriptional effects of Aza in primary MDS progenitors.
Methods: We selected 11 consecutive patients with higher-risk MDS (n=6), AML with 21-29% marrow blasts and multilinear dysplasia (n=4) and CMML type II (n=1). The patients were well characterized with regard to clinical data and mutations in recurrently mutated genes. CD34+ cells from these patients were cultured with or without Aza(1µM). Cells were harvested after 24h and 48h; treated and untreated cells were compared with regards to genome-wide DNA methylation using the Illumina 450k array (n=9), transcription levels using RNA sequencing (n=4), enrichment of the repressive chromatin mark H3K9me3 (n=6) and the activating mark H3K18ac (n=2) by ChIP seq.
Results: Cell growth and viability over 48 h showed little variation between untreated and treated cells. Gene expression increased over time in the control culture, while methylation changes patterns remained unchanged over time. We observed a strong correlation between gene expression, DNA promotor methylation (p=1.57e−22) and promotor H3K18ac (p=2.72e−61) in the untreated samples. Exposure to Aza for 24 h resulted in global DNA demethylation, with 18 657 and 908 promotors being demethylated and hypermethylated, respectively. The absolute change in methylation was however very modest with a median reduction in beta-value of 0.015 per gene. Similarly, the effects of Aza on the histone modifications H3K9me3 and H3K18ac were modest with 12 718 and 13 091 differentially enriched genes, respectively, whereof 52% and 44% being positively enriched in the Aza samples,; median fold change were 1.03 and 0.94, respectively. By contrast, the effect of Aza on gene expression was substantial with 8596 up- and 1804 downregulated; median fold change per gene was 1.61. Figure 1 illustrates global effects on gene expression and epigenetic changes with all genes plotted as bars on the X-axis sorted from most decrease to most increase. The relatively large changes observed in gene expression could not be explained by corresponding changes in promotor DNA methylation (p=1.88e−01), H3K9me3 (p=6.09e−01) or promotor H3K18ac (p=5.02e−01). GO analysis of the upregulated genes display enrichment in pathways involved in gene expression, protein synthesis and protein localization. Principal component analyses of epigenetic data displayed clustering based on patient identity while Aza-treatment had minor impact on clustering pattern.

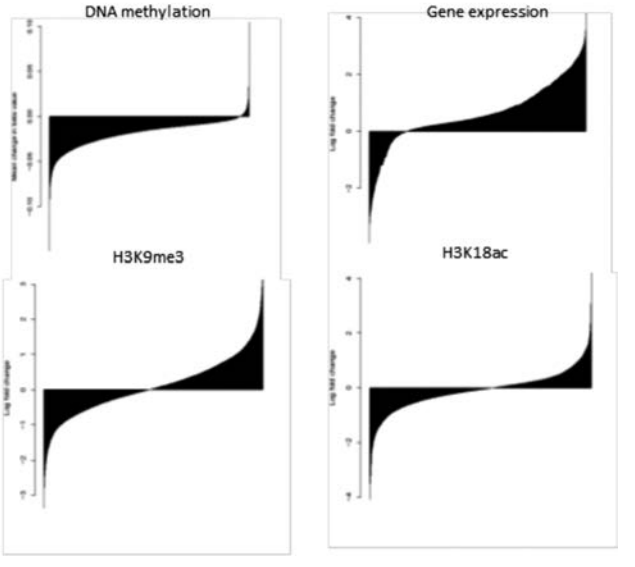

Figure 1.

Summary/Conclusions: *in vitro* Aza-treatment induces modest epigenetic changes in primary MDS progenitors. There are more pronounced effects on gene expression but since there is no correlation with changes in DNA methylation, H3K9me3 or H3K18ac, these effects must be a result of other mechanisms such as modulation of transcription factors. The limited epigenetic effect observed in the progenitor cells might be an explanation why Aza fails to achieve long-term remissions and the short-term remissions may be due to epigenetic effects on more differentiated cells.

Myelodysplastic syndromes - Clinical 1

P248

RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTER STUDY EVALUATING EPOETIN ALFA *VERSUS* PLACEBO IN ANEMIC PATIENTS WITH IPSS LOW- INT1 RISK MDS

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Background: Erythropoiesis stimulating agents (ESA) are first choice for treating anemia in lower-risk MDS patients (pts), but no ESA is approved for this indication. This international, Phase 3, randomized, double-blind, placebo (PBO)-controlled, multicenter study assessed the efficacy and safety of epoetin-alfa (epoetin-a; Eprex®) in pts with IPSS Low- or Int-1 risk MDS suffering from anemia.
Aims: To evaluate whether epoetin-a improves anemia (by erythroid response [ER]; IWG 2006) *vs* PBO over 24 weeks (w) of treatment. Duration of ER,% of pts maintaining ER up to w48, time to 1st RBC transfusion, transfusion-free interval, # of RBC transfusions, changes in PROs/ QoL and safety were also assessed.
Methods: Pts with *de novo* IPSS Low or Int-1 MDS with Hb ≤10.0 g/dl, transfusions ≤4 RBC units within 8-w before randomization, baseline serum EPO level <500 mU/mL, adequate iron and vitamin stores were eligible. The study included 3 phases: 3-w screening, 24-w double-blind treatment, and 24-w double-blind treatment extension in responders. Pts were randomized 2:1 to receive either epoetin-a 450 IU/kg (max tot. starting dose 40k IU) or matching PBO, once weekly sc, stratified according to transfusion need (yes/no) & serum EPO level (<200 mU/mL *vs* ≥200 mU/mL). Dose adjustments driven by ER and weekly Hb levels, included increase up to 1050 IU/kg (max tot. dose 80k IU) and dose reduction/withhold according to weekly Hb regardless of ER status. Primary endpoint was ER during first 24-w based on IWG 2006 criteria by investigators and an independent Response Review Committee (RRC). An *ad hoc* analysis was conducted in subjects who responded at any time of the study, regardless the IWG 2006 criteria, to allow assessment following dose adjustments and a longer period of observation.
Results: 130 pts were randomized, 85 to epoetin-a group; 54.6% were male; median age was 75 years. Baseline characteristics were comparable between groups. 70 (82.4%) epoetin-a pts completed the 24-w treatment; 39 (45.9%) epoetin-a pts entered the extension phase *vs* 1 (2.2%) PBO pt. 31.8% of epoetin-a pts achieved primary endpoint *vs* 4.4% of PBO pts, $p<0.001$. In the *ad hoc* analysis, 45.9% of epoetin-a pts *vs* 4.4% of PBO pts achieved ER, $p<0.001$. Median ER duration for epoetin-a pts was 197 days. Within 8-w prior to baseline, 51.8% of epoetin-a pts needed transfusions; this decreased to 24.7% by w-24. Transfusion need remained unchanged in the PBO pts (48.9% - 54.1%). Time to 1st transfusion was longer in the epoetin-a group ($p=0.046$). In a post-hoc analysis improvement in QoL scores was observed at w-24 in the epoetin-a arm in responders *vs* non responders (FACT-An p=0.025, EQ-5D p=0.007, EQ VAS p=0.037). Safety data were comparable up to w24; 77.6% of pts on epoetin-a reported ≥1 treatment-emergent adverse event (TEAE) *vs* 88.9% with PBO. Drug discontinuation due to AE was 10.6% in epoetin-a *vs* 13.3% in PBO. 4 pts in the epoetin-a arm (4.7%) and none in PBO reported ≥1 TVE. There were 4 TEAEs with fatal outcome in the epoetin-a arm *vs* 1 in PBO arm; none was reported to be related to the study drug. During the study progression to AML was similar between groups (3.5% in epoetin-a; 4.4% in PBO).
Summary/Conclusions: Epoetin-a significantly improved anemia by increasing Hb and reducing transfusion requirement in patients with Low or Int-1 MDS. Improved QoL was observed in responders. No new safety signals were detected in this study. These results confirm experience from clinical practice.

P249

OVERALL SURVIVAL (OS) AND SUBGROUP RESULTS FROM A RANDOMIZED PHASE 2 STUDY OF SGI-110 (GUADECITABINE) IN PREVIOUSLY TREATED MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Guadecitabine (formerly SGI-110) is a next generation small volume subcutaneous (SC) hypomethylating agent (HMA) with a differentiated PK profile and clinical activity reported in previously treated MDS and AML patients (pts) in Phase 1 (Issa et al, Lancet Oncology 2015). Responses were also seen in a Phase 2 study in both newly diagnosed and previously treated MDS and chronic myelomonocytic leukemia (CMML) pts randomized to guadecitabine SC 60 mg or 90 mg/m^2 QDx5 with no significant differences between the 2 dose groups (Garcia-Manero et al, ASH 2014).
Aims: To report the clinical activity, safety, long term OS, and subgroup analyses results for guadecitabine in previously treated MDS and CMML pts.
Methods: Intermediate (Int), or High Risk (HR) MDS by IPSS score, and CMML pts who were previously treated with azacitidine (AZA) and/or decitabine (DAC) were randomized to either 60 mg/m^2 or 90 mg/m^2 QDx5 every 28 days in this Phase 2 study. Clinical responses were assessed using the IWG 2006 criteria. Safety was assessed using CTCAE grades.
Results: 53 relapsed/refractory (r/r) MDS or CMML pts were randomized to either guadecitabine 60 mg/m^2 (26 pts) or 90 mg/m^2 (27 pts). There were no major differences between the 2 dose groups so data from both groups were combined for this report. Median age was 72 y (range 52-88y); ECOG PS was 0/1/2 in 21, 58, and 21% of patients. Patients were previously treated with AZA (77%) and/or DAC (32%) with 75% of patients receiving at least 6 months of prior treatment; 19% of patients had CMML, 47% had HR MDS; and 66% were transfusion-dependent. The median baseline bone marrow (BM) blasts were 8% (range 0-19%), baseline median Hb was 9.3 g/dL (range 7.1-13.5), median neutrophils were 0.81 x10^9/L (range 0.1-15.6), and median platelets count was 37 x10^9/L (range 7-328). Patients received a median number of 5 cycles of guadecitabine (range 1-29). Complete Response (CR) was achieved in 2 pts (4%), marrow CR in 15 pts (28%), and Hematological Improvement (HI) in 11 pts (21%). Median OS was 11.7 months (m). Patients who achieved a clinical response (CR, mCR, or HI) had longer OS than those with no response (p=.006). Median OS was 19.8 m for CMML; 12.3 m for Int MDS; and 10.1 m for HR MDS. Baseline BM blasts >5% and transfusion dependence predicted poor OS (p=0.07 and 0.06 respectively), with the worst OS in pts with both BM blasts >5% and transfusion dependence (p=0.007). Treatment was reasonably well tolerated. The most common Grade ≥3 AEs regardless of relationship to treatment were thrombocytopenia (55% of patients), anemia (51%), neutropenia (45%), febrile neutropenia (38%) and pneumonia (32%). All-cause 60-day mortality was reported in only 1 patient (1.9%).
Summary/Conclusions: SC guadecitabine (SGI-110) is a well-tolerated novel HMA with clinical activity and OS of almost 12 months in heavily HMA treated r/r Int and HR MDS and CMML pts that warrants further development. Clinical responses were associated with improved OS, and pts with both baseline BM blasts >5% and transfusion-dependence had the worst OS.

P250

MUTATIONS OF COHESIN COMPLEX GENES IN MYELODYSPLASTIC SYNDROME: DISTINCT CLINIC-BIOLOGICAL FEATURES AND PROGNOSTIC RELEVANCE

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Background: Cohesin, a multimeric protein complex, is essential for chromosomal stability and transcriptional regulation. Perturbation of cohesin complex genes may contribute to malignant transformation and clonal evolution of hematopoietic cells. Recently, mutations within the cohesin complex were found in patients with myeloid neoplasms, but the reports concerning their clinical implications in myelodysplastic syndrome (MDS) are limited.
Aims: We aimed to elucidate the clinic–biological features and prognostic relevance of cohesin mutations in a large cohort of *de novo* MDS patients.
Methods: The mutational status for cohesin complex genes, including *RAD21, STAG1, STAG2, SMC1A* and *SMC3*, and 18 other molecular genetic alterations were analyzed in 420 patients with *de novo* MDS based on the FAB classification. Among them, the disease of 324 patients fulfilled the criteria of MDS

according to the 2008 WHO classification. The clinical features, cytogenetics, molecular alterations, and treatment outcomes were compared between patients with and without cohesin mutations.
Results: Cohesin mutations were identified in 31 (7.4%) of 420 patients, including two (0.5%) with *STAG1* mutations, 27 (6.4%) with *STAG2* mutations, and 2 (0.5%) with *SMC1A* mutations, respectively. The patients with cohesin mutations were older (P=0.0368), and had lower absolute neutrophil counts (P=0.0361) at diagnosis. Cohesin mutations occurred frequently in patients with refractory anemia with excess blasts (RAEB1/RAEB2), those with higher-risk International Prognostic Scoring System (IPSS) and revised IPSS (P=0.0107, P=0.0207 and P=0.0011, respectively). Clonal chromosomal abnormalities were detected in 168 (42.5%) of 395 patients who had chromosomal data. There was no difference in the distribution of karyotypes between patients with cohesin mutations and those without. Twenty-seven (87.1%) of the cohesin-mutated patients had concurrently other gene mutations. The patients with cohesin mutations had significantly higher incidences of concurrent *RUNX1* (25.8% *vs* 11.3%, P=0.0403), *IDH1* (12.9% *vs* 0%, P<0.001), *ASXL1* (61.3% *vs* 19.5%, P<0.001), and *SRSF2* mutations (45.2% *vs* 11.1%, P<0.001), but a trend of lower incidence of *TP53* mutation (0% *vs* 10.5%, P=0.0587) than those with wild type cohesin. With a median follow up duration of 42.9 months, there was a close correlation between cohesin mutations and acute leukemia transformation (3-year leukemia transformation rate, 78.5% *vs* 25.1%, for patients with and without cohesin mutations; P<0.001). MDS patients, based either by the FAB or 2008 WHO classification, had a significantly shorter overall survival if they harbored cohesin mutations than those who did not (median, 22.5 months *vs* 34 months, P=0.004 and 28.3 months *vs* 48.1 months, P=0.003, respectively). The difference remained significant when the analysis was performed in the subgroup of patients with lower risk MDS defined by the FAB classification (including RA and RARS), the WHO classification (other than RAEB, subtypes with blasts <5%), IPSS (including low- and intermediate 1-risk groups) or IPSS-R (including very low-, low- and intermediate–risk groups). However, there was no prognostic impact of cohesin mutations in the patients with higher risk MDS.
Summary/Conclusions: Cohesin mutations can be detected in a portion of MDS patients and are closely associated with mutations of *RUNX1, IDH1, ASXL1* and *SRSF2.* The presence of cohesin mutations predicts shorter survival, especially in the patients with lower risk MDS.

P251

MOLECULAR PROFILING IN A POPULATION BASED COHORT OF NORDIC MYELODYSPLASTIC SYNDROME PATIENTS

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Background: High through-put sequencing studies have revealed a large number of mutated genes in patients with myelodysplastic syndromes (MDS), of which RNA splicing factors and epigenetic factors constitute the two most frequent groups. Several studies have suggested that mutational screening may be beneficial to predict prognosis and clinical outcome of MDS patients.
Aims: In the present study we investigated molecular profiles in Nordic MDS patients, asking the question whether these population-based cohorts differ from that of previously published patient materials.
Methods: A total of 495 consecutive samples from Sweden, Denmark, and Norway were included in this study, encompassing all MDS subgroups, CMML, mixed MDS-MPN and RARS-T. The respective regional ethical committees approved the study, and all patients provided informed consent. Clinical data was collected based on national health registry systems.
Fresh bone marrow MNCs were isolated using gradient centrifugation and genomic DNA was extracted using column based DNA purification kits. Targeted enrichment of protein coding genomic regions of 42-75 frequently mutated genes were carried out and analyzed by next generation sequencing.
Results: Samples were successfully sequenced with an average coverage over 500 reads, allowing us to set the mutation detection to an allelic burden of ≥3%. In addition, we set the detection limit to 1% for TP53, considering the putative prognostic role of mutations in this gene. In our cohort, only 28 patients (6%) showed no mutations. We found that eleven genes; TET2, SF3B1, ASXL1, SRSF2, DNMT3A, TP53, RUNX1, U2AF1, EZH2, IDH2 and ZRSR2 were mutated in more than 5% of the patients (Fig.1), as compared to 7 genes, in the study by Papaemmanuil, et al, (Blood, 2013, 122(22): 3616-3627). Correspondingly, 7 genes showed a mutation rate >10%, compared to 4 in that study. Although our top gene list was similar to those reported in previous large studies we noted some interesting differences. Mutations in AXSL1 (15%), RUNX1 (10%), and TP53 (10.7%) were more frequent than in the study reported by Papaemmanuil (12%, 6% and 5%, respectively). The higher TP53 mutation rate can to some extent be attributed to the higher sensitivity, since 7 mutations had an allelic burden between 1% and 5%. Moreover, 34% (174 Patients) of our cohort had ≥3-mutated genes, compared to 20% in the Papaemmanuil paper.

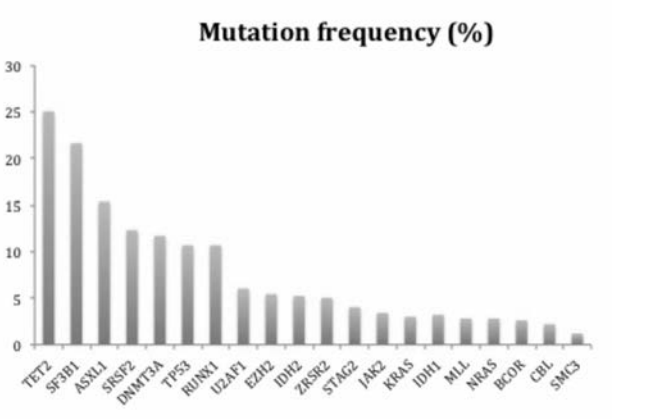

Figure 1.

Summary/Conclusions: The representativeness of reported studies based on selected patient cohorts constitutes a concern. Here, we show evidence that the mutational spectrum of a consecutive population-based Nordic cohort has an overall higher risk profile than previously published patient materials. This is valuable information, as mutation profiling will be soon part of standard clinical practice. In addition we believe that screening for TP53 mutations in MDS patients should be performed with sensitive methods, allowing for detection also of small subclones.

P252

CLINICAL BENEFIT AMONG LENALIDOMIDE (LEN)-TREATED PATIENTS (PTS) WITH RBC TRANSFUSION-DEPENDENT (RBC-TD) LOW-/INT-1-RISK MYELODYSPLASTIC SYNDROMES (MDS) WITHOUT DEL(5Q)

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Background: In the phase 3 MDS-005 study of LEN in RBC-TD lower-risk (LR) non-del(5q) MDS pts ineligible for/refractory to erythropoiesis-stimulating agents (ESAs), a statistically significant and clinically relevant higher proportion of LEN-treated pts achieved RBC transfusion independence (RBC-TI) ≥8 wks *vs* placebo (PBO) (*P* <0.001); other measures of efficacy collected included erythroid improvement and cytogenetic response (CyR).
Aims: To evaluate the relationship between LEN and clinically meaningful measures of response in pts from MDS-005.

Table 1.

Response, n (%)	LEN (n = 160)	PBO (n = 79)	OR (95% CI)
Clinical benefit	51 (31.9)	3 (3.8)	11.85 (3.57–39.38)
RBC-TI ≥ 8 wks	43 (26.9)	2 (2.5)	
Transfusion reduction ≥ 4 pRBC units ≥ 8 wks[1]	34 (21.3)	0	
Hb increase ≥ 1.5 g/dL (IWG 2006)	31 (19.4)	2 (2.5)	
CyR	9 (5.6)	0	

[1] Data normalized to 8 wks using data from a 112-day assessment period divided by 2.

Methods: Pts were randomized 2:1 to LEN 10 mg/day (n=160) or PBO (n=79) once daily. Clinical benefit ≥8 wks was defined as the composite endpoint of RBC-TI ≥8 wks, or transfusion reduction of ≥4 units packed RBCs (pRBCs) ≥8 wks, or hemoglobin (Hb) increase ≥1.5 g/dL at 8 wks (IWG 2006), or CyR. The rate of transfusion reduction was calculated using data collected during a 112-day assessment period to account for on-study differences in transfusion burden. CyR was also evaluated separately.

Results: Clinical benefit was higher in pts treated with LEN *vs* PBO (Table 1). In evaluable pts with ≥1 follow-up assessment (n=43), CyR with LEN was reported in 9 (33.3%) pts (major: n=5; minor: n=4). No CyR occurred in the PBO group. Among pts with CyR, 5 (55.6%) also achieved RBC-TI ≥8 wks.
Summary/Conclusions: In RBC-TD pts with LR non-del(5q) MDS ineligible for or refractory to ESAs, LEN was associated with significantly greater clinical benefit, as measured by this newly defined composite endpoint, *vs* PBO. This analysis provides evidence that other measures of response in addition to RBC-TI may be valuable in the management of LR non-del(5q) MDS. Clinically meaningful responses to LEN in non-del(5q) pts include CyR, decrease in transfusions, and increased Hb, in addition to RBC-TI.

P253

PHASE 1 DOSE-ESCALATION STUDY OF ORAL ASTX727, A COMBINATION OF ORAL DECITABINE WITH A CYTIDINE DEAMINASE INHIBITOR (CDAI) E7727, IN SUBJECTS WITH MYELODYSPLASTIC SYNDROMES (MDS)
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Background: Hypomethylating agents (HMA) are typically administered parenterally and adjusted to body surface area (BSA) in order to achieve target pharmacokinetic (PK) parameters associated with response. Much of the variation associated with administration of HMAs is related to the intrinsic variability of the activity of cytidine deaminase (CDA) an enzyme with highest levels in the gut and liver which rapidly metabolizes HMAs.
Aims: To assess PK comparability with the aim of matching PK parameters of BSA adjusted dosing with IV decitabine (DAC) with a novel fixed-dose oral combination (ASTX727), of DAC and E7727, a CDA inhibitor. Preliminary safety and clinical activity were previously reported (Savona M, Blood:126(23); 1683,2015).
Methods: Adult patients with Int-1/Int-2 or HR MDS or Chronic Myelomonocytic Leukemia (CMML) were enrolled in a Phase I, dose-escalation trial, aiming to achieve a mean AUC of DAC following oral ASTX727 comparable to IV DAC at the approved dose of 20mg/m^2. In the first cycle, each patient received an IV DAC dose of 20mg/m^2 on Day 1 as an internal comparator followed by oral ASTX727 on Days 2-5 escalated by cohort. Only one component at a time was escalated in each cohort and oral doses were not adjusted for weight or body surface area. Full PK assessments were performed on Days 1, 2 and 5.
Results: 43 patients were treated in 5 cohorts of 6 patients each plus a dose confirmation cohort of 13 patients. In cohorts 1-3 ASTX727 was given as a fixed oral DAC dose of 20mg with escalating doses of oral E7727 at 40, 60, and 100mg, respectively. In cohort 4, the oral DAC dose was escalated to 40mg while E7727 was kept at 100mg and Cohort 5 evaluated 30 mg DAC and 100mg E7727. DAC AUC increased with increasing doses of E7727. The AUC on Day 5 of dosing was higher than that on Day 2 of dosing due to residual levels of inhibitor E7727 present systemically 24 hours post dosing. Thirty patients were male, 13 female, their median weight was 87.8kg (range=51.1-204.1) and median BSA was 2.0m^2 (range 1.28-2.67). The Day 1 AUC for IV DAC 20mg/m^2 over all 5 cohorts was 188(+/-76) ng*hr/mL, CV= 41%. The Day 5 mean DAC AUC for ASTX727 increased with dose escalation from 31 to 148% of the Day 1 IV DAC AUC. (Table 1) Cohort 5 achieved ~90% oral DAC AUC exposures to IV with the acceptable variability of CV=50%. Linear regression between weight and DAC AUC for cohort 5 showed no correlation (p=0.7887).

Table 1. Mean Plasma DAC AUC and SD by Cohort for the Study afterASTX727 and IV DAC.

Cohort Dose mg/d DAC/E7727 (Days 2-5)	20/40 N=5	20/60 N=6	20/100 N=6	40/100 N=6	30/100 N=19	Total N=43
	AUC ng*h/mL	AUC ng*h/mL	AUC ng*h/mL	AUC ng*h/mL	AUC ng*h/mL	AUC ng*h/mL
IV DAC (20 mg/m^2) Day 1						188(76)[41%]
Oral DAC Mean(SD) [CV]	60.1(21.7) [36%]	79.8(28.2) [35%]	112(53.7) [48%]	285(149) [52%]	169(84.4) [50%]	
Weight (Kg) Mean(SD) [CV]	82.6(7.2) [9%]	88.5(11) [12.4%]	107.9(43) [40%]	117.2(55.4) [47%]	93.5(31.8) [34%]	96.6(34) [35%]
BSA (m^2)	1.85(0.31) [17%]	2.1(0.12) [5.7%]	1.9(0.34) [18%]	2.0(0.32) [16%]	2.0(0.23) [11.5%]	2.0(0.26) [13%]

Summary/Conclusions: Despite wide ranges in weight and BSA, oral ASTX727 administered in a fixed low dose of decitabine and E7727 delivers an AUC comparable to BSA adjusted IV decitabine in both exposure and variation. The low PK variability is attributed to the elimination of variations due to CDA activity through CDA inhibition by E7727 which appears to compensate for body mass disparities. An ongoing randomized cross-over Phase 2 trial is currently studying the PK and pharmacodynamic effects of full 5-day cycles of IV DAC and ASTX727.

P254

THE USE OF A HIGH DENSITY, 2.6 MILLION MARKER, SNP ARRAY AS THE PRIMARY GENETIC TEST FOR MYELODYSPLASTIC SYNDROME AT DIAGNOSIS: INTERIM FINDINGS OF THE WEST MIDLANDS, UK STUDY
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Background: Myelodysplastic syndrome (MDS) prognosis, risk stratification and management is currently dictated by the IPSS-R with metaphase chromosome analysis being the only current contributing genetic factor. The UK NHS genetic service offers metaphase cytogenetics (MC) as the only routine test, which is not only time consuming but ultimately only detects an abnormal karyotype in approximately 45% samples. Although there is no single technology that can identify the various genomic and genetic aberrations observed in MDS in a single test, the cytogenetic aberrations seen in MDS are mostly copy number changes (with the exception of 3q26 *MECOM* gene rearrangements) and therefore microarray analysis offers an alternative approach to MC. High density single nucleotide polymorphism microarrays (SNP-A) offer genome wide copy number analysis at a resolution beyond that of MC analysis, and often down to the single gene, and even exon, and can identify regions of copy number neutral loss of heterozygosity (CN-LOH) that may harbour biallelic gene mutations. Consequently microarray analysis has the potential to increase genetic abnormality detection rates thereby improving diagnosis.
Aims: The West Midlands Regional Genetic Laboratory has undertaken a two year study to use SNP-A analysis as a complementary tool to MC in patients with confirmed or highly suspected MDS both at presentation and during the disease course. The aim of the first arm of the study is to determine the utility of high density SNP-A as the primary test for MDS patients to replace MC analysis at diagnosis. This includes comparison of abnormal karyotype detection rate and aberrational yield.
Methods: SNP-A analysis was performed on DNA extracted from marrow samples of 102 MDS patients. All 102 patients' samples were, in parallel, subjected by MC analysis. DNA was interrogated using Affymetrix CytoScan HD SNP arrays which contain 2.6 million markers including approximately 750,000 SNPs. Data was analysed using Affymetrix Chromosome Analysis Suite (ChAS).
Results: Forty four cases (43%) were found to have no reportable abnormalities by both MC and SNP-A analysis, and 40 cases (39%) were found to be abnormal by both techniques. The remaining 18 cases (15%) were discrepant: 15 cases were found to be abnormal by SNP-A but normal by MC analysis, whereas 3 cases were found to be abnormal by MC analysis but normal by SNP-A analysis. The karyotype of 33 cases were discrepant between MC and SNP-A. A total of 39 additional abnormalities in 30 cases were detected by SNP-A that were not seen or identified by MC analysis, including: 18 regions of CN-LOH; gain of chromosome 4; loss of chromosome 7; loss of 7q; two *TET2* deletions, two *CUX1* deletions; a partial deletion of *RUNX1*; and a partial tandem duplication of *KMT2A*. Regions of CN-LOH (>10 Mb, extending to the telomere) were found in 17 patients (18%), with 7q being the region most affected (5 patients). Three aberrations were identified by MC that were not detected by SNP-A: a low level trisomy 8 clone (~1% clone); an insertion resulting in a *RUNX1* rearrangement as the sole abnormality; and a 3q translocation resulting in a *MECOM* rearrangement as part of a complex karyotype.
Summary/Conclusions: SNP array analysis increased the number of cases identified as genetically abnormal from 43% to 54% and identified an additional 39 aberrations compared to MC analysis. Additional aberrations detected by SNP array, including gene deletions and CN-LOH, have the potential to refine risk stratification and prognosis in MDS. The detection of cytogenetically cryptic and potentially prognostically significant abnormalities in these MDS patients highlights the need for higher resolution genomic detection methods in the diagnosis of this disease.
Acknowledgements: This work was funded by Affymetrix.

P255

PHASE 1B STUDY OF GLASDEGIB (PF-04449913) IN COMBINATION WITH AZACITIDINE IN PATIENTS WITH HIGHER RISK MYELODYSPLASTIC SYNDROME, OLIGOBLASTIC ACUTE MYELOID LEUKEMIA, OR CHRONIC MYELOMONOCYTIC LEUKEMIA
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Background: The Hedgehog signaling pathway (HhP) is known to be aberrantly activated in leukemias and myelodysplastic syndrome (MDS), promoting cancer stem cell maintenance. Glasdegib is a potent, selective, oral HhP

inhibitor which acts by inhibition of Smoothened (SMO), with activity in preclinical models and antitumor efficacy *in vivo*. A Ph.1 study of glasdegib monotherapy in patients (pts) with hematologic malignancies suggested the drug is well tolerated and active. When glasdegib was combined with low-dose cytarabine, decitabine or standard induction chemotherapy in 7 previously untreated high-risk MDS pts, 2 achieved complete responses (CR) and 3 had marrow (m)CRs. Further, blocking the HhP with SMO inhibitors in combination with azacitidine (aza) demonstrated synergy *in vitro* and inhibited primary malignant myeloid cells *ex vivo*.

Aims: Primary objective of the open-label Ph.1b part of this study (NCT02367456) was to assess the safety of combining glasdegib with aza in pts with previously untreated higher risk MDS (IPSS score risk: high or Int-2), oligoblastic acute myeloid leukemia (oAML) with 20-30% blasts and multi lineage dysplasia (WHO 2008 criteria), or non-proliferative chronic myelomonocytic leukemia (NP-CMML) with white blood cells <13x10^9/L.

Methods: Adult pts gave informed consent and received glasdegib 100mg once daily in combination with standard aza 75mg/m^2 days 1-7 every 28 days for as long as pts received clinical benefit. Patients were assessed for safety, tolerability and efficacy.

Results: As of Dec 30, 2015, 12 pts (7 males) with a mean age of 72 years (range 59-89) were enrolled. Primary baseline diagnosis was MDS: 7 (IPSS-risk Int-2: 5, high risk: 2), oAML: 3, NP-CMML: 1, other (erythroleukemia): 1. Across all cycles the median dose intensity was 80% for glasdegib and 97% for aza. At data cutoff, 8 pts (67%) discontinued treatment due to an adverse event (AE) (n=5), physician decision (n=2) or patient decision (n=1). Plasma exposure of both agents was unchanged when coadministered. The most common, all-cause AEs included anemia and constipation (75% each), nausea (67%), fatigue (58%), dysgeusia and neutropenia (50% each). Non-hematologic grade (G)3-4 AEs of sepsis occurred in 2 pts (17%); additional G3-4 AEs occurring in 1 pt (8%) are listed in the Table 1. Eight pts (67%) experienced serious AEs (SAEs); SAEs occurring in 2 pts (17%): cellulitis, febrile neutropenia, and pyrexia. Four of the 12 pts had an investigator-assessed response by IWG criteria: 3 CR and 1 mCR; 4 pts had stable disease. Three pts died from disease progression, sepsis with multi-organ failure, and myocardial infarction due to underlying CAD. Six-month survival was 67%.

Table 1. Non-hematologic G3-4 AEs (n=1).

G3: Fatigue, prolonged QT, muscle spasms, hyponatremia, urinary tract infection, hematoma, hypophosphatemia, hypoxia, bacteremia, diverticulitis, hemarthrosis, hypokalemia, lung malignancy, mental status changes, musculoskeletal chest pain, neutropenic sepsis, osteomyelitis, procedural hypotension, respiratory tract infection, and tachycardia
G4: Dyspnea, cellulitis, hypotension, pneumonia, acute kidney injury, acute respiratory distress syndrome, ischemic hepatitis, respiratory distress, and septic shock

Summary/Conclusions: The addition of glasdegib to standard-of-care aza in the treatment of pts with MDS, CMML or oAML appears to have an acceptable safety profile. The number of CRs observed so far appears favorable in the context of the 15-17% CR rate seen with aza alone. A randomized trial of glasdegib in combination with aza is planned in MDS.

P256

RESULTS FROM PHASE I/II STUDY OF THE COMBINATION OF ORAL RIGOSERTIB AND AZACITIDINE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Rigosertib is a Ras-mimetic (Athuluri-Divakar, Cell 2016, in press) that inhibits the PI3K and PLK cellular signaling pathways by binding directly to the Ras-binding domain found in Ras effector proteins. *In vitro*, the combination of rigosertib with azacitidine synergistically inhibits growth and induces apoptosis of leukemic cells in a sequence-dependent fashion (rigosertib administered prior to azacitidine) (Skidan, AACR 2006). Phase I results of this study in pts with MDS or AML showed the combination of oral rigosertib and standard-dose azacitidine to be well-tolerated with evidence of efficacy (Navada, Blood 2014).

Aims: Phase II was initiated to further study the combination in pts with MDS.

Methods: Oral rigosertib was administered twice daily on Day 1-21 of a 28-day cycle at the recommended Phase II dose (560 mg qAM and 280 mg qPM). Azacitidine 75 mg/m^2/d was administered SC or IV for 7 days starting on Day 8. A complete blood count was performed weekly and a bone marrow aspirate and/or biopsy at baseline, Day 29, and every 8 weeks thereafter.

Results: Pts with MDS previously untreated with an HMA or progressing/failing to respond to a prior HMA are presented. Oral rigosertib/azacitidine was administered to 40 pts with MDS. Demographic data are available on 37 pts, among whom 73% were male; median age was 64 years (range: 25-85); ECOG performance status was 0, 1, and 2 in 24%, 73%, and 3%, respectively. Fourteen pts (38%) had received prior HMA therapy: 10 (27%) azacitidine, 3 (8%) decitabine, and 1 (3%) both. Patients received 1-27+ months of treatment with the doublet, with a median duration of 4 months. Seven pts have been treated >1 year and 1 for >2 years. Thirty pts were evaluable for response: Phase I (N=8) or Phase II (N=22). Among these 30 pts, hematologic response per the International Working Group (IWG: Cheson, Blood 2006) criteria was seen in 23 (77%) of pts in Phase I/II: 6 (20%) with complete remission (CR), 16 (53%) marrow CR (mCR), and 1 (3%) hematologic improvement (HI). Six (20%) pts had stable disease (SD) and 1 (3%) had progressive disease (PD). Among the 27 of 30 pts with sufficient follow-up for a peripheral blood lineage response evaluation (3 were too early to evaluate), 13 (48%) had an HI: 11 erythroid, 12 platelet, 7 neutrophil. The table shows response by International Prognosis Scoring System (IPSS-R; Greenberg, Blood 2012) risk categories for the 30 evaluable MDS pts. Among the 11 evaluable pts with MDS who had failed to respond/progressed/relapsed on prior treatment with an HMA, 7 (64%) had a response after rigosertib was added: CR (1), mCR (4), mCR plus HI (2). For HMA-naïve patients (N=19); 16 (84%) responded per IWG. The most frequent adverse events (AEs) in Cycle 1 were nausea and fatigue (27% each) and pyrexia and thrombocytopenia (24% each). The most frequent AEs for ≥Cycle 2 were constipation and neutropenia (22% each). Three deaths due to adverse events have been reported.

Table 1.

	IPSS-R Risk Category			
	Low/ Intermediate	High	Very High	Unknown
Hematologic response*				
N = 30	6	9	10	5
CR	2 (33)	1 (11)	3 (30)	0
mCR	3 (50)	4 (44)	6 (60)	3 (60)
SD	1 (17)	2 (22)	1 (10)	2 (40)
PD	0	1 (11)	0	0
HI**	0	1 (11)	0	0
Hematologic improvement*				
N = 27	6	8	9	4
Erythroid response	3 (50)	3 (38)	5 (56)	0
Platelet response	3 (50)	3 (38)	6 (67)	0
Neutrophil response	0	2 (25)	5 (56)	0

*Per 2006 IWG
**HI based on peripheral blood only; no bone marrow response

Summary/Conclusions: The combination of oral rigosertib and standard-dose azacitidine was well tolerated in repetitive cycles in pts with MDS. Among the 30 evaluable MDS pts in Phase I/II, hematologic response was seen in 23 (77%), with responses seen in 64% of patients who failed HMA therapy. These results suggest potential synergistic interaction of the combination and the capability to reverse clinical HMA resistance, and supports continued study of this unique combination in pts with MDS.

P257

FREQUENT MUTATIONS IN EPIGENETIC REGULATORS IN CYTOPENIA OF UNDETERMINED SIGNIFICANCE: ASSOCIATION WITH RISK OF PROGRESSION TO MYELODYSPLASTIC SYNDROME

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Background: Peripheral blood cytopenia is a common finding in the elderly population, and an extensive diagnostic work up is often done trying to unravel its causality. Next generation sequencing may be a valuable new diagnostic tool in these patients, and clonal cytopenia of undetermined significance (CCUS) has recently been introduced to describe patients with cytopenia of unknown significance who carry a mutation characteristic of myeloid malignancies.

Aims: The purpose of the present study was to investigate the frequency of mutations in patients with persistent cytopenia and an undetermined diagnosis after routine workup.

Methods: We included 60 patients between 2008-2015, who all had persistent cytopenia for more than six months, and an undetermined diagnosis after routine workup. Median (range) age was 70 years (25-89) (Table). Diagnosis according to bone marrow morphology was reviewed in a blinded fashion by two hematopathologists. Patients with aberrant cytogenetics, except deletion of the Y-chromosome, were excluded. DNA from peripheral neutrophils or from mononuclear cells from the bone marrow were sequenced with a targeted 20 gene panel, including genes frequently mutated in myelodysplastic syndrome (MDS).

Results: Twenty-one (35%) patients had only one cytopenia, 30 (50%) had two cytopenias and nine(15%) had pancytopenia. Patients with a mutation had a lower level of neutrophils compared to non-clonal patients, otherwise the characteristics were very similar between the two groups (Table). At time of inclusion mutations in 15 of the 20 genes included in the sequencing panel was identified. Thirty-seven (62%) patients carried at least one mutation. *TET2* was the most frequently mutated gene, found in 26 (43%) patients. There was no association with the number of cytopenias and the risk of having a mutation detected at time of inclusion. Follow-up was 1-8 years and during this period six patients (10%) progressed to MDS or chronic myelomonocytic leukemia (CMML). Among these patients, five had mutations at the time of inclusion. At time of progression to MDS or CMML four had a new co-occurring mutation in *NRAS*, *TP53, GATA2* or *ASXL1*.

Table 1.

CHARACTERISTICS	TOTAL (n=60)	CCUS (n=37)	Cytopenia – non-clonal (n=23)	P-value
Sex Women Men	 22 38	 14 23	 8 15	0.81
Age, years (range)	70 (25-89)	70 (60-75)	70 (60-80)	0.89
Hemoglobin mmol/l, median (IQR)	7.0 (6.2-7.8)	6.9 (6.1-7.8)	7.3 (6.2-8.1)	0.51
Leukocytes 10^9/L, median (IQR)	4.3 (1.7-22.4)	3.9 (3.2-6.1)	5.5 (2.9-7.8)	0.32
Neutrophils, median 10^9/L (IQR)	2.3 (0.5-18.1)	1.93 (1.1-3.55)	3.3 (1.7-6.8)	0.025
Thrombocytes 10^9/L, median (IQR)	110 (30-383)	111 (78-163)	103 (88-142)	0.96
MCV, median (IQR)	92 (87-99)	93 (87-102)	89.5 (85-98)	0.34
Ferritin µg/L, median (IQR)	246 (77-420)	283 (91-542)	138 (59-340)	0.037
LDH U/l, median (IQR)	183 (161-222)	186 (161-236)	180 (164-219)	0.565
One Cytopenia, n (%)	21 (35)	12 (33)	9 (39)	0.597
Two Cytopenias, n (%)	30 (50)	19 (51)	11 (48)	0.791
Pancytopenia, n (%)	9 (15)	6 (16)	3 (13)	0.738

IQR=inter quartile range
CCUS=clonal cytopenia of undetermined significance
MCV=mean corpuscular volume
LDH=lactate dehydrogenase

Summary/Conclusions: Patients with persistent cytopenia and an undetermined diagnosis often carry mutations in genes associated with myeloid malignancies. Next generation sequencing is a promising supplement in the diagnostic workup as it may identify patients with increased risk of disease progression.

Molecular characterization of MM

P258

MOLECULAR AND CLINICAL TYPES OF PLASMA CELL DYSCRASIAS ARE ASSOCIATED WITH DISTINCT EXPRESSION PATTERNS OF LONG NONCODING RNAS

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Background: Multiple myeloma (MM) is a malignant proliferation of antibody-secreting bone marrow plasma cells (PCs) characterized by a wide clinical spectrum ranging from the presumed pre-malignant condition called monoclonal gammopathy of undetermined significance (MGUS), to smoldering MM (SMM), truly overt and symptomatic MM, and extra-medullary myeloma/plasma cell leukemia (PCL). Although many efforts have recently contributed to improve our knowledge of molecular pathogenesis of MM, the role and significance of long non-coding RNAs (lncRNAs) in PC malignancies remains virtually absent.
Aims: We investigated the lncRNA expression profiles in a large and representative cohort of PC dyscrasia, with the aim of identifying deregulated lncRNAs putatively associated with MM pathogenesis and possibly linked to the multi-step process underlining the different stages of the disease.
Methods: We developed a custom annotation pipeline on Gene 1.0 ST microarray data, to investigate lncRNA expression in highly purified bone marrow PCs from 20 MGUS, 33 SMM, 170 MM, 36 PCL and 9 healthy donors, collected from proprietary and publicly available datasets.
Results: Our study identified 31 lncRNAs deregulated in pathological samples compared to the healthy condition. In particular, the upregulation of MALAT1 in symptomatic patients was associated with molecular pathways involving cell cycle regulation, p53-mediated DNA damage response, and mRNA maturation processes. Furthermore, we found 21 lncRNAs whose expression was progressively deregulated trough the more aggressive stages of PC dyscrasia, suggesting a possible role in the progression of the disease. In the context of molecular heterogeneity of MM, we identified a transcriptional fingerprint in hyperdiploid (HD) patients, characterized by the upregulation of lncRNAs/pseudogenes related to ribosomal protein genes. These findings are in line with the global up-regulation of the translational machinery, including genes involved in protein biosynthesis, characterizing HD group. To gain evidences of lncRNAs that may potentially act on gene expression, we evaluated the correlation of each of the differentially expressed lncRNAs with all the transcripts unambiguously detectable by the arrays. By focusing on high correlation coefficient, we identified five lncRNAs suggestive of an *in cis* rather than an *in trans* interaction. Finally, based on several evidences that have suggested the interplay of the various non-coding species, we investigated the liaison between lncRNAs and miRNAs in 125 samples out of our series for which miRNA expression profiling was available. We identified 290 lncRNA-miRNA couples significantly anti-correlated in our database. Hence, to add confidence to the anti-correlation connection, we inspected which of the 290 lncRNAs anti-correlated to miRNAs could also be miRNAs targets. Based on RNA22 prediction algorithm, we identified nine lncRNA-miRNA couples with transcripts resulting anti-correlated and for which lncRNA is a predicted miRNA target. Particularly interesting is lnc-MAP1LC3B2-2, found upregulated in MM with MAF translocation and anti-correlated with miR-222 and miR-221.
Summary/Conclusions: Our study reported many lncRNAs deregulated in different forms of PC dyscrasia, providing an important source for future functional studies on lncRNAs in MM disease.

P259

RPL5 ON 1P22 IS RECURRENTLY DELETED IN MULTIPLE MYELOMA AND ITS EXPRESSION IS LINKED TO BORTEZOMIB RESPONSE

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Background: Deletions of chromosomal region 1p22 are found in ~20% of multiple myeloma patients and have been identified as a negative prognostic

factor for progression-free survival (PFS) and overall survival (OS) in newly diagnosed patients (Hebraud *et al.*, Leukemia, 2013). These findings suggest the presence of an unidentified tumor suppressor gene on 1p22.
Aims: We aimed to delineate a minimal deleted region (MDR) on 1p22 to identify potential tumor suppressor genes in this region. Additionally, we were interested in the clinical relevance of lowered expression of genes in the MDR on 1p22.
Methods: We investigated 1p22 deletions using high resolution copy number arrays in a cohort of 35 multiple myeloma patients. The expression of the genes in the MDR and their correlation with clinical outcome was further investigated in data of the MMRC, the phase III HOVON-65/ GMMG-HD4 trial and the APEX trial. *In vitro* experiments in mouse B-cells were done to investigate the correlation between expression levels of one of the genes (*RPL5*) in the MDR with sensitivity to proteasome inhibitors.
Results: Using high resolution genomic profiling, we delimit a 58 kb minimal deleted region on 1p22.1 encompassing two genes: ectopic viral integration site 5 (*EVI5*) and ribosomal protein L5 (*RPL5*). Although mutations in 1p22 genes are rare in multiple myeloma, a tumor suppressor role for *EVI5* and *RPL5* may also be supported by the fact that these genes show the highest frequency of mutations predicted to impair protein function on 1p22. Interestingly, inactivation of *RPL5* was also recently described in T-cell acute lymphoblastic leukemia (T-ALL) and glioblastoma. We also found that 1p22 deleted patients have significantly lower levels of *EVI5* and *RPL5* mRNA expression. Whereas 1p22 deletion status correlates well with *EVI5* expression in all patients, it is a bad predictor of *RPL5* expression level in some, suggesting that other mechanisms besides 1p22 deletion can downregulate *RPL5* expression in multiple myeloma. In agreement with what is known for deletion of 1p22, we saw that low mRNA expression of *EVI5* and *RPL5* is associated with worse PFS and OS in diagnostic patients. Interestingly, reduction of *RPL5* expression in B-cells caused 2-fold sensitization to the proteasome inhibitors bortezomib and carfilzomib. In addition, *RPL5* but not *EVI5* mRNA expression levels were significantly lower in multiple myeloma patients showing clinical response to bortezomib compared to patients that are not responding. Furthermore, PFS of newly diagnosed patients with low *RPL5* expression was significantly longer when they were treated on a bortezomib containing protocol (median PFS bortezomib protocol 29.8 months *versus* 18.7 months for non-bortezomib, p=0.03, data phase III HOVON-65/ GMMG-HD4 trial). In contrast, PFS of *RPL5* high patients was not influenced by bortezomib. These associations between *RPL5* expression level and bortezomib response were confirmed when performing the same analyses on the PFS data from relapse patients in the APEX trial. For low *EVI5* expressing patients, there was a non-significant trend towards benefit from bortezomib which was not present in the *EVI5* high cases.
Summary/Conclusions: We provide genetic data that point towards a tumor suppressor role for *EVI5* and *RPL5* on 1p22. Although the exact role of these genes in promoting MM progression remains to be determined, we identify *RPL5* mRNA expression as a relevant biomarker for initial response to bortezomib and subsequent survival benefit after long-term treatment.

P260

COMPREHENSIVE CHARACTERIZATION OF THE GENOMIC LANDSCAPE OF MULTIPLE MYELOMA WITH A DELETION OF CHROMOSOME 17P REVEALS A HIGH PREVALENCE OF SOMATIC TP53 MUTATIONS, WHICH CORRELATE WITH A POOR PROGNOSIS

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Background: A deletion of chromosome 17p (del17p) is detected in 10% of multiple myeloma (MM) patients at diagnosis and is associated with a dismal prognosis, classifying it as high-risk disease. Moreover, its prevalence is known to increase during disease progression. Even though this suggests that it may be a driver of disease, little is known about the genetic defects that are involved in its pathology. Del17p in MM usually involves a hemizygous loss of a commonly deleted region (CDR) on 17p13.1, in which several potential tumor suppressor genes reside that could be bi-allelically inactivated by the deletion itself and a concomitant mutation. Of these, *TP53* is the most studied gene in del17p MM, but previous targeted sequencing efforts failed to identify a high prevalence of mutations herein. We therefore hypothesized that relevant genetic lesions might have been missed in either *TP53* itself, in other genes in the CDR on chromosome 17p (Chr17p), or in disease-relevant pathways, that could explain the aggressive clinical behavior of this type of MM.
Aims: With this study, we sought to systematically analyze the genomes of del17p MM tumors, in order to identify recurrent genetic aberrations and to assess their prognostic significance within this disease subgroup.
Methods: DNA was isolated from peripheral blood and CD138+ enriched bone marrow mononuclear cells from chemotherapy-naive MM patients with a del17p in ≥50% of plasma cells, as detected with fluorescent *in situ* hybridization (FISH). Libraries were generated using a custom capture (SeqCap EZ Exome Plus, Nimblegen) of 111 Mb, comprising the whole exome, Chr17p and the *IgH*, *Igk*, *IgL* and *MYC* regions. Paired-end sequencing was performed on a HiSeq2500 platform, followed by data analysis using an in-house bioinformatics pipeline at Erasmus MC, including SNPExpress, MutSigCV and GISTIC2. Single nucleotide variants in *TP53*, *FAM46C*, *KRAS*, *NRAS*, *DIS3* and *BRAF* were validated using a custom amplicon panel (TruSeq Custom Amplicon Assay v1.5, Illumina), followed by deep sequencing on a MiSeq System.
Results: We captured and sequenced tumor and germline DNA from 44 patients, which resulted in an average coverage of 90x and overall coverage of 94%. Clinical data were available from 40/44 patients, with a median follow-up time of 18 months. We identified a CDR on Chr17p of 235 kb, in which one or more somatic, nonsynonymous aberrations in *TP53* were detected in 25/44 (57%) patients (adj. $p<1x10^{-10}$; n=17 missense, n=9 nonsense, n=2 splicing). All other genes in the CDR on Chr17p lacked any nonsynonymous mutations. Besides *TP53*, we found *FAM46C* and *KRAS* to be significantly mutated. *RB1*, *TRAF3* and *FAM46C* were significantly deleted in our patient cohort. *TP53* mutated patients had a worse overall survival (OS) than *TP53* wildtype patients (p=0.008). Patients with *TP53* missense mutations showed the worst OS ($p<1x10^{-3}$), as well as progression-free survival (PFS) (p=0.015).
Summary/Conclusions: (1) *TP53* is somatically mutated in the majority of del17p MM patients; others lack any nonsynonymous somatic event in the CDR on Chr17p. (2) Particularly *TP53* missense mutations have a significantly negative impact on both OS and PFS in del17p MM patients. (3) The rest of the genomic landscape of del17p MM is characterized by significant mutations in *FAM46C* and *KRAS*, as well as deletions of *RB1*, *TRAF3* and *FAM46C*.

P261

DEPENDENCE ON GLUTAMINE UPTAKE OF MYELOMA CELLS DELINEATES A NEW ATTRACTIVE THERAPEUTIC STRATEGY

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Background: The importance of glutamine (Gln) metabolism in multiple myeloma (MM) cells and its potential role as a therapeutic target are still under investigation, although it has been reported several years ago that human myeloma cell lines (HMCL)s are highly sensitive to Gln depletion. Moreover, MM cells produce an excess of ammonium (NH_4^+) in the presence of Gln and hyperammonemia is a possible rare clinical manifestation in relapsed/refractory MM patients.
Aims: The aim of this study was to investigate the relationship between NH_4^+ production and Gln-dependence in MM cells, as well as the mechanisms involved therein, actually unknown, in order to identify a new potential target in MM patients.
Methods: A total cohort of 64 patients with Plasma cell (PC) disorders were included in the study: 6 patients with monoclonal gammopathy of undetermined significance (MGUS), 12 with smoldering myeloma (SMM), and 46 with active MM including 28 newly diagnosed MM (ND-MM) and 18 relapsed MM (R-MM). NH_4^+ levels in bone marrow (BM) plasma and produced by HMCLs (JJN3, OPM2, RPMI 8226, XG1, KMS-12-BM) and primary $CD138^+$ cells purified from BM aspirates in the presence or in the absence of Gln were assessed by the Roche Cobas NH3 Ammonia assay. BM plasma content of amino acids was determined by HPLC. The expression of the main enzymes involved in Gln metabolism (Glutaminase (GLS); Glutamine Synthetase (GS) and Asparaginase) and Gln transporters were evaluated. The effect on MM cell viability of *E. chrysanthemi* L-Asparaginase (ASNase), with glutaminolytic activity, was tested alone or in combination with Bortezomib (0-10 nM). The intracellular levels of Gln, Glutamate (Glu), and 2-oxoglutarate (2-OG) were measured with mass spectrometry. Furthermore, gene expression profiles of glutamine transporters were evaluated in two PC dyscrasia datasets, generated using 3 proprietary (GSE13591, GSE6205 and GSE66293) and 3 publicly available (GSE6477, GSE6691 and GSE47552) datasets. Anti-SLC1A5 shRNA lentiviral vector was used for ASCT2 stable knockdown in HMCLs, whereas the scramble lentiviral vector was used as control. For the *in vivo* experiment 8 SCID-NOD mice for group were injected subcutaneously with JJN3 stably transfected with anti-SLC1A5 containing plasmid vectors or with JJN3 stably transfected with the empty vector and then tumor growth was monitored.
Results: We found that both HMCLs and primary BM $CD138^+$ cells produced large amounts of NH_4^+ in the presence of Gln. Active MM patients have higher BM plasma NH_4^+ and Glu and lower Gln levels in comparison with patients with indolent monoclonal gammopathies. Interestingly, HMCLs expressed GLS and were sensitive to its inhibition with BPTES and CB-839, while exhibited negligible

expression of GS. High GLS and low GS expression were also observed in primary CD138+ cells. Gln-free incubation or treatment with the glutaminolytic enzyme ASNase depleted the cell contents of Gln, Glu and the anaplerotic substrate 2-OG, inhibiting MM cell growth. Moreover, ASNase effect on HMCL viability was potentiated in the presence of Bortezomib. In line with the dependence of MM cells on extracellular Gln, the gene expression profile analysis showed an increased expression of the Gln transporters SNAT1, ASCT2, and LAT1 by CD138+ cells across the progression of monoclonal gammopathies. Interestingly, among these transporters, only ASCT2 inhibition in HMCLs caused a marked decrease in Gln uptake. Consistently, ASCT2 stable down-regulation inhibited HMCL growth both *in vitro*. Finally, in the *in vivo* mouse model we found that mice inoculated with the ASCT2-silenced cells developed significantly smaller plasmacytomas than animals injected with the scramble-transfected cells.
Summary/Conclusions: In conclusion, MM cells strictly depend upon extracellular Gln and show features of Gln-addiction. Therefore, the inhibition of Gln uptake is a new attractive therapeutic strategy for MM.

P262

LOSS OF PRIMING OR MCL-1 DEPENDENCY CONFERRED ACQUIRED RESISTANCE TO VENETOCLAX IN MULTIPLE MYELOMA
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Background: Venetoclax/ABT-199 is the first in class Bcl-2 specific BH3 mimetic and among the most promising targeted therapy in oncology. Venetoclax has already demonstrated its efficacy in chronic lymphoid leukemia and is currently under investigation in multiple myeloma. Multiple myeloma is heterogeneous and includes patients with translocation of the IgH locus on chromosome 14 with different chromosomes (4, 6, 11 or 16) and patients with a hyperdiploidy involving odd chromosomes. Venetoclax induces apoptotic cell death in a subgroup of myeloma patients that carried the t(11;14) translocation and expressed a high Bcl-2/Mcl-1 gene expression ratio. In contrast, resistance to venetoclax is mediated by a high Mcl-1 expression in other myeloma subgroups.
Aims: The expected use of venetoclax alone or in combination with other agents in the treatment of myeloma leads us to explore the mechanisms of acquired venetoclax resistance. We investigated whether the acquired resistance to venetoclax might be different from the intrinsic resistance.
Methods: We derived resistant sublines from initially KMS12-PE and XG5 t(11;14) myeloma venetoclax highly sensitive cell lines (LD50 15 and 5nM respectively). Cell lines were cultured during several months with increasing venetoclax concentrations until cells were able to maintain viability with 2mM venetoclax. Patterns of Bcl-2 family gene and protein expression were compared between parental and resistant sublines by quantitative PCR and western blotting analysis. BH3 profiling approach by flow cytometry was used to analyze the mitochondrial priming of parental and subline.and their dependence on individual anti-apoptotic family member. Bax exon sequencing was performed to analyze potential mutations.
Results: We first provided evidence that venetoclax resistant sublines (XG5-199R and KMS12PE-199R) displayed cross-resistance to bortezomib. We then demonstrated that both resistant sublines exhibited a strong increase in both Mcl-1 mRNA and protein levels. Beside Mcl-1, the other Bcl-2 anti-apoptotic proteins were not significantly modified. Strikingly, a total lack of both Bax and Bak effector proteins characterized the XG5-199R subline. According to its strong sensitivity to venetoclax, XG5 cells exhibited a primed Bcl-2 profile. In contrast, we found that XG5-199R cells were unprimed. The XG5-199R unprimed status could be explained by the absence of Bax and Bak effector proteins. Finally, by sequencing *BAX* gene, we identified a mutation at position Q52 leading to a stop codon, which resulted in a very short Bax truncated protein missing all the BH domains. KMS12PE-199R was characterized by a decline in several pro-apoptotic proteins (*i.e.*, Bak, Bim, Bik and Puma). Like XG5, KMS12-PE had a primed Bcl-2 profile. Of interest, a switch in the dependence on Bcl-2 in KMS12PE cells towards Mcl-1 was demonstrated in KMS12PE-199R cells. Finally, we found that KMS12PE-199 acquired resistance could be overcome by A1210477, a recently discovered potent Mcl-1 inhibitor.
Summary/Conclusions: The XG5199R and KMS12PE199R acquired models of venetoclax resistance in myeloma reflect two main ways to escape to apoptosis, either by a reduction of BH3 only proteins or by a loss of effector multidomain proteins both being accompanied by an increase of Mcl-1 level of expression. Both models of acquired resistance affect the mitochondrial priming and/or the dependency on individual anti-apoptotic protein of the cells.

P263

LOSS OF THE INHIBITORY RECEPTOR CD85J/LILRB1 ON MALIGNANT PLASMA CELLS: FROM MGUS TO SYMPTOMATIC MULTIPLE MYELOMA
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Background: Immune regulation may control proliferation of aberrant plasma cells (PCs) in the asymptomatic monoclonal gammopathy of undetermined significance (MGUS). Loss of inhibitory pathways could lead to progression from MGUS to active multiple myeloma (MM). CD85j (LILRB1) molecule is an inhibitory immunoreceptor containing four immunoreceptor tyrosine-based inhibitor motifs (ITIM). Binding of HLA-G to CD85j suppresses B cell responses. The role of CD85j in MM pathogenesis remains to be elucidated.
Aims: The aim of this study was to investigate CD85j contribution to immune regulation in monoclonal gammopathies and its role in progression from MGUS to MM.
Methods: CD138+ PCs from bone marrow (BM) aspirates from individuals with MGUS (n=20) and patients with active MM (n=20) were isolated with immunomagnetic beads. Both fractions CD138+ and CD138^{neg} were analyzed by real-time PCR. CD85j expression and cell proliferation assays were assessed by multi-parameter 8-color flow cytometry. Effects of ligation of CD85j with specific anti-CD85j monoclonal antibodies were analyzed on myeloma cell lines and *ex vivo* BM functional assays.
Results: Gene expression of CD85j (*lilrb1*) and its ligand S100A9 was significantly downregulated in BMPCs from patients with MM compared to PCs from individuals with MGUS. Consistently, flow analysis showed that the inhibitory molecule CD85j was mainly expressed on the BMPCs and B cells in MGUS. In contrast, patients with MM showed significantly lower levels of CD85j compared to those with MGUS. On the other hand, the expression of this inhibitory checkpoint was recovered in patients in complete remission but not in progression (Fig.1). In asymptomatic MGUS, flow cytometry analysis identified PCs at distinct stages of the malignant transformation: (i) normal PCs were CD85j^{high}, (ii) PCs positive for CD85j and with abnormal expression of CD56 or CD117 and, finally, (iii) PCs expressing CD56 or CD117 that lost CD85j expression like malignant PCs observed in MM. In MGUS, CD85j expression was significantly decreased when the proportion of aberrant PC was higher than 40%, suggesting that loss of CD85j may be an early event promoting tumor immune escape. Furthermore, our functional assays showed that ligation with anti-CD85j antibody decreased the number of normal *ex vivo* BMPCs from patients with MGUS. In contrast, malignant PCs from patients with MM and MM cell lines were resistant to treatment with anti-CD85j. Finally, we wanted to investigate whether other inhibitory members of the LILRB family were also downregulated in MM. Consistent with our results on CD85j expression, *lilrb2* (CD85d) and *lilrb3* (CD85a) were significantly decreased in PCs from patients with MM compared to PCs from individuals with MGUS.

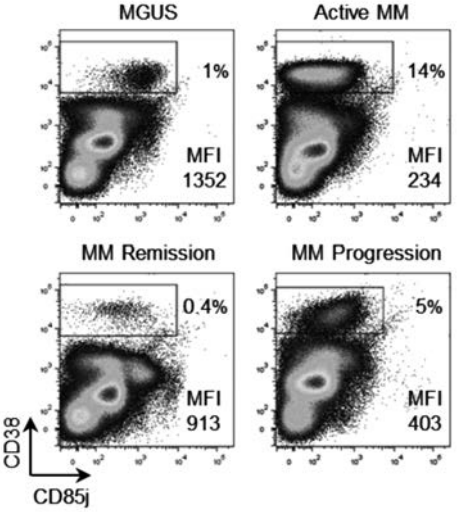

Figure 1. Flow cytometry analysis of bone marrow samples. Frequency of plasma cells and CD85j MFI are shown.

Summary/Conclusions: Similar to loss of tumor suppressor function, loss of inhibitory immune checkpoints such as CD85j may provide a selective advantage for malignant cell clones promoting immune escape and tumor survival. In this emerging era of cancer immunotherapies, it is crucial to better understand relevant immune checkpoint signaling playing a role in tumor progression. Further investigation is needed to discover whether downregulation of inhibitory receptors on malignant PC may provide a novel mechanism of immune escape associated with myeloma progression.

P264

PROTEIN KINASE CK1A INACTIVATION IN MULTIPLE MYELOMA EMPOWERS LENALIDOMIDE INDUCED CYTOTOXICITY AND CELL CYCLE ARREST
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Background: Multiple myeloma (MM) is a malignant plasma cell neoplasm, accounting for about 10% of all blood tumors. Despite the introduction of proteasome inhibitors and immunomodulatory drugs (such as lenalidomide) in the therapy of MM, it remains still incurable. Recently, it was shown in myelodysplastic syndromes that lenalidomide determines the degradation of Protein Kinase CK1α. CK1α is a serine/threonine kinase essential for the function of signaling pathways that could be involved in MM pathobiology: the canonical Wnt/β-catenin cascade, the Hedgehog and the NF-κB pathways and the p53-driven response (here by stimulating the binding to Mdm2 and p53 inhibition). Recent evidence indicates that protein kinase CK1α may play a key role in myeloma cell growth and its inactivation determines MM cell death.
Aims: In this study we have investigated CK1α expression, cellular localization and its contribution to MM survival. We investigated the therapeutic potential of the combination of lenalidomide and CK1α inactivation analyzing whether lenalidomide could cause CK1α degradation and whether CK1α could take part in lenalidomide-induced MM cell apoptosis and cell cycle arrest. We next studied whether blocking CK1α αcould influence pro-survival signalling pathways, accounting for resistance to lenalidomide.
Methods: CK1α expression and activity were evaluated in MM cells and controls; the consequences of CK1α inhibition, with a small ATP-competitive compound (D4476) or by RNA interference in association with lenalidomide, on MM cell survival and proliferation were investigated with Annexin V/PI and Ki-67/PI staining and FACS analysis or PARP and caspase cleavage, p21 and Mdm2 protein expression level detection. CK1a-dependent signalling events were analyzed by WB.
Results: We found that CK1α is overexpressed in a fraction of MM patient and cell lines. CK1α inactivation caused MM apoptosis and cell cycle arrest. Treatment of cells with lenalidomide not only caused MM death and cell cycle impairment, but strikingly determined a time-and dose-dependent reduction of CK1α expression and activation (monitored with a reduction of its target Ser 45 β-catenin). A therapeutic potential of the combination of lenalidomide and CK1α inactivation was established. Remarkably, the association of lenalidomide and D4476 increased MM cell apoptosis and reduced the S-G2/M and G1 phases of the cell cycle, suggesting a cooperative effect of the two compounds. Ki-67 staining showed a clear reduction of active proliferating cells in the combination therapy. Activation of apoptosis and derangement of the cell cycle were also confirmed by the raise of p21 with concomitant decline of Mdm2 protein levels (indicating an activation of p53 axis) in the combination treatment compared to the single treatments. Most importantly these results were confirmed by RNA interference using siRNAs targeting the α isoform of CK1.
Summary/Conclusions: Our findings suggest that the combination of lenalidomide and CK1α inactivation could be a promising therapeutic approach for MM therapy. The identification of the exact mechanism of action and of the pathways targeted by CK1α/lenalidomide in MM plasma cells is the subject of future research.

P265

QUANTIFICATION OF CYCLIN D1, CYCLIN D2, IKAROS, AIOLOS AND CEREBLON PROTEINS BY CAPILARY IMMUNOASSAY IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA AND ANALYSIS OF THEIR IMPACT ON PATIENT SURVIVAL

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Background: Multiple myeloma has been comprehensively analyzed using high-throughput genomic technologies. Although a large number of biomarkers have been described, most of them were not subsequently validated at the protein level. In fact, the unresolved difficulties in studying the proteome have made the quantification of messenger RNA (mRNA) an indirect measure of protein expression. However, many studies have shown that levels of mRNA cannot be used as surrogates for protein levels. The amount of myeloma cells obtained after purification of patient samples is usually very limited, which precludes the posibility of quantify protein levels using standard Western Blot analysis.
Aims: To quantify the steady state levels of cyclin D1, cyclin D2, ikaros, aiolos and cereblon proteins, and correlate them with the corresponding gene expression (mRNA). To analyze the impact of the protein expression compared to gene expression on MM prognosis.
Methods: Bone marrow aspirates from 64 newly diagnosed MM patients treated with lenalidomide- and bortezomib-containing regimens were included in the study. Myeloma cells were purified by anti-CD138 magnetic microbeads using the AutoMACs separation system (purity was above 90%) and next, were stored in RLT+ buffer at -80^{0}C. We developed and optimized a protocol for extraction of DNA, RNA and proteins from the same single sample. Total protein quantification and particular proteins expression analysis were performed using the capillary electrophoresis with immunoassay methodology (patented by the ProteinSimple company as WES system). mRNA expression was quantified using Taqman assays by q-RT-PCR.
Results: After the analysis of total protein content, 61 MM samples fulfilled the quantity and quality requirements. Cyclin D1 protein levels were strongly correlated with mRNA levels (R^2 *0.804, p<0.01)*. The highest cyclin D1 protein expression was found in MM samples with t(11;14), whereas intermediate cyclin D1 levels were observed in those patients with polysomy 11. Lower correlations between protein and mRNA levels were observed for cyclin D2/*CCND2* (R^2 *0.409, p<0.01),* aiolos/*IKZF3* ($R^2$0.383, p<0.01) and ikaros/*IKZF1* (R^2 *0.337, p<0.01).* On the contrary, no significant correlation was seen for cereblon protein levels and the corresponding *CRBN* expression. Higher protein levels of cereblon and ikaros were significantly associated with longer progression free survival (PFS) and overall survival (OS). Interestingly, no impact on prognosis was observed when survival analysis was carried out using the mRNA levels of *CRBN, IKZF1* and *IKZF3.*

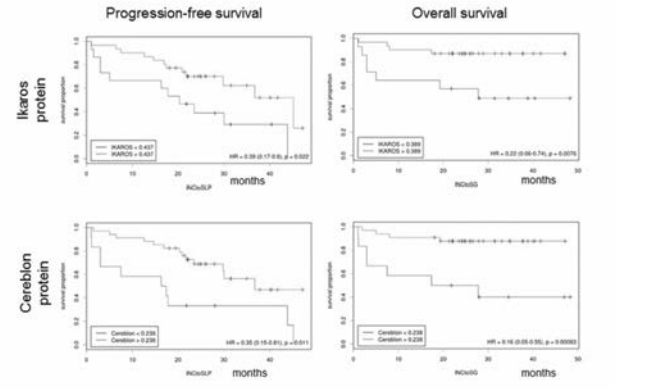

Figure 1.

Summary/Conclusions: The capillary electrophoresis with immunoassay methodology allowed us to analyze the steady state expression of mRNA and protein obtained from the same one MM sample. The preliminary results showed that protein measurement of cereblon and ikaros discriminate prognosis of patients with MM better than mRNA levels. The Capilary Immunoassay offers the novel opportunity to automatically analyze, for the first time, the expression of proteins encoded by key genes in the development of MM, with high levels of sensitivity and reproducibly.
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P266

DEFERASIROX INDUCES APOPTOSIS THROUGH INHIBITION OF ONCOGENIC PYK2/BETA-CATENIN SIGNALING IN MULTIPLE MYELOMA CELLS

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Background: Iron is an element essential for many indispensable cellular functions, including proliferation and DNA synthesis. Accumulating evidence suggests that abnormal iron metabolism plays an important role in carcinogenesis and in the progression of many cancers. In cancer cells, the demand for iron increases in response to sustained, accelerated cell proliferation and DNA synthesis. Multiple myeloma (MM) is characterized by clonal proliferation of long-lived plasma cells within the bone marrow. Despite recent advances in its treatment, MM remains an incurable disease, underlining the need to continue exploring its molecular characteristics. Recently, two independent groups reported that serum ferritin, which is used as a marker for iron overload, can be a negative prognostic indicator in MM. However, the underlying mechanisms of this effect have not been clarified, and the significance of iron metabolism in MM cells remains unclear.
Aims: Our study was aimed to investigate iron metabolisms and iron chelation therapy with deferasirox (DFX) in MM cells.
Methods: We analyzed the prognostic relevance of iron metabolism-related genes by integrating a data set in the public domain (MAQC-II Project MM data set). We also evaluated mRNA expression of iron metabolism-related genes in MM cell lines and primary cells. Intracellular iron concentrations were measured with fluorescent probes FeRhoNox-1. The effect of DFX, an orally active, long-acting iron chelator approved for iron overload, on MM cell lines was examined using a WST-1 cell proliferation assay. To elucidate the cytotoxic mechanism of DFX, immunoblotting and flow cytometry analysis were performed. To clarify

the mechanisms of DFX-induced apoptosis in MM, we analyzed the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway using PCR arrays. Subsequently, Pyk2 downstream target molecules were analyzed with immunoblotting and qPCR. To test the therapeutic potential of DFX, we evaluated its ability to suppress tumor growth *in vivo* using the subcutaneous RPMI8226 murine xenograft model of human MM.
Results: Overall survival was significantly longer MM patients with high ferroportin levels, a cellular iron exporter. qRT-PCR studies showed lower *ferroprtin* expression in both cell lines and primary cells compared to control cells. Increased intracellular iron concentrations were noted in MM cells. DFX induced apoptosis in MM cells and overcame the survival advantage conferred by the bone marrow microenvironment. The FAK transcript was substantially suppressed following DFX treatment in PCR array. However, no expression of FAK protein in three of four MM cell lines was observed. Next, we investigated Pyk2, which is a member of the FAK family and has a 48% amino acid sequence identify with FAK. We assessed Pyk2 activation after treatment with DFX. DFX significantly reduced phosphorylation of Pyk2(Tyr402), resulting in inactivation of Pyk2 activity. beta-catenin protein levels were decreased by DFX treatment, accompanied by decreased levels of phosphorylated-GSK-3beta (inactive form). Treatment with DFX reduced the mRNA levels of *Axin2* and *c-Myc*, which are Wnt target genes; in particular, *Axin2* is a robust and specific Wnt target gene. We detected an increase in apoptotic tumor cells in animals treated with DFX compared to vehicle-treated mice, as evaluated by Cleaved PARP staining and immunoblotting.
Summary/Conclusions: This study has demonstrated that low expression of *ferroportin* is associated with a poor prognosis in MM, and had been revealed that increased intracellular iron contributes to MM pathogenesis. Our results suggest the potential clinical application of DFX for iron overloaded and/or Pyk2/beta-catenin driven MM treatment.

P267

DEVELOPMENT AND IMPLEMENTATION OF A HIGH-THROUGHPUT SEQUENCING BASED, CLINICALLY-FOCUSED, COMPREHENSIVE GENOMIC CHARACTERISATION OF MYELOMA WITHIN A MOLECULAR DIAGNOSTIC SERVICE

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Background: In the genomic context, treatment decisions in patients with myeloma are currently influenced by (i) the presence of copy number changes (*e.g.* TP53) and balanced chromosomal translocations (*e.g.* t(4;14)) (ii) response to therapy as assessed by minimal residual disease (MRD) testing (*e.g.* with IGVH MRD) and (iii) the presence of potentially targetable mutations (*e.g.* BRAF V600E). In addition, there are numerous novel genomic factors that may influence treatment including gene expression based prognostic scores, gene expression signatures indicating sensitivity to novel therapeutics and response to therapy as measured by circulating tumour DNA (ctDNA) monitoring.
Aims: We aimed to develop, validate and implement a suite of molecular tools and workflow in a molecular diagnostic laboratory setting to enable comprehensive molecular characterisation of multiple myeloma biosamples, prioritising genetic lesions that now or may in the future influence patient management. Moreover, we aimed to utilise this molecular workflow to establish a local compendium of genomic data on cases of myeloma.
Methods: DNA and RNA is extracted from CD138+ MACS selected plasma cells from patients undergoing bone marrow biopsies for either newly diagnosed or relapsed myeloma at Peter MacCallum Cancer Centre (Melbourne, Australia). Amplicon library preparation is performed using a Fluidigm Access Array IFC. Whole transcriptome RNA-seq RNA libraries are prepared using Illumina TruSeq RNA. Low-coverage Whole Genome Sequencing (LC-WGS) is performed using DNA libraries prepared by Kapa Hyper Library Prep (Kapa Biosystems). Sequencing is performed on an Illumina NextSeq (RNA-seq and LC-WGS) and an Illumina MiSeq (amplicon panel).
Results: Targeted amplicon sequencing is performed using an in-house designed panel which includes the most frequently mutated genes in myeloma (BRAF, NRAS, KRAS and TP53). This provides relatively rapid feedback to clinical teams on the presence of genetic lesions which may direct management. Assessment of myeloma samples is aligned with other next-generation sequencing amplicon assays within the laboratory in order to minimise turnaround times and achieve efficiencies in laboratory workflow. Whole transcriptome RNA-seq is performed and used for (i) variant calling to correlate with the mutations identified from the targeted-amplicon panel and also as a discovery platform for novel mutations (ii) assessment against established gene expression profile classifiers for cytogenetic lesions and prognosis (*e.g.* GEP70) and (iii) assessment of expression of therapeutic targets (*e.g.* CD38, SLAMF7, CRBN). LC-WGS is then used to detect copy number changes. During the validation phase, findings from RNA-seq and LC-WGS were validated using orthogonal methods including conventional metaphase cytogenetics and FISH. Novel bioinformatics and clinical informatics tools including a copy number calling pipeline, RNA-seq variant calling and differential expression pipeline, copy number browser and an IGVH analysis interface have been developed to analyse and report samples. Approximately 50 samples have been processed with this workflow to date with the results used to inform patient management.
Summary/Conclusions: We have successfully developed and implemented a suite of molecular testing for multiple myeloma within a molecular diagnostic laboratory to detect key genomic lesions which affect patient management as well as establishing a local discovery-level data set to answer future research questions.

P268

DYNAMIC EVOLUTION OF COPY NUMBER ABERRATIONS ACCOMPANIES DISEASE RELAPSE IN MULTIPLE MYELOMA

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Background: Despite novel and effective therapies, the clinical course of multiple myeloma is that of a relapsing-remitting disease. In general, remission times shorten with each subsequent therapy, finally resulting in refractory disease. Changes in the predominance of molecular subclones have been implicated in disease relapse.
Aims: To identify the longitudinal changes in predominant copy number aberrations in myeloma patients from new diagnosis to first relapse.
Methods: Matched presentation and relapse CD138 immunomagnetically selected bone marrow tumour material from 69 patients treated in the NCRI Myeloma XI trial were analysed. Median time between samples was 19 months (range 2 – 39 months). Cases were molecularly profiled using a combination of MLPA and qRT-PCR (Boyle EM, *et al.* Gen Chrom Canc 2015; Kaiser MF, *et al.* Leukemia 2014). Copy number by MLPA is inferred by comparison of target regions against control regions.
Results: Three regions showed frequent changes in copy number from diagnosis to relapse: TP53 at 17p, CKS1B at 1q, and MYC at 8q. In 20% of cases, copy number of at least one of the regions changed between the timepoints. The frequency of predominant clones with TP53 deletion increased from 10.1% at presentation to 15.9% at relapse with five patients showing a novel TP53 deletion at first relapse. Copy number of CKS1B changed in 16% of cases between presentation and relapse: eight cases demonstrated novel gain or amplification of CKS1B, whereas in three cases copy number gain of CKS1B reverted to normal at relapse. The frequency of gain(1q) was 34.7% at presentation and 42.0% at relapse. A novel, predominant clone with gain of MYC was detected in four cases at relapse. Gain of MYC reverted to normal in three cases from presentation to first relapse.
Summary/Conclusions: We found dynamic changes in predominant clones between presentation and first relapse. Deletion of TP53/del(17p) and gain(1q) were more frequent at relapse than at presentation, indicative of clonal advantage under therapeutic selective pressure early in the disease course. Although novel gains of 8q suggest a clonal advantage for MYC, this appears to be context-dependent and further longitudinal studies are required to assess its impact over time.

Innovative therapies for MM 1

P269

IMPACT OF CYTOGENETIC RISK STATUS ON EFFICACY AND SAFETY OF IXAZOMIB-LENALIDOMIDE-DEXAMETHASONE (IRD) VS PLACEBO-RD IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS IN THE GLOBAL TOURMALINE-MM1 STUDY

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Background: The phase 3 TOURMALINE-MM1 study (NCT01564537) showed a significant 35% improvement in progression-free survival (PFS) with IRd *vs* placebo-Rd (hazard ratio [HR] 0.742, p=0.012) in 722 patients (pts) with relapsed/refractory multiple myeloma (RRMM) (Moreau et al, ASH 2015). Data from this study led to the approval by the US FDA of ixazomib, in combination with Rd, for the treatment of pts with MM who have received at least one prior therapy.
Aims: A key secondary endpoint was overall survival (OS) in patients with del(17p), and another secondary endpoint was outcome in patients with high-risk cytogenetic abnormalities. Here, we analyze the efficacy and safety of IRd *vs* placebo-Rd by cytogenetic risk status.
Methods: Pts with RRMM were randomized 1:1 to receive IRd or placebo-Rd (ixazomib 4 mg or matching placebo on days 1, 8, and 15, plus lenalidomide 25 mg on days 1–21 and dexamethasone 40 mg on days 1, 8, 15, and 22, in 28-day cycles) until disease progression or unacceptable toxicity. Stratification factors were number of prior therapies (1 *vs* 2 or 3), proteasome inhibitor-exposed *vs* -naïve status, and ISS stage I or II *vs* III. Cytogenetic risk status was assessed using FISH at a CLIA-certified central laboratory. High-risk abnormalities were defined as del(17p), t(4;14) or t(14;16); cut-off values for these abnormalities were, per protocol, based on the false-positive rates (technical cut-offs) of the Kreatech FISH probes used, which were 5%, 3%, and 3% positive cells, respectively. The standard-risk group consisted of all other patients with known baseline cytogenetics. Post-hoc analyses were performed using different cut-off values for del(17p) and t(4;14).

Table 1.

	Median PFS, mos		
Pts	IRd	Placebo-Rd	HR
All (N=722)	20.6	14.7	0.742
Standard-risk cytogenetics (n=415)	20.6	15.6	0.640
High-risk cytogenetics (n=137)	21.4	9.7	0.543
del(17p) alone or with t(4;14) and/or t(14;16) (n=69)	21.4	9.7	0.596
t(4;14) alone (n=61)	18.5	12.0	0.645

Using cut-offs of 5% positive cells for del(17p), 3% for t(4;14), and 3% for t(14;16)

Results: A total of 552 patients (76% of the 722 pts enrolled) had cytogenetic results (97% central laboratory-confirmed), of whom 137 had high-risk abnormalities, 75 in the IRd arm and 62 in the placebo-Rd arm. After a median follow-up of ~15 mos, PFS was improved with IRd *vs* placebo-Rd in both pts with high-risk and pts with standard-risk cytogenetics (Table); in high-risk pts, the median PFS with IRd was similar to that in all pts and in standard-risk pts. The PFS benefit with IRd *vs* placebo-Rd was consistent using different cut-off values for defining del(17p) and t(4;14) positivity. For del(17p), median PFS with IRd *vs* placebo-Rd was 21.4 *vs* 6.7 mos (HR 0.611) with a 20% cut-off (n=59), and 15.7 *vs* 5.1 mos (HR 0.49) with a 60% cut-off (n=33). For t(4;14) alone using a cut-off of 10% (n=59), median PFS was 18.5 *vs* 12.0 mos (HR 0.444) with IRd *vs* placebo-Rd. Median time to progression with IRd *vs* placebo-Rd was 21.4 *vs* 12.0 mos and 20.6 *vs* 15.9 mos in high-risk and standard-risk pts. Overall response rate with IRd *vs* placebo-Rd was 79% *vs* 60% in high-risk pts and 80% *vs* 73% in standard-risk pts, including CR+VGPR rates of 45% *vs* 21% and 51% *vs* 44%, respectively. At data cut-off, OS data were not mature, with 8 (11%) deaths in the IRd arm *vs* 13 (21%) in the Rd arm for high-risk pts, and 21 (11%) deaths in the IRd arm *vs* 32 (15%) in the Rd arm for standard-risk pts. At a 23-month analysis, rates of grade ≥3 adverse events (AEs) with IRd *vs* placebo-Rd were 66% *vs* 73% in high-risk pts and 75% *vs* 65% in standard-risk pts; rates of serious AEs were 42% *vs* 52% and 45% *vs* 47%, respectively.
Summary/Conclusions: IRd showed substantial benefit *vs* placebo-Rd in RRMM pts with high-risk cytogenetic abnormalities, irrespective of the cut-offs used, with limited additional toxicity. Molecular studies, including analyses of the impact of p53 mutation on outcomes, will also be presented.

P270

HIGH CUT-OFF HAEMODIALYSIS (HCO-HD) DOES NOT IMPROVE OUTCOMES IN MYELOMA CAST NEPHROPATHY: RESULTS OF EUROPEAN TRIAL OF FREE LIGHT CHAIN REMOVAL BY EXTENDED HAEMODIALYSIS IN CAST NEPHROPATHY (EULITE)

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Background: Severe acute kidney injury (AKI) secondary to myeloma cast nephropathy (MCN) and requiring acute dialysis is associated with poor patient survival. MCN is caused by the pathogenic (involved) immunoglobulin serum free light chain (sFLC). High cut-off haemodialysis (HCO-HD) can remove large quantities of sFLC and retrospective uncontrolled clinical trials using HCO-HD have reported renal function recovery (from dialysis) in over 60% of patients. This is compared to historic reports of 20% renal recovery. Recovery of renal function is associated with improved survival. However, it is not known if these better outcomes were associated with HCO-HD or novel chemotherapy regimens. There have been no RCTs of the efficacy of HCO-HD or the efficacy of bortezomib based chemotherapy in patients with MCN and severe AKI requiring dialysis treatment.
Aims: To carry out a prospective multi-centre RCT in patients with newly diagnosed MM and associated MCN who required acute dialysis treatment, comparing HCO-HD to standard high flux (HF)-HD, and using bortezomib based chemotherapy as the standard of care.
Methods: Ninety patients with a first diagnosis of MM & MCN confirmed by kidney biopsy, sFLC levels of the involved LC >500 mg/l (Freelite™), and a requirement for acute dialysis, were randomised to receive HCO-HD or standard HF-HD. HCO-HD was carried out with two 1.1 m^2 Baxter-Gambro HCO 1100 dialyser in series. The dialysis regimen was based on studies that identified an optimal protocol for FLC removal. All patients received bortezomib, doxorubicin, and dexamethasone chemotherapy to a maximum of 8 cycles. Primary outcome was independence of dialysis at 3-months. Patients were followed for 2-years. Other pre-defined outcomes included; 3-week sFLC levels, overall renal recovery, stem-cell transplantation, 2-year OS. All results are reported as intention to treat (ITT). Where statistical tests are not stated the chi-squared test is reported.

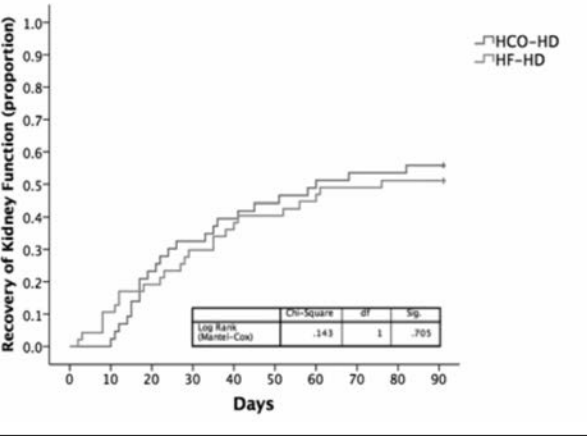

Figure 1.

Results: There was no difference between groups for sFLC levels (mg/L, median, range (Mann-Whitney test)) by isotype at randomisation. These were: kappa LC; HCO- HD 12000 (1839-61900), HF-HD 11800 (1200-76322), lambda LC; HCO-HD 6031 (1950-33196), HF-HD 8400 (574-38400). There was no difference at randomisation between HCO-HD and HF-HD for age, ethnicity, gender, myeloma type (light chain only *vs* intact Ig). Serum FLC levels levels on the first full protocol dialysis decreased for kappa LC and lambda LC by 75.6% and 71.2% for HCO-HD, and 20.2% and 9.1% for HF-HD, respectively (P<0.001). However, there was no difference in the percentage reduction of sFLC level from baseline at 3 weeks between HCO-HD and HF-HD. 24/43 patients (55.8%) in the HCO-HD group and 24/47 patients (51.6%) in the HF-HD group recovered renal function by 3 months (not significant, NS). Kaplan–Meier analysis is shown in the figure. Overall recovery of renal function was 62%; 58.1% in the HCO-HD group, 66% in the HF-HD group (NS). 35% in the HCO-HD group and 32.2% in the HF-HD received a stem-cell transplant (NS). OS at 2-years was 24 (55.8%) in the HCO-HD group and 36 (76.6%) in the HF-HD group (P=0.037; odds ratio (95% confidence interval), 1.583 (1.048-2.392)). There were more lung infections in the first 3-months in the HCO-HD group (12 *vs* 3, P=0.014).

Summary/Conclusions: In this RCT, where all patients received bortezomib based chemotherapy, 62.2% of patients recovered renal function and overall survival at 2-years was 66.7%. HCO-HD, compared to HF-HD, did not improve renal recovery rates in patients with severe AKI secondary to MCN. There was worse overall survival in the HCO-HD group.

P271

OPROZOMIB, POMALIDOMIDE, AND DEXAMETHASONE (OPOMD) IN PATIENTS (PTS) WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (RRMM): INITIAL RESULTS OF A PHASE 1B STUDY (NCT01999335)

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Background: Oprozomib (OPZ), an irreversible proteasome inhibitor has demonstrated activity in pts with RRMM (Vij, *Haematologica* 2015;100[suppl 1]:abst P646; Hari, *Haematologica* 2015;100[suppl 1]:abst P653).

Aims: The primary objective of the phase 1b portion of this study was to determine the maximum tolerated dose (MTD) and recommended phase 3 dose (RP3D) of OPZ in the OPomd regimen and to evaluate safety and tolerability. Secondary objectives included estimating overall response rate (≥partial response) and clinical benefit rate (≥minimal response), and evaluating OPZ pharmacokinetics (PK).

Methods: Pts with RRMM (≥2 prior regimens) were eligible; pts must have received ≥2 consecutive cycles of both bortezomib and either lenalidomide or thalidomide. Prior carfilzomib (CFZ) was allowed if CFZ was not discontinued due to an adverse event (AE). Pts received OPZ orally once-daily on days (D) 1-5 and 15-19 (*i.e.*, 5/14 schedule) or on D1, 2, 8, 9, 15, 16, 22, 23 (*i.e.*, 2/7 schedule) of 28-day cycles. Pts received pomalidomide (POM) orally on D1–21 and dexamethasone (DEX) 20 mg orally on D1, 2, 8, 9, 15, 16, 22, 23. The starting OPZ dose in the 5/14 schedule was 150mg/day and in the 2/7 schedule was 210mg/day; subsequent escalation was in a standard 3+3 dose-escalation schema. The starting POM dose for both schedules was 4mg.

Results: Thirty-one pts were enrolled (5/14 schedule, n=4; 2/7 schedule, n=17 and n=10 for the expansion arm). In the 5/14 schedule, pts received OPZ 150 mg/day + POM 4 mg/day (n=3); or OPZ 150 mg/day + POM 2 mg/day (n=1). In the 2/7 schedule, pts received OPZ 210 mg/day + POM 4 mg/day (n=17 including expansion arm patients); or OPZ 240 mg/day + POM 4 mg/day (n=10). Median age was 57 yr (5/14 schedule), 61 yr (2/7 schedule OPZ 210 mg/d) and 71 yr (2/7 schedule OPZ 240 mg/d). Pts received a median (range) of 7 (3-22) prior regimens in the 5/14 schedule, 3 (2-8) in the 2/7 schedule OPZ 210 mg/d and 3.5 (1-11) in the 2/7 schedule OPZ 240 mg/d. At data cutoff (10/26/15), pts in the 5/14 and 2/7 schedules had been on treatment for a median duration of 17.8 wk (range, 3.3-38.6) and 19.3 wk (range, 3.3-50.9) for all study drugs, respectively. Three dose-limiting toxicities (DLTs) were reported in 2 pts in the 5/14 schedule: 1 pt grade (Gr) 3 mucositis and rash; 1 pt Gr 3 abdominal distention and impaired cognitive function. One DLT occurred in the 2/7 schedule (OPZ 210 mg/d): Gr 3 gastric hemorrhage with severe erosive gastritis identified in endoscopy. One pt in the 5/14 schedule (25%) and 1 pt (5.9%) in the 2/7 schedule OPZ 210 mg/d discontinued treatment due to an AE. No pts died on study. The most common hematologic and of clinical interest AEs (any Gr and Gr ≥3) are shown in the table. In the 2/7 schedule, a partial response or better was reported in 71% of the OPZ 210 mg/d patients (and in 4 of 5 CFZ-refractory patients) and 50% of the OPZ 240 mg/day patients; 50% of patients in the 5/14 schedule also achieved partial response or better. Considering current measures of variability and sample size, the PK of OPZ, when given in combination with POM and DEX, were generally consistent with previous OPZ studies.

Table 1. Adverse events of any grade occurring in >25% and grade ≥3 adverse events occurring in ≥10% of patients.

AE, n (%)	5/14 Schedule OPZ150 mg/d (n= 4)		2/7 Schedule OPZ 210 mg/d (n= 17)		2/7 Schedule OPZ240 mg/d (n= 10)	
	Any grade	Grade ≥3	Any grade	Grade ≥3	Any grade	Grade ≥3
Hematologic						
Anemia	2 (50)	2 (50)	10 (59)	8 (47)	3 (30)	2 (20)
Neutropenia	1 (25)	1(25)	6 (35)	6 (35)	2 (20)	0
Thrombocytopenia	1 (25)	0	6 (35)	4 (24)	1 (10)	0
Decreased platelet count	0	0	0	0	3 (30)	2 (20)
Decreased neutrophil count	0	0	4 (24)	3 (18)	2 (20)	1 (10)
Febrile neutropenia	0	0	3 (18)	3 (18)	0	0
Clinical interest						
Nausea	2 (50)	0	10 (59)	0	10 (100)	1 (10)
Diarrhea	2 (50)	0	14 (82)	2 (12)	9 (90)	4 (40)
Vomiting	3 (75)	0	7 (41)	0	8 (80)	1 (10)
Constipation	2 (50)	0	6 (35)	0	6 (60)	0
Peripheral neuropathy	0	0	1 (6)	0	4 (40)	1 (10)
Acute kidney injury	0	0	1 (6)	0	1 (10)	1 (10)
Gastric hemorrhage	0	0	1 (6)	1 (6)	0	0

AE, adverse event; OPZ, oprozomib

Summary/Conclusions: Initial results from this ongoing phase 1b study suggest that the combination of OPomd has significant anti-myeloma activity, is active in a highly refractory patient population, and is generally well tolerated. The most common grade ≥3 AEs observed were anemia (47%) and diarrhea (11%) with 210 mg OPZ in the 2/7 schedule. The MTD of OPZ in combination with POM/DEX was not reached on either schedule.

P272

PHASE 1B STUDY OF VENETOCLAX COMBINED WITH BORTEZOMIB AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: BCL-2 and MCL-1 promote multiple myeloma (MM) cell survival. Bortezomib (BTZ) inhibits MCL-1 activity, and venetoclax (VEN), an oral, selective BCL-2 inhibitor, enhances its efficacy in MM xenograft models.

Aims: The objectives of the study are to evaluate safety and preliminary efficacy of VEN with BTZ and dexamethasone (Dex) in relapsed/refractory (RR).

Methods: Patients received daily VEN, per assigned dose escalation cohorts (50-1200mg), combined with BTZ (1.3mg/m^2 SC) and Dex (20mg PO) for 11 cycles, and then VEN alone thereafter.

Table 1.

Best response by prior B status, n (%)	B Naïve (n=6)	B Sensitive (n=17)	B Refractory (n=18)	All (n=41)
ORR	6 (100)	12 (71)	3 (17)	21 (51)
≥VGPR	5 (83)	6 (35)	0	11 (27)
sCR	1 (17)	1 (6)	0	2 (5)
CR	1 (17)	1 (6)	0	2 (5)
VGPR	3 (50)	4 (24)	0	7 (17)
PR	1 (17)	6 (35)	3 (17)	10 (24)
MR	0	0	2 (11)	2 (5)
SD	0	2 (12)	4 (22)	6 (15)
PD	0	3 (18)	8 (44)	11 (27)
DC before assessment	0	0	1 (6)	1 (2)
Median (range) time on study*, months	9.8 (0.6–16.5)	5.5 (0.5–18.3)	1.3 (0.3–13.5)	4.9 (0.3–18.3)

*All patients (n=45): 1st dose to cut-off (active patients) or last dose (DC patients)

Results: 45 patients were enrolled as of 9/17/15. Median age was 65, with 29 males and 16 females; 24 patients had ISS stage II/III. The median (range) number of prior lines of therapy was 4 (1-13). 38 patients received prior BTZ (19 refractory), 36 had lenalidomide (26 refractory), and 32 had prior SCT. Adverse events occurring in ≥30% of patients were constipation (40%), diarrhea (38%), thrombocytopenia (33%), and insomnia (29%). Grade 3/4 adverse events in ≥10% were thrombocytopenia (22%), and anemia (16%). Serious adverse events in ≥2 patients were pneumonia (n=3), cardiac failure, embolism, pyrexia, respiratory failure, sepsis, and thrombocytopenia (n=2 each). One dose limiting toxicity was observed, and maximum tolerated dose was not reached. 30 patients discontinued (DC): 24 related to disease progression (3 died), 2 due to adverse events, and 4 withdrew consent. Dose-normalized PK for VEN with BTZ+Dex was similar to VEN monotherapy. 41 out of45 patients were evaluable for efficacy (Table 1). Preliminary results also indicate that

patients who had received 1-3 prior lines of therapy (N=18) had higher ORR (83.3%) as compared to patients who had received 4-6 (N=13, 38.5% ORR) or ≥7 prior lines of therapy (N=10, 10% ORR).
Summary/Conclusions: Venetoclax with bortezomib and dexamethasone has an acceptable safety profile and evidence of anti-tumor activity in RR MM; response rates were highest in patients who were naïve or sensitive to prior bortezomib, or had received 1–3 prior therapies. Currently, dose escalation is complete and safety expansion is ongoing at 800 mg.

P273

A PHASE IB STUDY OF THE AKT INHIBITOR AFURESERTIB IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: FINAL RESULTS WITH LONG TERM FOLLOW-UP

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Background: The PI3K/AKT pathway is constitutively active in multiple myeloma (MM), providing proliferative and anti-apoptotic signals, and contributing to drug resistance in preclinical models. Afuresertib is an orally bioavailable, ATP-competitive, reversible inhibitor of all 3 AKT kinases. Single-agent activity has been seen in patients (pts) with heavily pretreated MM. Preclinical studies have shown that combining afuresertib with bortezomib results in downstream target inhibition and synergistic cell death in myeloma cell lines.
Aims: The aims of the current study were to determine the safety, tolerability, and MTD of afuresertib when dosed in combination with bortezomib and dexamethasone for pts with relapsed/refractory (R/R) MM. Secondary endpoints included pharmacokinetics (PK), pharmacodynamics (PD), and clinical activity.
Methods: In this Phase Ib open-label study, pts (aged ≥18 years, ECOG PS ≤2) with MM who had failed ≥1 line of systemic therapy were enrolled into the dose escalation part of the study (part 1). The initial dose of afuresertib (75 mg QD) was increased in 25 mg increments until the MTD was reached. Bortezomib was dosed at 1.0 mg/m^2 IV in the first cohort and 1.3 mg/m^2 IV for all other cohorts. Dexamethasone was administered at 20 mg (40 mg for the MTD cohort) on the same days as bortezomib. Pts received induction with afuresertib, bortezomib, and dexamethasone for eight 21-day cycles, and then continued single-agent afuresertib until treatment discontinuation criteria were met. Pts were enrolled into the dose expansion part of the study (part 2) at the MTD. Two cohorts were explored in part 2: a PK/PD and a safety/efficacy cohort. Pts with bortezomib-refractory disease were eligible for part 1, but were excluded in part 2.

Table 1. Clinical activity of afuresertib in combination with bortezomib category.

Cohort	Summary of overall response rate by prior bortezomib category			
	Bortezomib naïve (n=17)	Bortezomib relapsed (n=49)	Bortezomib refractory (n=24)	All patients (N=90)
Part 1 dose escalation cohort (N=34)	- sCR: 0/3 - CR: 0/3 - VGPR: 0/3 - PR: 2/3 (67%) sCR+CR+VGPR+PR: 2/3 (67%), 95% CI: [9.4%,99.2%]	- sCR: 0/17 - CR: 1/17 (6%) - VGPR: 1/17 (6%) - PR: 6/17 (35%) sCR+CR+VGPR+PR: 8/17 (47%), 95% CI: [23.0%,72.2%]	- sCR: 0/14 - CR: 0/14 - VGPR: 2/14 (14%) - PR: 2/14 (14%) sCR+CR+VGPR+PR: 4/14 (29%), 95% CI: [8.4%,58.1%]	- sCR: 0/34 - CR: 1/34 (3%) - VGPR: 3/34 (9%) - PR: 10/34 (29%) sCR+CR+VGPR+PR: 14/34 (41%), 95% CI: [24.6%,59.3%]
Part 2 PK/PD cohort (N=10)	NA	NA	- sCR: 0/10 - CR: 0/10 - VGPR: 1/10 (10%) - PR: 3/10 (30%) sCR+CR+VGPR+PR: 4/10 (40%), 95% CI: [12.2%,73.8%]	- sCR: 0/10 - CR: 0/10 - VGPR: 1/10 (10%) - PR: 3/10 (30%) sCR+CR+VGPR+PR: 4/10 (40%), 95% CI: [12.2%,73.8%]
Part 2 safety/efficacy cohort (N=46)	- sCR: 0/14 - CR: 2/14 (14%) - VGPR: 3/14 (21%) - PR: 3/14 (21%) sCR+CR+VGPR+PR: 8/14 (57%), 95% CI: [28.9%,82.3%]	- sCR: 1/32 (3%) - CR: 3/32 (9%) - VGPR: 6/32 (19%) - PR: 12/32 (38%) sCR+CR+VGPR+PR: 22/32 (69%), 95% CI: [50.0%,83.9%]	NA	- sCR: 1/46 (2%) - CR: 5/46 (11%) - VGPR: 9/46 (20%) - PR: 15/46 (33%) sCR+CR+VGPR+PR: 30/46 (65%), 95% CI: [49.8%,78.6%]

CI, confidence interval; CR, complete response; NA, not available; PD, pharmacodynamics; PK, pharmacokinetics; PR, partial response; SCR, stringent complete response; SD, stable disease; VGPR, very good partial response.

Results: 90 pts were enrolled (34 in part 1, 10 in the PK/PD cohort, and 46 in the safety/efficacy cohort). Pts had a median age of 64 years and had received a median of 3 prior lines of treatment (range: 1-10). Pts received a median of 105 days (range: 5-1050) of afuresertib treatment and a median of 5 cycles (range: 0-8) of bortezomib plus dexamethasone. In the safety/efficacy cohort, median time on study was 119 days (range: 5-757). DLTs were reported in the 100 mg cohort (Grade [G] 2 ALT elevation, n=1), the 125 mg cohort (G3 erythema multiforme, n=1), and the 175 mg cohort (G3 rash, n=1; G3 rash/G3 diarrhea/G3 thrombocytopenia, n=1). The MTD and recommended dose for Phase 2 was established as 150 mg QD afuresertib plus 1.3 mg/m^2 bortezomib and 40 mg dexamethasone on Days 1, 4, 8, and 11. In the part 2 safety/efficacy cohort, the most common AEs were diarrhea (65%), fatigue (59%), nausea (52%), thrombocytopenia (37%), constipation (35%), hyperglycemia (35%), and anaemia (35%). G3/4 AEs occurring in >10% of pts were thrombocytopenia (26%), diarrhea (22%), hyperglycemia (15%), and orthostatic hypotension (13%). The overall response rate across all cohorts was 53% (95% CI: 42.5%,63.9%) and 65% (95% CI: 49.8%,78.6%) in the safety/efficacy cohort. Responses were observed in 8/24 pts who were refractory to prior bortezomib (Table 1). Afuresertib PK profiles were generally dose proportional and mean afuresertib exposure was similar alone or in combination with bortezomib and dexamethasone. Survival data will be presented at the meeting.
Summary/Conclusions: Treatment of R/R MM with afuresertib plus bortezomib and dexamethasone demonstrated a manageable safety profile and showed promising clinical activity. The PK profile of afuresertib was not affected by bortezomib or dexamethasone.

P274

UPDATED DATA FROM A PHASE II DOSE FINDING TRIAL OF SINGLE AGENT ISATUXIMAB (SAR650984, ANTI-CD38 MAB) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Isatuximab (ISA) is a humanized anti-CD38 monoclonal antibody with multiple modes of action for killing tumor cells through direct tumor targeting and immune cell engagement. We report updated data from an ongoing Phase II dose finding study of ISA monotherapy in patients with RRMM (NCT01084252).
Aims: Primary objective: evaluation of ISA activity (overall response rate [ORR; IMWG]).
Methods: Patients with RRMM (≥3 lines of anti-MM therapy or refractory to immunomodulatory drugs [IMiDs] and proteasome inhibitors [PIs]) were randomized to ISA 3 mg/kg Q2W, 10 mg/kg Q2W x 2 cycles then Q4W, or 10 mg/kg Q2W. Randomization stratified by prior pomalidomide and/or carfilzomib therapy. Emerging PK data prompted enrollment of a 4th treatment arm at 20 mg/kg QW x 4 doses then Q2W. 1 cycle=28 days. All patients signed an IRB approved informed consent.
Results: 97 patients treated: median age, 62.5 (38-85) y; median time from diagnosis, 5.9 (1.2-24.1) y; ISS stage 3, 37%; median prior lines of therapy, 5 (2-14); 86%, 61%, 80%, 57%, 88% refractory to lenalidomide, pomalidomide, bortezomib, carfilzomib, or IMiD+PI, respectively. 24/56 cytogenetics-evaluable patients (43%) had t(4;14) and/or del(17p). Median treatment duration, 13.1 wks; 22 patients remain on treatment (cut-off Nov 2015). ORR: 9% (2/23), 20% (5/25), 29% (7/24) & 24% (6/25) at ISA 3 Q2W, 10 Q2W/Q4W, 10 Q2W, & 20 mg/kg QW/Q2W, respectively; 14/20 responders continue without progression. At ≥10 mg/kg: ORR was 24% (18/74), similar in subgroups (age, CrCl, prior lines of therapy), and 44% (8/18) in patients with abnormal cytogenetics. Median time to 1st response, 1.35 mo; median duration of response at data cut-off, 6.6 mo. Most common adverse events (AEs) were nausea (33%), fatigue (30%), dyspnea (26%), and cough (24%), which were typically grade ≤2. Infusion-associated reactions (IARs) occurred in 49% of patients, mostly grade ≤2, 94% during the 1st infusion. 6 patients discontinued therapy due to AEs, 2 due to IARs.
Summary/Conclusions: ISA monotherapy is active and generally well tolerated in heavily pretreated RRMM, with efficacy greatest at ≥10 mg/kg (ORR

24%). ORR was similar in subgroups, including high-risk cytogenetics. Progression-free survival and overall survival data will be presented.

P275

CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE VS LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA: ANALYSIS OF RESPONSE AND PROGRESSION-FREE SURVIVAL HAZARD RATIO OVER TIME

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Background: As previously reported, treatment with carfilzomib, lenalidomide, and dexamethasone (KRd) significantly improved progression-free survival (PFS) and treatment responses *versus* lenalidomide and dexamethasone (Rd) in patients (Pts) with relapsed multiple myeloma (ASPIRE; NCT01080391). Per trial protocol, carfilzomib (CFZ) was discontinued after 18 cycles (28 days/cycle) so optimal duration of KRd treatment was not determined; all Pts continued to receive Rd treatment until disease progression.
Aims: To evaluate post hoc the time to cumulative complete response or better (≥CR) and PFS hazard ratio (HR) at 18 months from randomization for KRd *vs* Rd treatment.
Methods: Cumulative rates of ≥CR overtime were evaluated from start of treatment for KRd (CFZ starting dose 20 mg/m^2 and target dose 27 mg/m^2, lenalidomide 25 mg, and dexamethasone 40 mg) *vs* Rd (same doses lenalidomide and dexamethasone) arms This analysis was done on treated patients. PFS HR at 18 months for the intent-to-treat population was evaluated using piecewise Cox regression analyses based on the method of Collett (2003).
Results: KRd (n=396) and Rd (n=396) Pts were followed for a median of 31 and 30 months for PFS, respectively. A total of 126 and 37 Pts in the KRd and Rd groups achieved ≥CR with sample median time from treatment start to ≥CR of 6.7 and 8.3 months, respectively. The increase in rate of ≥CR Pts over time was greater in the KRd group than the Rd group, most notably in the first 15 months; cumulative ≥CR rates increased steadily thereafter (Table). The overall PFS HR in ASPIRE for KRd *vs* Rd was 0.69 (95% CI, 0.57 to 0.83). For the first 18 months, the PFS HR was 0.58 (95% CI, 0.46 to 0.72).

Table 1.

	Cumulative ≥CR rates* (% Pts)		
Treatment (Months)	KRd (n=392)	Rd (n=389)	KRd-Rd
3	3.1	0.8	2.3
6	13.5	3.1	10.4
9	20.9	4.9	16.0
12	24.2	6.7	17.5
15	27.3	7.2	20.1
18	28.6	7.7	20.9
21	29.6	8.2	21.4
24	30.6	9.0	21.6
27	31.1	9.3	21.9
30	31.9	9.3	22.6

*Safety population: time from treatment start

Summary/Conclusions: CFZ combined with Rd provides greater response and improvements/benefits in PFS compared with Rd alone. Cumulative ≥CR rates increased over time in the KRd arm with rates rising rapidly in the first 15 months. The 18 month PFS HR was lower than the overall study PFS HR, possibly related to KRd Pts receiving CFZ for a maximum of 18 months.

P276

THE SKY92 PROGNOSTIC MARKER IS VALIDATED IN EIGHT MULTIPLE MYELOMA CLINICAL DATASETS

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Background: Prognostic markers for Multiple Myeloma (MM) are recommended in most guidelines and consensus papers for clinical use and for risk stratified trials. A prognostic marker is considered suitable for clinical use when it identifies a reasonable fraction of all patients with sufficiently differential outcome (hazard ratio), and is reproducible in multiple independent clinical studies. SKY92 risk stratification was performed in eight independent clinical datasets for which gene expression profiling was available along with overall survival (OS). Three datasets also had progression free survival (PFS), which is an important measure to identify the patient's response to therapy and which is often a relatively early indication of the severity of the disease. Patients with a shorter PFS require care at a higher frequency, possibly a different treatment strategy and likely need higher intensity treatment than patients with longer progression free survival. It would therefore be useful to reliably identify patients that are expected to have a shorter OS and PFS in an early stage. The SKY92 signature has been reported to be a robust predictor for OS as well as PFS (Kuiper *et al.* 2012, Kuiper *et al.* 2015).
Aims: To validate the prognostic value for PFS and OS of the SKY92 high risk marker in newly diagnosed and relapsed refractory Multiple Myeloma across a wide variety of clinical trial datasets.
Methods: OS data and gene expression profiling (GEP) data, either publicly available or obtained by analysis of the CE IVD MMprofiler assay (that includes the SKY92 signature), of eight clinical datasets (Figure 1) were used to evaluate the proportion of cases, hazard ratios and *p*-values associated with the SKY92 risk marker. The datasets included both newly diagnosed MM (TT2, TT3, MRC-IX, MMGI, HOVON87) and relapsed refractory MM (APEX and TT6) and also elderly (>65 yrs) MM (HOVON87 and about half of MRC-IX). The Cox proportional hazards model was used to calculate Hazard Ratios (HR), and associated p-values.
Results: Figure 1 shows Kaplan Meier curves for eight datasets comparing cases that are high risk for SKY92 *versus* those that are standard risk for SKY92. The proportion of high risk cases was very similar in all cohorts (range 15.4% - 20.7%). For OS the (high risk) / (standard risk) hazard ratios range from HR=2.2 (MRC-IX) to HR=10.3 (TT6) and are all significant, see Figure 1A, including the relapsed stage cohorts TT6 and APEX. For PFS, the hazard ratios range from HR=1.7 (APEX) to HR=2.2 (HOVON87) and are all significant, see Figure 1B. The hazard ratios for OS (Figure 1A) are larger than for PFS (Figure 1B), which seems to suggest that the SKY92 signature is prognostic beyond the first line of treatment.

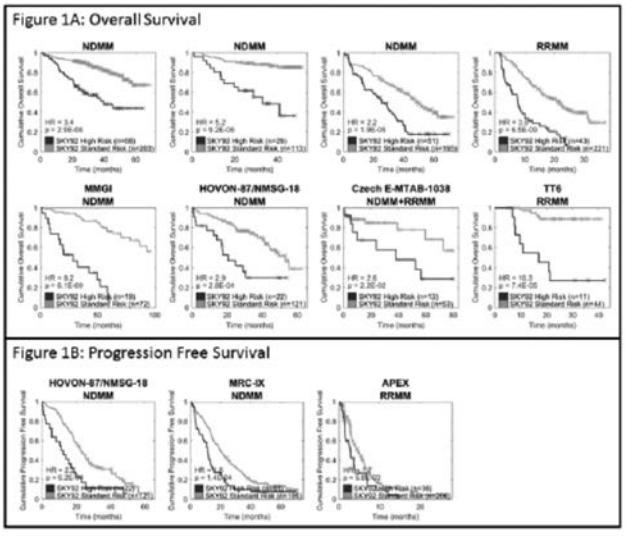

Figure 1. A) Prognostic value for OS of SKY92 in eight clinical datasets including NDMM and RRMM; B) for PFS (bottom) in three clinical datasets including NDMM and RRMM. TT2 |n=351|, TT3 |n=139|, MRC-IX |n=246|, APEX |n=264|, MMGI |n=91|, HOVON-87/ NMSG-18 |n=143|, Czech E-MTAB-1038 |n=66|, TT6 |n=55|. Note that follow up time (x-axis) differs between studies.

Summary/Conclusions: SKY92 is a powerful and robust prognostic marker, not only for OS but also for PFS in younger, elderly, newly diagnosed, relapsed (refractory) MM patients across various treatments including transplant, thalidomide, lenalidomide and bortezomib. It can therefore be used reliably as a predictor for survival to optimize follow-up and treatment strategies in an early stage.

P277

A PHASE 1B/2 STUDY OF SELINEXOR IN COMBINATION WITH BACKCONE THERAPIES FOR TREATMENT OF RELAPSED/REFACTORY MULTIPLE MYELOMA

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Background: The nuclear export protein exportin 1, (XPO1) is overexpressed in a wide variety of cancers including multiple myeloma (MM). Selinexor is an oral Selective Inhibitor of Nuclear Export (SINE), specifically a XPO1 antagonist. Selinexor forces nuclear retention and reactivation of tumor suppressor proteins (TSPs; NF-κB, p53 and FOXO) and reduction of many proto-oncogenes, including MDM2, MYC and Cyclin D. Preclinical activity for selinexor has been demonstrated in combination with pomalidomide (pom), bortezomib (bort) or lenalidomide (len). There is *in vitro* and *in vivo* evidence of selinexor synergy with bort (*e.g.* proteasome inhibitors) through repression of NF-κB pathway and induction of autophagy in MM. In MM xenografts model, selinexor in combination with IMiDs have shown synergy in tumor growth inhibition and increased overall survival when compared to single agents alone. In addition, phase 1 and 2 clinical studies demonstrated selinexor single agent anti-myeloma efficacy.
Aims: To independently assess the efficacy and safety of three combination therapies for the treatment of patients (pts) with relapsed/refractory MM. The combinations are selinexor + dexamethasone (dex) + either Pom (SdP), Bort (SdB), or Len (SdL).
Methods: The dose escalation phase, conducted via the standard 3+3 design, consists of 3 treatment arms (SdP, SdB, and SdL), each arm including two treatment cohorts (once-weekly (80 mg, 100 mg) *vs* twice-weekly (60 mg, 80 mg) selinexor dosing). These 3 treatment arms are evaluated in parallel. Pom (4 mg), len (25mg) and dex (20mg with each selinexor dose) will not be dose escalated. For the SdB arm, bort (1.3mg/m^2 given SC) will be dose escalated from once-weekly to twice-weekly dosing (consistent with its approved, labeled dose).
Results: As of 10-Feb-2016, 4 pts (2M/2F, median of 8 prior regimens, median age 61 yrs) have been enrolled in the SdP arm. Ten pts (5M/5F, median of 8 prior regimens, median age 65 yrs) have been enrolled in the SdB arm. Common grade 1/2 adverse events (AEs) in the SdP arm are nausea (2 pts, 50%) and dysgeusia (2 pts, 50%). One grade 4 AE, neutropenia, was reported in the SdP arm. The most common related grade 1/2 AEs in the SdB are fatigue (2 pts, 20%), nausea (1 pt, 10%), and diarrhea (1 pt, 10%). Thrombocytopenia (5 pts, 50%) is the only Grade 3 AE reported in the SdB arm. Two pts were evaluable for response in SdP arm and achieved a very good partial response (VGPR, n=1) and a minor response (MR, n=1). Seven pts were evaluable for responses in the SdB arm including 3 partial responses (PR, 2 pts were bort refractory and 2 with del17p in >60%), 2 MR, 1 stable disease and 1 progressive disease.
Summary/Conclusions: Significant responses are observed in both treatment combinations of selinexor + dex + pom and selinexor + dex + bort, even in heavily pretreated patients with del17p and/or prior refractoriness to bortezomib. In patients with relapsed/refractory MM whose disease has progressed after PI and/or IMIDs based regimen, the addition of selinexor to bortezomib or pomalidomide is well tolerated. The early response rate observed is promising given the history of extensive pretreatment in this patient group. Updated results from this ongoing phase I/II trial will be presented at the meeting. (clinicalTrials.gov #NCT02343042)

Innovative therapies for MM 2

P278

A PHASE IB DOSE-ESCALATION TRIAL OF ISATUXIMAB (SAR650984, ANTI-CD38 MAB) PLUS LENALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): INTERIM RESULTS FROM 2 NEW DOSE COHORTS

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Background: Isatuximab (ISA), a humanized anti-CD38 monoclonal antibody, has demonstrated activity in patients with RRMM in combination with lenalidomide and dexamethasone (Len/Dex) in an ongoing Phase 1b study (NCT01749969). Overall response rate (ORR) was 63% at ISA 10 mg/kg Q2W (n=24). Here, we report data from 2 new cohorts evaluating a higher ISA dose and a different administration schedule.
Aims: Primary objective: to determine the maximum tolerated dose (MTD).
Methods: Patients with RRMM (≥2 prior MM therapies) were sequentially enrolled to ISA 10 or 20 mg/kg (weekly × 4 doses, then every 2 wks; initial infusion rate 175 and 250 mg/h at 10 and 20 mg/kg, respectively) plus Len 25 mg (days [D]1-21) and Dex 40 mg (D1, 8, 15, and 22), in 28-day cycles. All patients signed an IRB approved informed consent.
Results: An additional 26 patients were treated in the 2 cohorts: median age 65 (42-76) yrs; median yrs from diagnosis 4.5 (1.8-16.6). Median 4.5 (1-8) & 6 (3-10) prior lines of therapy at 10 & 20 mg/kg, respectively; Len-refractory (67% & 86%), pomalidomide+carfilzomib-refractory (25% & 64%), immunomodulatory drug+proteasome inhibitor-refractory (50% & 86%). At data cut-off (Dec 2015), median duration of dosing was 21.5 wks (10 mg/kg) and 9.9 wks (20 mg/kg); 13 patients remain on treatment. 4 patients discontinued therapy due to adverse events (AEs) (grade [Gr] 3 infusion-associated reactions [IARs] [n=3], dose-limiting toxicity of Gr 3 pneumonia [n=1]), all at 20 mg/kg in Cycle 1; these patients were excluded from the efficacy analysis. Most frequent AEs were fatigue (46%), pyrexia (35%) and diarrhea (31%). IARs occurred in 65% of patients, mostly Gr ≤2, and >90% during 1st infusion. MTD has not been reached. In evaluable patients, ORR (IMWG criteria; confirmed responses) was 50% in both cohorts (10 mg/kg [n=12]: VGPR 25%; PR 25%. 20 mg/kg [n=10]: VGPR 20%; PR 30%). Clinical benefit rate (≥MR) was 83% and 50% in the 10 and 20 mg/kg cohorts, respectively. Median time to 1st response was 4 (4-16) wks.
Summary/Conclusions: The combination of ISA ≥10 mg/kg and Len/Dex was generally tolerated and clinically active in heavily pretreated RRMM. Responses were observed after approximately 4 wks. PK, biomarker, and longer term follow-up data will be presented.

P279

MOR202 ALONE AND IN COMBINATION WITH POMALIDOMIDE OR LENALIDOMIDE IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA: DATA FROM CLINICALLY RELEVANT COHORTS FROM A PHASE I/IIA STUDY

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Background: MOR202, a HuCAL-derived, human IgG1 CD38 monoclonal antibody induces potent ADCC and ADCP; preclinical models show high activity of single-agent MOR202 and synergy in combination with immunomodulatory drugs (IMiDs), lenalidomide (LEN) or pomalidomide (POM). Unlike other CD38 antibodies, MOR202 does not induce complement-dependent cytotoxicity thought to be a major contributor to infusion-related reactions (IRRs).
Aims: The primary objectives of the study were to evaluate the safety, maximum tolerated dose (MTD)/recommended phase II dose of MOR202 in patients with relapsed or refractory multiple myeloma.

Methods: This is an interim analysis of a multicenter, dose-escalation phase I/IIa study of MOR202. Preliminary safety and efficacy data from 3 cohorts of patients treated with MOR202 alone or with an IMiD are presented: MOR202 4, 8 and 16 mg/kg weekly; MOR202 8 or 16 mg/kg weekly with either LEN or POM.
Results: As of January 29, 20 patients were treated: 11 patients with MOR202 alone, 5 patients with MOR202+LEN and 4 patients with MOR202+POM. Patients receiving MOR202 alone or with POM were relapsed or refractory to prior bortezomib and LEN with a median of 4 prior regimens. Patients given MOR202 with LEN had a median of 2 prior regimens, mainly bortezomib, cyclophosphamide and autologous stem cell transplant. The MTD has not been reached. MOR202 alone or with an IMiD was well tolerated with mainly hematological toxicity. No MOR202-related study discontinuations or deaths were recorded. A 2-hour MOR202 infusion was feasible in all patients. IRRs (grade 1) were seen in only 1/20 patients. Responses were reported in 8/18 evaluable patients: 3/10 responses (1 very good partial response [VGPR] and 2 partial responses [PR]) in the MOR202 alone, 3/4 responses (all PR) in the MOR202+LEN and 2/4 responses (1 complete response and 1 VGPR) in the MOR202+POM cohorts. In total, 7/8 responses were still ongoing, the longest duration of response was >10 months in a patient given MOR202 alone. Preservation of high CD38 levels on MM cells under MOR202 therapy was shown.
Summary/Conclusions: In this analysis, MOR202 given as a 2-hour infusion showed excellent infusion tolerability and overall safety profile. Promising preliminary efficacy and long-lasting tumor control was seen for MOR202 +/- IMiDs.

P280

ELOTUZUMAB + LENALIDOMIDE/DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: ELOQUENT-2 POST-HOC ANALYSIS OF PFS AND TUMOR REGROWTH BY TIME FROM DIAGNOSIS AND PRIOR LINES OF THERAPY

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Background: Elotuzumab, an immunostimulatory monoclonal antibody targeting SLAMF7, kills myeloma cells by both directly activating natural killer cells and mediating antibody-dependent cell-mediated cytotoxicity. ELOQUENT-2 (NCT01239797) is a Phase 3 study comparing elotuzumab plus lenalidomide and dexamethasone (ELd) with lenalidomide and dexamethasone (Ld) in patients (pts) with relapsed/refractory multiple myeloma (RRMM). Pts had a median time from diagnosis of 3.5 yrs. In a 3-yr follow-up, ELd showed a 27% reduction in the risk of disease progression/death (hazard ratio [HR] 0.73; 95% CI 0.60–0.89; p=0.0014), with durable efficacy across key subgroups.[1] Pts in the ELd group also demonstrated a delay of 1 yr in time to next treatment (TTNT) *vs* the Ld group, with a median (95% CI) TTNT of 33 (26.2–40.2) months in the ELd group *vs* 21 (18.1–23.2) months in the Ld group (HR 0.62; 95% CI 0.50–0.77).[1] Interim overall survival (OS) analysis indicated a strong trend in favor of long-term benefit of ELd *vs* Ld.[1]
Aims: To examine the effect of time from diagnosis and number of prior lines of therapy on progression-free survival (PFS), and to assess tumor shrinkage/regrowth, indicated by serum M protein, via dynamic modeling.
Methods: RRMM pts with 1–3 prior therapies were randomized to ELd or Ld in 28-day cycles until disease progression/unacceptable toxicity. PFS was a co-primary endpoint. An exploratory, post-hoc Kaplan-Meier analysis evaluated the impact of time from diagnosis and prior lines of therapy on PFS. Serum M protein dynamic, non-linear, mixed-effect modeling assessed the rate of serum M protein suppression as well as tumor regrowth, based on longitudinal serum M protein data from baseline.
Results: 646 RRMM pts were randomized (ELd n=321; Ld n=325). Baseline demographics: median age 66 yrs; 32% of pts had del(17p), 9% had t(4;14); 35% of pts were refractory to their last therapy. In pts with ≥median time from diagnosis, median PFS in the ELd group was 26.0 months *vs* 17.3 months in the Ld group (HR 0.60; p=0.0004). In the ELd group, pts with ≥median time from diagnosis and 1 prior line of therapy (n=48) had a 53% reduction in the risk of progression/death (HR 0.47; p=0.013) *vs* the Ld group (n=61). PFS HR in pts with ≥median time from diagnosis and >1 prior line of therapy was 0.59 (p=0.004; ELd n=111; Ld n=96). Serum M protein dynamic modeling showed the pattern of tumor regrowth; specifically, regrowth rate was slower for pts in the ELd *vs* the Ld group regardless of whether pts had ≥or <median time since diagnosis. The slowest rate of tumor regrowth (0.00145 g/dL.Day^{-1}) was in pts in the ELd group with ≥median time from diagnosis (vs 0.00234 g/dL.Day^{-1} in Ld group; <median time from diagnosis: ELd, 0.00279 g/dL.Day^{-1}; Ld, 0.00389 g/dL.Day^{-1}).
Summary/Conclusions: ELOQUENT-2 data show a PFS benefit with elotuzumab plus Ld *vs* Ld across key subgroups. Data from the current analyses indicate that PFS is favorable for ELd *vs* Ld across pt subgroups according to time from diagnosis, including in pts with a longer time from diagnosis and with either 1 or >1 prior therapy. This finding is further supported by serum M protein dynamic modeling, which suggests that elotuzumab may differentially slow the rate of tumor regrowth, thus providing sustainable and long-term survival benefits for pts, as an immuno-oncology agent. Further investigations to identify pt subgroups with increased PFS and OS benefit from elotuzumab are ongoing.
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Reference

1. Dimopoulos M *et al.* ASH Annual Meeting, 2015; Oral 28.

P281

FINAL PHASE 2 STUDY DATA OF MELFLUFEN AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED-REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Melflufen is a peptidase potentiated therapy with an alkylating payload, designed for efficient targeting of tumor cells with a unique mechanism of action. As a highly potent anti-angiogenic compound, melflufen triggers rapid, robust and irreversible DNA damage and exerts its cytotoxicity through alkylation of DNA. The lipophilicity of melflufen leads to rapid and extensive distribution into cells where it is readily metabolized by intracellular peptidases (often over-expressed in malignant cells) into hydrophilic alkylating metabolites leading to 50-fold enrichment in MM cells of these metabolites.
Aims: To study the efficacy and safety of melflufen in combination with dexamethasone (dex) in patients (pts) with RRMM.
Methods: 40 mg melflufen was given in 28-day cycles in combination with 40 mg weekly dex in RRMM pts with ≥2 prior lines of therapy, including lenalidomide and bortezomib, and who progressed on or within 60 days of last therapy.
Results: As of 11 Feb 2016, recruitment was complete (N=40). Median time from initial diagnosis to first dose of melflufen was 5.0 years (range 1-20) and the median number of prior therapies was 4 (range 2-9). 12 patients had ISS stage I, 16 stage II, 10 stage III and 2 unknown. 12 pts (30%) had high cytogenetic risk (del17p, t(4;14) or t(14;16)). 62% were double-refractory and 56% alkylator-refractory. Median duration of treatment was 4.0 months (m), (range 0.7-15) with 4 pts still ongoing. The best response achieved in the 30 efficacy evaluable pts (defined as received ≥2 cycles with appropriate assessments) was VGPR in 3 and PR in 9 for an overall response rate (ORR, ≥PR) of 40%. An additional 7 pts achieved MR for a clinical benefit rate (≥MR) of 63%. 10 pts achieved SD and 1 PD. Similar results were seen regardless of refractory status with an ORR of 35% in double-refractory pts and 53% in alkylator-refractory pts. The ORR was 33% in pts with high-risk cytogenetics. Time to first response (≥MR) was rapid with a median of 1.0 m (range 0.6-5.5). ORR in all 40 treated pts was 30%. The median Progression-Free Survival (PFS) was 8.0 m (95% conf. int. [CI] 4.1-13.6) based on 21 events in 30 pts in the efficacy evaluable population. Median PFS was 4.5 m (95% CI 3.7-11.0) based on 30 events in all 40 treated pts. Median duration of response was 8.8 m and overall survival was not yet measurable. Hematologic toxicity was common but manageable with cycle prolongations, dose modifications and supportive therapy. Melflufen treatment-related Grade 3/4 adverse events (AE) were reported in 85% of patients; thrombocytopenia 63%, neutropenia 58%, anemia 43% and neutrophil count decreased 10% followed by febrile neutropenia, asthenia, pyrexia, fatigue and pneumonia that each occurred in 5% of patients. 16 patients (40%) experienced serious AEs and 12 patients (30%) had melflufen treatment-related serious AEs. 58% of the pts required RBC transfusions, 43% platelet transfusions and 53% G-CSF.
Summary/Conclusions: Melflufen produces rapid and durable responses in MM pts refractory to the major classes of therapy and with high-risk cytogenetics. Clinical benefit (≥MR) was achieved among 63% of these advanced and heavily pretreated pts. Median PFS was 8.0 m in efficacy evaluable pts. Hematological AEs were common but manageable and non-hematological AEs were infrequent. Results support continued development of melflufen with its unique mechanism of action. Recruitment continues to a cohort of single-agent melflufen. Updated information will be presented at the time of the conference.

P282

PRECISION AS PART OF THE ANALYTICAL VALIDATION OF THE SKY92 HIGH RISK SIGNATURE AND THE MMPROFILER ASSAY

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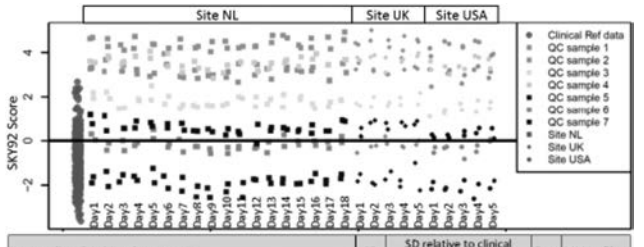
Background: The SKY92 signature is a tool to identify high risk Multiple Myeloma (MM) patients. This signature has been clinically validated on eight independent cohorts, spanning patients from newly diagnosed to the relapsed/refractory setting. For clinical application and patient management, analytical validation is required on top of the clinical validation. That is, the precision should be such that given the technical variability of the assay its prognostic value can be guaranteed. Here the results of the MMprofiler precision studies, Repeatability, Intermediate Precision, and Reproducibility are reported.
Aims: To demonstrate the precision as part of the analytical validation of the SKY92 high risk signature and the MMprofiler assay.
Methods: The CE-IVD MMprofiler assay employs an Affymetrix Gene Expression Profiling (GEP) microarray, and standardized reagents and lab procedures. The assay reports the qualitative SKY92 result, *i.e.* an MM patient can either be standard risk or high risk. This result depends on an underlying quantitative SKY92 score and cut point, which was therefore used to assess the precision. Seven standard QC samples were created using representative samples covering the entire detection range of the SKY92 score. The repeatability (successive measurements of the same sample under the same conditions, also known as within run precision) and intermediate precision were assessed at the site in The Netherlands, by performing 18 runs of the seven samples in duplicate, while alternating three microarray lots, three reagent lots, three technicians, and two Affymetrix DX2 machines, providing a total of 252 MMprofiler analyses. For the reproducibility study, two external sites (UK, and USA) performed five runs of the same seven samples in duplicate (70 MMprofilers per site). In accordance with CLSI standard EP5-A2, the precision close to the cut point was estimated as the 95% confidence interval upper limit of the standard deviation (SD). Prior to the execution of the precision studies, the maximal tolerable SD at which the SKY92 signature would remain prognostic was established to be 0.80.
Results: A total of 392 MMprofiler assays were performed at three different sites (UK, USA, NL), for which the SKY92 scores were plotted, see Figure 1. The variation due to Repeatability was found to be SD=0.22, and the other sources of variation amount to SD=0.19. Combined, the Intermediate Precision, was found to be SD=0.28, which is only 4.4% of the range of SKY92 scores seen in a reference cohort of samples. Next, the Reproducibility was assessed across the three participating sites (SD=0.34), which corresponds to 5.4% of the reference range. All SD estimates were well below the acceptance criterion of 0.80, and thus the prognostic value of the SKY92 signature can be guaranteed.



Intermediate Precision Component	SD	SD relative to clinical reference samples range	Days	MMprofilers
Intermediate Precision (95% Confidence Interval Upper Limit)	0.28	4.4%	18	252
Intermediate Precision	0.25	3.9%		
Variation due to days, reagents, machines, microarrays, operators	0.19	3.0%		
Repeatability	0.22	3.5%		

Reproducibility Site (for all sites 95% Confidence Interval Upper Limits are reported)	SD	SD relative to clinical reference samples range	Days	MMprofilers
Site NL (* day 1 to 5 from the Intermediate Precision reused here)	0.33	5.2%	5	70*
Site UK	0.34	5.4%	5	70
Site USA	0.29	4.6%	5	70
Sites combined	0.34	5.4%	15	210

Figure 1. Scatterplot showing the SKY92 scores from seven QC samples performed on 18 days using alternating reagent lots/machines/technicians at the NL site. An additional five runs have been performed at two external sites in the UK and USA. The Tables below shows the SDs, also expressed relative to the range of SKY92 scores observed in a clinical reference cohort.

Summary/Conclusions: The precision estimates of the SKY92 signature and MMprofiler were found to be small, at most 5.4% of the range of SKY92 scores seen in a clinical reference cohort. This shows that the MMprofiler SKY92 marker provides reliable results for determination of prognosis across different sites. This allows worldwide comparison of the MMprofiler results for clinical (research) purposes.

P283

RISK STRATIFICATION BY SKY92+ISS OUTPERFORMS IFISH MARKERS T(4;14) AND DEL(17P) IN MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a disease with a heterogeneous genetic makeup across patients, and differing survival. Various prognostic markers have been identified, which may be employed in risk stratified treatment paradigms. A large comparison has unveiled that the combination of SKY92 + ISS is the best prognostic marker for identifying high risk and low risk patients. However, a recurring question is how this marker model relates to the currently established iFISH abnormalities such as t(4;14), and del(17), which have earlier been associated with poor overall survival (OS). Here, we demonstrate that iFISH is outperformed by the SKY92 + ISS marker combination for risk stratification.
Aims: To show the prognostic relevance of iFISH markers in perspective of the SKY92 + ISS risk stratification model for prognosis of MM patients.
Methods: Four datasets were gathered, HOVON-65/GMMG-HD4 (n=329), HOVON-87/NMSG-18 (n=143), MRC-IX (n=246), and a Czech cohort (E-MTAB-1038, n=66). Pooled, this provided n=559 MM patients for which GEP data, iFISH annotation, Overall Survival (OS), and ISS were available. The pooled dataset was split into three risk strata as previously proposed (Kuiper *et al.*, 2015): SKY92 standard risk (SR) and ISS1 (low risk), SKY92 Standard Risk and ISS2/3 (intermediate risk), SKY92 high risk (HR). Subsequently, each of these three risk strata were further refined into two groups: those that had either iFISH t(4;14) and/or del(17p) characteristics and those without. The Cox proportional Hazards model was applied to those two groups in each of the three strata separately, to calculate hazard ratios and associated *p*-values. Since groups would become too small, splitting the groups into all possible FISH subgroups was not performed, *i.e.* positive for both t(4;14) and del(17p), and those positive for one and negative for the other.
Results: The four datasets were pooled and then split into three risk strata, providing n=140 low risk (25.0%, SKY92 SR and ISS1), n=289 intermediate risk (51.7%, SKY92 SR and ISS2/3), and n=130 high risk (23.3%, SKY92 HR) MM patients, see Figure 1A. Out of the 140 low risk patients 28 (20%) had a t(4;14) and/or del17, whose survival was equivalent to those without iFISH aberrations (HR=2.4, p=0.054, see Figure 1B). Moreover, their survival was much longer than those in the SKY92 HR group, signifying that they would be at risk for overtreatment when iFISH would be used for risk stratification. Similarly, 55 (19%) out of 289 intermediate risk patients were found to harbor either iFISH t(4;14) or del(17p), but with no impact on their survival (hazard ratio=1.3, *p*=0.17, see Figure 1C). Finally, the group of SKY92 high risk patients were clearly overrepresented for t(4;14) and del(17p), with 65 out of 130 cases (50%). However, those 50% of patients that did not bear a t(4;14) and/or del(17p) aberration (*i.e.* non high risk by iFISH) had the same poor OS as those with them (hazard ratio=1.0, *p*=0.82, see Figure 1D). As a consequence, applying the current prognostic models using iFISH, those 50% HR patients not detected by iFISH would be at risk of undertreatment.

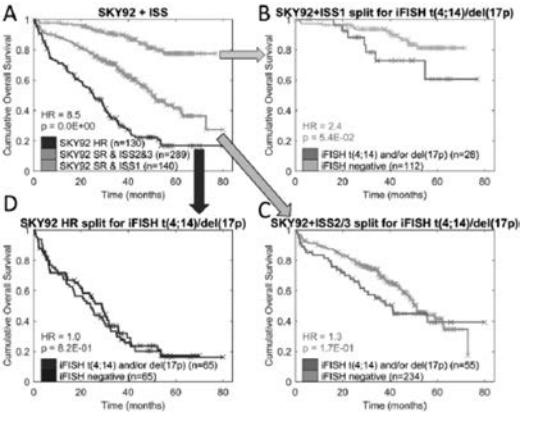

Figure 1. Kaplan Meier plots for the SKY92 + ISS risk stratification scheme on the four pooled datasets (top left). Each of the three strata were subsequently split based on the presence of at least one of the iFISH markers t(4;14) or del(17p) or absence thereof.

Summary/Conclusions: Risk stratification based on SKY92 and ISS is a powerful tool to identify both high risk MM patients and low risk MM patients. Patients identified to be iFISH t(4;14) and/or del(17p) are only high risk when they co-occur with SKY92 HR. Moreover, when they are SKY92 SR, their prognosis is better defined by their ISS stage.

P284

CONSOLIDATION WITH BORTEZOMIB AND LENALIDOMIDE POST-ASCT WITHOUT DEXAMETHASONE AND BISPHOSPHONATES: FINAL ANALYSIS OF A PROSPECTIVE STUDY IN NEWLY DIAGNOSED MYELOMA PATIENTS

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Background: Consolidation therapy with the combination of bortezomib, lenalidomide and dexamethasone (VRD) is an effective therapy in patients with multiple myeloma (MM) post autologous stem cell transplantation (ASCT). Bisphosphonates (BPs) are given for at least for 12 months after diagnosis in these patients.
Aims: The primary endpoint of the study was to explore the efficacy of VR consolidation, without dexamethasone, in newly-diagnosed MM patients who received bortezomib-based induction treatment and then underwent ASCT. Secondary endpoints included: safety, time to progression (TTP), time to next treatment (TtNT), overall survival and effects of VR on bone metabolism, in the absence of BPs.
Methods: Patients who achieved at least stable disease post-ASCT were eligible for participation in the study. Consolidation consisted of 4 cycles of VR, which started on day 100 post-ASCT. Bortezomib was given at a dose of 1.3 mg/m^2, on days 1, 4, 8 and 11 of a 21-day cycle, while lenalidomide was given at a dose of 25 mg, on days 1-14. Patients did not receive any BP during or post-ASCT and during VR consolidation. The following bone remodeling markers were measured on the day of SC collection, before and after VR consolidation: i) osteoclast regulators RANKL and OPG, ii) osteoblast inhibitors Dkk-1 and sclerostin, iii) bone resorption markers CTX and TRACP-5b and iv) bone formation markers bALP and osteocalcin (OC).
Results: Fifty-nine patients (30M/29F, median age 54 year, range 37-68 years) participated in the study. After induction, one (1.7%) patient achieved sCR, one (1.7%) CR, 30 (50.8%) vgPR, 22 (37.3%) PR, while 5 (8.5%) patients had stable disease. After ASCT, 34 (57.6%) patients improved their status of response; in total, 14 (23.7%) achieved sCR, one (1.7%) CR, 35 (59.3%) vgPR and 9 (15.3%) PR. After VR consolidation, 23/59 (39%) patients further improved their response; overall, 30 (50.8%) patients achieved sCR, one (1.7%) CR, 26 (44.1%) vgPR and two (3.4%) PR. The most common adverse events included neutropenia (68%, grade 3/4 23%), thrombocytopenia (59%, grade 3/4 7%), peripheral neuropathy (56%, grade 3/4 2%), anemia (50%, grade 3/4 4.5%) and skin rash (34%, grade 3/4 9%). Fifteen patients (34.1%) experienced at least one infectious episode (grade 1 and 2). Post-VR consolidation there was a reduction of sRANKL/OPG ratio and sclerostin ($p<0.001$) in all patients. Patients who achieved at least vgPR showed higher reductions of sRANKL/OPG and sclerostin compared to all others ($p<0.01$). There was no reduction of bone resorption markers throughout the study period, possibly due to the dramatic reduction of these markers during induction therapy, when zoledronic acid was given. Furthermore, there were no alterations in Dkk-1, bALP and OC, despite the use of bortezomib which has bone anabolic effects. This may be due to the enhanced expression of Dkk-1 caused by lenalidomide and the short period of observation. No skeletal-related events (SREs) were observed during the study period. The median follow-up after ASCT was 35 months and 54% of patients have progressed. The median TTP after ASCT was 42 months (95% CI 29-54 months). There was a trend for longer TTP in patients achieving sCR (48 *vs* 35 months, p=0.145). The median TtNT has not been reached.
Summary/Conclusions: Four cycles of VR consolidation without dexamethasone improves the quality of response in approximately 40% of patients and produces long TTP. In the absence of bisphosphonates, VR consolidation has beneficial effects on bone metabolism and is related with no SREs.

P285

LOW INCIDENCE OF SKELETAL-RELATED EVENTS AT THE TIME OF FIRST RELAPSE IN PATIENTS WITH MULTIPLE MYELOMA WHO RECEIVED BORTEZOMIB-BASED REGIMENS AS FIRST LINE TREATMENT

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Background: Skeletal-related events (SREs) which include pathological fractures, spinal cord compression (SCC) and need for radiotherapy or surgery to bone are frequent complications of multiple myeloma (MM)
Aims: The aim of the study was to evaluate SRE rate in MM patients who received frontline therapy with bortezomib or IMiD-based therapies and explore possible correlations with disease or genetic features
Methods: We studied MM patients who received frontline therapy with novel agents in a single center, since 2007. All patients had a whole body skeletal survey using conventional radiography (WBXR) at diagnosis and then at the time of relapse or whenever clinically indicated; MRI of the spine and pelvis at diagnosis was recorded if available. SNPs in genes that are involved in bone destruction were also evaluated: LRP5 (rs4988321), GC vitamin D (rs4588), TNFRSF11A (rs3018362), DKK1 (rs1569198), RANKL (rs9594759), OPG (rs6469804) and ERS1 (rs1038304).
Results: Since 2007, 463 consecutive patients with symptomatic MM (237M/226F, median age: 68 years) were studied. At diagnosis, the skeletal survey detected osteolytic disease in 328 (71%) patients. MRI was available in 243 patients: 36% of patients had focal, 40% diffuse, 20% normal, and 4% variegated pattern of marrow involvement. SREs were observed in 194 (42%) patients at diagnosis: 120 (26%) patients presented with pathological fractures (89 with vertebral fractures, 21 with rib fractures and 17 with fractures of the long bones; 28 patients had both vertebral and long bone or rib fractures), while 22 (4.7%) patients needed surgery to bone, 21 (4.5%) radiotherapy and 20 (4.3%) patients presented with SCC. The incidence of SREs was higher in patients with osteolytic lesions (52% *vs* 19%, $p<0.001$) or abnormal MRI pattern (51% *vs* 22%, p=0.001). However, we noted that approximately 1/4 patients without lytic lesions in WBXR or with normal MRI pattern presented with a SRE at diagnosis. No correlation was found between the presence of SREs and a specific polymorphism of those studied. Frontline therapy with IMiD-based regimens was given in 38% of patients; 36% patients received bortezomib-based regimens and 26% both IMiD and bortezomib (VTD or VRD). BPs were given in all but 86 patients (18.5%) at diagnosis, mainly due to renal insufficiency; however, almost 60% of them (n=51) received BPs later in the course of their therapy. The vast majority (91%) of patients received zoledronic acid. During first line treatment, 8 (1.7%) patients developed a SRE: 2 on bortezomib- and 6 on IMiD-based regimens. The rate of SREs was higher in patients who did not receive upfront BPs (5% *vs* 1%; p=0.021). The median follow-up was 63 months. At the time of first relapse (data available for 218 patients), 12 patients presented with fractures and 35 patients required local radiotherapy to bone (SRE rate: 21.5%). Patients who had received only bortezomib-based regimens had lower SRE rate (8% *vs* 24%, p=0.06). In total, during the course of their disease, 52.8% of the patients presented with at least one SRE. Presentation with SREs at diagnosis did not predispose for SREs during the disease course.
Summary/Conclusions: Our data, from the first systematic report on the incidence and characteristics of SREs in the era of novel agents, indicate that SREs remain a significant complication in MM at diagnosis. Importantly, despite high response rates after first line therapy more than 20% of patients develop an SRE, which was lower in patients who received first line therapy with bortezomib-based regimens

P286

PHARMACODYNAMIC RELATIONSHIP BETWEEN NATURAL KILLER CELLS AND DARATUMUMAB EXPOSURE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Daratumumab (DARA), a human IgG1 monoclonal antibody that targets CD38, was recently approved in the United States for patients with relapsed/refractory multiple myeloma (RR MM) who have received ≥3 lines of prior therapy, including a proteasome inhibitor (PI) and immunomodulatory drug (IMiD), or who are double refractory to PI/IMiD. High levels of CD38 are expressed on MM cells, and to a lesser extent on immune cells, including natural killer (NK) cells. We assessed the exposure-response relationship of DARA and NK cells, and the impact on clinical outcomes of RR MM patients treated with DARA.
Aims: To characterize the relationship between DARA exposure, NK cell kinetics, and patient clinical outcomes.
Methods: ADCC and CDC assays were performed following DARA treatment of NK cells isolated from peripheral blood mononuclear cells of normal, healthy donors. Samples were pooled from 2 DARA monotherapy studies in patients with RR MM (GEN501, N=104; SIRIUS, N=124). We explored the kinetics of total NK cells ($CD16^+CD56^+$) and activated NK cells ($CD16^+CD56^{dim}$) after DARA infusion. Associations between changes in NK cells post-treatment and DARA exposure, safety, and efficacy were evaluated.
Results: High levels of CD38 were expressed on the surface of NK cells, making these cells sensitive to DARA-mediated ADCC and CDC *in vitro*. After infusion of DARA in patients, the numbers of total and activated NK cells were rapidly and significantly reduced in a dose-dependent manner in whole blood and bone marrow, but recovered post-treatment (Figure 1). A hyperbolic maximum effect (E_{max}) dose- or concentration-response relationship was observed for the maximum reduction in NK cells in the peripheral blood. While baseline levels of NK cells (total or activated) between responders and nonresponders were similar, a trend towards higher overall response rate was associated with greater reduction in NK cells following DARA treatment, suggesting that depletion of NK cells did not interfere with the clinical activity of DARA. No correlations were observed between reductions in the number of NK cells and onset of grade ≥3 adverse events (AEs), infections of any grade, grade ≥3 infections, or herpes zoste.

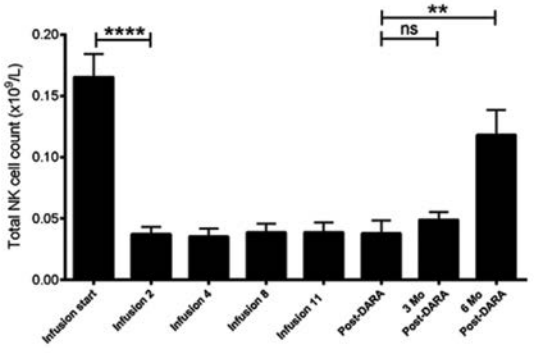

Figure 1. Rapid reduction of total NK cell counts and recovery after DARA treatment.

Summary/Conclusions: CD38 is expressed on NK cells, which are sensitive to DARA treatment. The peripheral NK cells decreased with increasing DARA exposure, exhibiting an E_{max}-type exposure-response relationship. This finding suggests that NK cells can be used as a pharmacodynamic marker for DARA. However, no apparent relationship was observed between NK cell reduction during treatment and incidence of grade ≥3 AEs or infections of any grade.

Myeloproliferative neoplasms - Biology

P287

CONCURRENT INHIBITION OF PI3K/MTOR AND JAK1/JAK2 PATHWAYS PROMPTS COMPLETE DEPHOSPHORYLATION OF STAT5 PROVIDING SYNERGISTIC ACTIVITY IN MPN MODELS

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Background: The constitutive activation of the JAK/STAT pathway by JAK2V617F (VF) and other driver mutations in myeloproliferative neoplasms (MPN) on turn promotes deregulation of other intracellular signaling pathways including the PI3K/mTOR. We previously reported that drugs targeting the PI3K pathway induce cell cycle arrest and attenuate dissemination of JAK2VF cells infused in immunodeficient mice (JCellMolMed 2013;17:1385). Also, the mTOR inhibitor RAD001 demonstrated clinical efficacy in a phase I/II trial in MPN patients (Blood 2011;118:2069).
Aims: Herein we have addressed the molecular mechanisms of the efficacy of PI3K inhibitors, using as relevant drugs the pan-PI3K inhibitor BKM120 and the mTORC1 complex inhibitor RAD001, that were employed as single agents or combined with the JAK1/JAK2 inhibitor Ruxolitinib (Ruxo).
Methods: As MPN models, we used *in vitro*, JAK2VF-mutated SET2 and HEL cell lines, *ex vivo*, the primary $CD34^+$cells from MPN patients and *in vivo*, JAK2V617F knock-in (KI) mice (Blood 2013;122:1464).
Results: Treatment of JAK2VF SET2 and HEL cell lines and JAK2V617F KI mice with BKM120 caused a marked decrease of p-mTOR and its target p-4EBP1, that were accompanied by reduction of p-STAT5 levels. We found that cells treated with BKM120 showed marked dephosphorylation of serines (Ser) residues of STAT5, specifically Ser193/Ser731, while p-tyrosine (Tyr) residues, particularly p-Tyr694, were unaffected. Conversely, Ruxo caused reduction of p-Tyr694 with irrelevant effects on Ser residues. To understand the mechanisms of BKM120-induced Ser dephosphorylation, we sought to identify involved protein phosphatases. We found that only the downregulation of serine/threonine protein phosphatase 2A (PP2A) effectively abrogated drug-induced dephosphorylation of Ser residues. In addition, the mRNA and protein levels of the cancerous inhibitor of protein phosphatase 2A (CIP2A) were strongly reduced by PI3K-inhibitors both in cell lines and primary samples from patients who showed responsiveness to RAD001 treatment. Also, downregulation of the PI3K downstream effector SNAI1 induced by BKM120 treatment leads to upregulation of miR-375, which is involved in the stability of CIP2A mRNA (MolBiolCell 2013;24:1638-48). Therefore, inhibition of p-STAT5 by PI3K inhibitors in MPN cells results from a microcircuit involving miR-375, CIP2A and PP2A overall controlling phospho status of STAT5 Ser residues. Owing that PI3K/mTOR and JAK2 inhibitors induced dephosphorylation at different STAT5 residues, we evaluated the combination of BKM120, RAD001 and Ruxo to induces complete STAT5 inactivation, both *in vitro* and *in vivo* models. *In vitro,* triple drug combination showed the highest synergic activity (CI<0.25; Chou and Talalay combination index) in reducing proliferation of JAK2VF cell lines and colonies formation from patients' cells, as compared to double combinations (BKM120+Ruxo CI<0.65; RAD001+Ruxo CI<0.3). Immunoblots of cells treated with triple-combo showed a concomitant dephosphorylation of both Tyr694 and Ser193/Ser731 reflecting stronger STAT5 inhibition. *In vivo,* JAK2V617F KI mice treated with triple-combo showed dramatic reduction of splenomegaly and control of blood cell counts much more effectively than single-drug treated ($p<0.5$) and control ($p<0.1$) animals.
Summary/Conclusions: Overall, these data reinforce the functional cross-talk between the JAK/STAT and PI3K/mTOR pathways in MPN cells and, by providing evidence of drug synergism mediated by differential targeting of STAT5 phosphoresidues, supporting the combined use of JAK1/2 and PI3K/mTOR inhibitors for the treatment of MPN.

P288

ACE-1332 (TGFB LIGAND TRAP) INHIBITS ELEVATED TGFB1 SIGNALING AND REDUCES FIBROSIS IN A MURINE MODEL OF MYELOFIBROSIS

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Background: Myeloproliferative neoplasms (MPN) are group of clonal stem cell disorders that originate from acquired mutations in the hematopoietic lineage cell leading to abnormal kinase signaling, cell proliferation, cytokine expression, splenomegaly and bone marrow (BM) fibrosis. Primary myelofibrosis (PMF), post-polycythemia vera (PV) MF and post-essential thrombocythemia MF are categorized under myelofibrosis (MF) with overlapping disease phenotypes including progression to BM fibrosis. Mutations in JAK2 have been highly correlated with MF and currently, JAK2 kinase inhibitor therapy is

the only available treatment that offers symptomatic benefit, but does not alter the natural history of the disease or improve BM fibrosis. It is known that TGFβ1 is a critical regulator of fibrosis in many disease states and may be an important therapeutic target for improving bone marrow fibrosis in MPN.
Aims: Elevated TGFβ1 levels were reported to be important for fibrosis in patients with MF. We hypothesize that inhibition of TGFβ1 signaling may prevent fibrosis and help reduce secondary morbidities associated with disease in MF patients. Therefore, we evaluated this hypothesis using ACE-1332 in a murine model of MF.
Methods: Transgenic JAK2 (*V617F*) mutant mice (MF model) and age matched wild-type controls were used in the studies. Mice were dosed with RAP-1332 (murine ortholog of ACE-1332) twice weekly (10 mg/kg) by subcutaneous injection. Expression of TGFβ and inflammatory cytokines at different ages (2-12 months) during disease progression was determined by serum ELISA and qRT-PCR. Bone marrow and spleen cells were analyzed for different cell lineages by flow cytometry. BM sections were stained with H&E and reticulin to determine cellularity or degree of fibrosis respectively.
Results: To understand the onset and progression of MF disease in JAK2 (*V617F*) mice, we initially analyzed the complete blood counts and degree of fibrosis at various ages (2, 3, 4, 5, 8, 10 and 12 months) compared to wild-type mice. These data were then correlated with the expression of TGFβ and inflammatory cytokines. As expected, red blood cells (RBC) and platelets were elevated in JAK2 mutant mice at all ages compared to wild-type mice, although a trend towards a progressive increase was observed between 2 to 5 months followed by a decrease from 8 to 14 months. BM fibrosis was detected starting at 5 months and worsened with age. JAK2 mutant mice also displayed splenomegaly that increased as the disease progressed. Interestingly, serum levels of TGFβ1 and TGFβ3 increased at earlier ages (2-5 months) compared to the latter ages, a trend similar to RBC levels. These levels peaked during the initiation of fibrosis at 5 months. In contrast, inflammatory cytokines (such as IL6, IL-1β, and TNFα) were elevated at later ages consistent with disease progression. We initiated treatment with RAP-1332 in JAK2 (*V617F*) mice (N=8/treatment group) at 4 months of age, the age corresponding to elevated serum TGFβ1 levels and prior to the onset of fibrosis (at 5 months of age). Following 6 months of treatment, vehicle (VEH) treated JAK2 mutant mice displayed elevated RBC (+37.1%, P<0.001), platelets (+74.5%, P<0.001) and spleen weights (+9.5 fold, P<0.001) compared to wild-type mice. BM and spleen sections from VEH treated JAK2 mutant mice revealed severe fibrosis. TGFβ1 antagonism by RAP-1332 treatment of JAK2 mice displayed moderate effect on RBC (-8.4%, N.S) without any effect on platelet counts compared to VEH treatment. Flow-cytometry identified a reduced proportion of $Ter119^+$ erythroid precursors in BM and spleen (-15%, P<0.05) and no change in $CD41^+$ megakaryocytes. RAP-1332 treated mice displayed reduced spleen weights (-29%, P<0.01), and marked reduction in bone marrow (Figure) and spleen fibrosis compared to VEH. Consistent with the reduction in fibrosis, RAP-1332 treated JAK2 mice displayed reduced IL-6 levels (-48.9%, P<0.05) compared to VEH treatment.

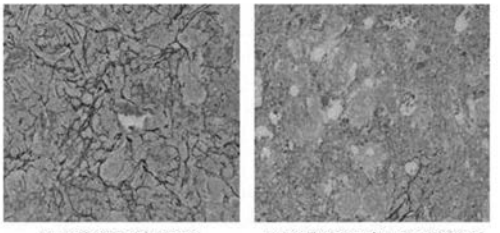

Figure 1.

Summary/Conclusions: These data demonstrate that TGFβ1 levels were correlated with bone marrow fibrosis in a murine model of MF disease. Inhibition of TGFβ1 using RAP-1332 reduces fibrosis, splenomegaly and inflammation in this the JAK2 V617Fmurine model of myelofibrosis.

P289

TARGETING OF BRD4 AS A NOVEL THERAPEUTIC CONCEPT IN JAK2 V617F+ MPN

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Background: Myeloproliferative neoplasms (MPN) are characterized by clonal expansion and accumulation of myeloid cells, erythrocytes, and/or platelets in the bone marrow (BM) and peripheral blood. Classical MPN are polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In patients with MPN, the *JAK2* V617F mutation is frequently detected. Although MPN are chronic and indolent diseases in most patients, fatal progression may occur. So far, the only curative approach for these patients is hematopoietic stem cell transplantation. Therefore, current research is evaluating new therapeutic targets. The epigenetic reader bromodomain-containing protein 4 (BRD4) has recently been identified as a promising target in acute myeloid leukemia.
Aims: The aim of the present study was to investigate the potential value of BRD4 as a molecular target in MPN.
Methods: We employed two *JAK2* V617F+ cell lines, SET-2 and HEL, as well as BM samples obtained from 19 patients with *JAK2* V617F+ MPN (ET: n=6; PV: n=7; PMF: n=6). Three BRD4 inhibitors were applied: JQ1, BI2536, and BI6727.
Results: As assessed by qPCR, SET-2, HEL, and primary MPN cells were found to express BRD4 mRNA and mRNA of the BRD4-downstream target MYC. In ^{3}H-thymidine uptake experiments, all three BRD4 blockers were found to suppress the proliferation of both cell lines and all tested MPN patient samples (7/7). The effects of these drugs were dose-dependent with reasonable IC_{50} values (Table 1). In addition, we analyzed the effects of the BRD4 inhibitors on the putative (neoplastic) stem cells (CD34+/CD38-). In one PMF and one PV patient, exposure to JQ1 was followed by a decrease in the percentage of CD34+/CD38- cells (PMF: control: 0.16% *vs* JQ1: 0.045%; PV: control: 0.078% *vs* JQ1: 0.056%). In the PV patient, the effects of BI2536 and BI6727 were also tested and found to be comparable to JQ1. To confirm the role of BRD4 as a potential target in MPN cells, we performed target-knockdown experiments in SET-2 and HEL cells using two different BRD4 shRNAs. Knockdown of BRD4 was found to block proliferation in transfected cells when compared to untransfected or random shRNA-transfected cells. In a next step, we examined the effects of the BRD4 inhibitors on cell cycle progression. BI2536 and BI6727 were found to induce a G2/M phase arrest in both cell lines. In contrast, JQ1 induced a G1-arrest in HEL cells, but did not show a significant effect in SET-2 cells. In addition, we found that all three BRD4 blockers induced apoptosis in SET-2 and HEL cells in a dose-dependent manner (ED_{50} values: Table 1). Finally, we examined whether exposure to BRD4 inhibitors is associated with modulation of MYC mRNA expression. JQ1, BI2536, and BI6727 were found to downregulate MYC mRNA levels in SET-2 and HEL cells after 2 hours. As assessed by Western blotting, protein levels of MYC were found to be reduced in the MPN cell lines after 24 hours of treatment with BRD4-targeting drugs.

Table 1.

		JQ1	BI2536	BI6727
IC_{50} (nM)	SET-2	50 - 100	20 - 40	50 - 75
	HEL	100 - 500	20 - 40	30 - 50
	primary cells	500 - 1000	500 - 1000	500 - 1000
ED_{50} (nM)	SET-2	100 - 250	10 - 25	25 - 50
	HEL	250 - 500	10 - 25	10 - 25

Summary/Conclusions: In conclusion, our data show that BRD4 is expressed in *JAK2* V617F+ MPN cells and that BRD4 inhibition is associated with decreased proliferation and survival of neoplastic cells. The clinical value of BRD4 as a therapeutic target in *JAK2*-mutated MPN remains to be determined in clinical trials.

P290

LNK MUTATIONS IN FAMILIAL MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN), including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) have in most instances a sporadic occurrence. However, familial clustering of MPN has been reported. Common mutations involved in the pathogenesis of MPN such as *JAK2* V617F, *CALR*, and *MPL* mutations are not inherited, but somatically acquired also in familial cases. The SH2B adaptor protein 3 (SH2B3) gene, also known as *LNK* gene, encodes a negative regulator of cytokine signaling: Lnk negatively regulates erythropoietin receptor signaling and thrombopoietin receptor signaling, by attenuating Jak2 activation and thus reducing erythropoiesis and megakaryopoiesis, respectively. *LNK* mutations have been described in some patients with sporadic MPN and in a small number of cases with idiopathic erythrocytosis and subnormal Epo levels. The majority of mutations occur in exon 2 and is acquired although rare cases of germline *LNK* mutations have been reported. To date, there are no data regarding the occurrence of *LNK* mutations in familial myeloproliferative neoplasms.
Aims: In an attempt to identify the germline genetic factors that underlie familial clustering of MPN we applied next generation sequencing to our MPN families.
Methods: Our cohort of 94 MPN families was analyzed with two strategies. First we applied whole exome sequencing (Illumina instrument) in a subgroup of 16 families with MPN. As this approach resulted in the identification of a exon 2 *LNK* mutation in one family, next we screened for exon 2 *LNK* mutations by Sanger

sequencing the remaining 93 families. Variants were validated by Sanger sequencing. All samples were collected after subjects gave their written informed consent.
Results: Exome sequencing resulted in the identification of an E208Q *LNK* mutation in a patient with familial PV belonging to family 36. Then we screened for exon 2 *LNK* mutations the remaining 93 families. Of 149 patients affected with familial MPN, two patients (1.4%) showed E208Q *LNK* mutation (including the initial case, identified through exome sequencing). The pedigrees and the *LNK* sequences of the two mutated cases (MeF and MPC12_294, belonging to family 36 and family 38 respectively) are reported in Figure 1. Both patients were affected with *JAK2* V617F mutated polycythemia vera. Unfortunately DNA from healthy relatives of family 36 and 38 was not available. The two patients carried the mutation both in granulocyte and T lymphocyte DNA, thus suggesting a germline mutation. In both families the other family member affected with MPN (MeR and MPC07_24) did not carry any mutation of the *LNK* gene, thus excluding segregation of the E208Q mutation with the disease phenotype. This raised the suspicious that the mutation might be an early mutation occurred in the hematopoietic system instead of a real germline mutation. To test this hypothesis we recalled patient MeF to collect DNA from his hair. E208Q mutation was found also in DNA from hair roots, thus confirming that the E208Q mutation was a real germline mutation.

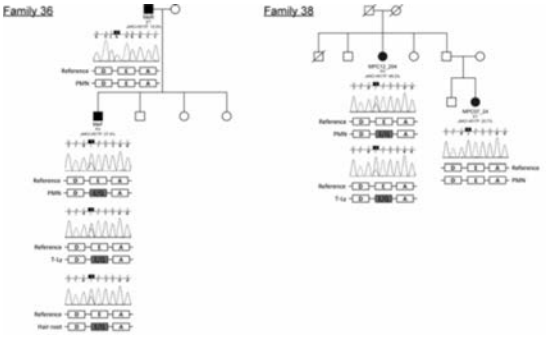

Figure 1.

Summary/Conclusions: *LNK* germline mutations may rarely occur in familial MPN, also in the presence of *JAK2* V617F mutation, but do not segregate with the disease phenotype.

P291

HOMOZYGOUS CALRETICULIN MUTATIONS IN PATIENTS WITH MYELOFIBROSIS LEAD TO ACQUIRED MYELOPEROXIDASE DEFICIENCY
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Background: The pathogenesis of acquired myeloperoxidase (MPO) deficiency, a rare phenomenon observed in patients with Philadelphia chromosome-negative myeloproliferative neoplasms (MPN), is unknown. MPO is a glycoprotein chaperoned by Calreticulin (CALR) in the endoplasmic reticulum. Mutations in *CALR* are frequently found in patients with myelofibrosis (MF) and essential thrombocythemia (ET) with nonmutated *Januskinase 2* (*JAK2*).
Aims: We aimed to determine whether acquired MPO deficiency in MPN is associated with the presence of *CALR* mutations.
Methods: A cohort of 317 MPN patients (142 polycythemia vera (PV), 94 ET and 81 MF) was screened for MPO deficiency using an ADVIA hematology analyzer. Molecular and cell biological analyses were performed in order to determine the underlying mechanism implicated in cases with MPO deficiency.
Results: MPO deficiency was observed in 6/81 MF patients (7.4%), but not in PV or ET patients. Susceptibility to infections had been documented in 2/6 (33%) MPO deficient patients. Five out of six patients with MPO deficiency carried a homozygous *CALR* mutation and were also deficient in eosinophilic peroxidase (EPX). In contrast, one MF patient with a *JAK2*-V617F mutation and MPO deficiency carried two previously reported *MPO* mutations and showed normal EPX activity. Importantly, we did not find patients with homozygous *CALR* mutations and normal MPO activity or patients with heterozygous *CALR* mutations and MPO deficiency in our cohort. Although additional MPN-associated mutations were detected in patients with homozygous *CALR* mutations, none of them was recurrent within these patients. Patients with homozygous *CALR* mutations had reduced MPO protein, but normal *MPO* mRNA levels supporting a post-transcriptional defect in MPO production. Finally, we demonstrate *in vitro* that in the absence of CALR immature MPO protein precursors are degraded in the proteasome.
Summary/Conclusions: Four decades after the first description of acquired MPO deficiency in MPN we provide the molecular correlate associated with this phenomenon and evidence that *CALR* mutations can affect the biosynthesis of glycoproteins.

P292

CHARACTERISATION OF THE INFLAMMATORY MICROENVIRONMENT IN MYELOPROLIFERATIVE NEOPLASMS AND ITS FUNCTIONAL IMPACT ON STEM AND PROGENITOR CELLS
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Background: The immune microenvironment in the bone marrow can have tumor-promoting activity in leukaemia. However, the role of the microenvironment in the initiation and evolution of early myeloid malignancies in not well understood.
Aims: The aim of this study is to characterise the inflammatory microenvironment in chronic myeloproliferative neoplasms (MPNs), including essential thrombocythaemia (ET), polycythaemia vera (PV), and myelofibrosis (MF). We undertook a cell surface marker screen and secreted molecule screen in an effort to identify disease biomarkers and to functionally characterise their impact on pre-malignant stem and progenitor cells.
Methods: We performed a screen of cell surface markers (n=244 markers in 7 patients, followed by 7 candidate markers in 18 patients) and cytokine levels (n=38 cytokines, 193 patients) in MPN patient mononuclear cells and peripheral blood serum (and in 12 normal controls). Statistical analyses to identify candidates for disease subtype and/or progression was undertaken. Colony assays were performed in patient samples with and without candidate cytokines.
Results: A cell surface marker screen of 244 antigens identified an increase in the frequency of markers associated with activated T- and NK-cells in myelofibrosis patients. We therefore undertook a screen of inflammatory cytokines in the serum of 193 patients to determine if specific cytokines were driving disease progression. Using multi-plexed cytokine bead arrays (Millipore) with 38 analytes, we identified a range of serum cytokines that displayed differential serum concentration across the MPN subtypes. Higher levels of IL-8, IP-10, IFN-gamma and TNF-alpha all associated with myelofibrosis. Distinct cytokine profiles were also seen for PV and ET, incl. increased EGF in ET and increased TGF-alpha in PV patients. In colony assays, a direct effect on the growth of stem and progenitor cells was observed with IFN-gamma treatment. Single HSCs from ET, PV and MF patients were treated with IFN-gamma, and progenitor expansion was decreased in ET, unaffected in PV and increased in MF. Further analyses are now underway to determine the longitudinal changes in serum cytokines over the course of disease and transformation as well as additional functional data in single stem and progenitor cell assays.
Summary/Conclusions: Together these data implicate the immune microenvironment as a major player in MPN biology with several cytokines showing differential levels across subtypes of MPN.

P293

MESENCHYMAL STROMAL CELLS (MSC) FROM HIGH-GRADE BONE MARROW FIBROSIS PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS (PH-NEG MPN) DISPLAY REDUCED CLONOGENICITY AND PROLIFERATION CAPACITY
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Background: MSC play an important role in the structure of the bone marrow (BM) niche and regulation of stem cells homeostasis. In Ph-neg MPN, including Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Myelofibrosis (MF), there is a substantial alteration of the BM niche, characterized by massive deposition of fibrous tissue, neoangiogenesis and osteosclerosis, with concomitant loss of the hematopoietic tissue.
Aims: The aim of this work is to study possible alterations in the mesenchymal compartment in Ph-neg MPN in relationship to the BM fibrosis grade.
Methods: MSC were characterized after isolation from BM biopsies of 23 patients with Ph-neg MPN and BM harvests of 6 healthy donors (HD), from washouts of transplant bags, as previously described (Pievani A. *et al.* Cytotherapy. 2014 Jul;16(7):893-905). BM fragments were digested using collagenase

soltion and repeatedly washed with a syringe with 25-Gauge needle using fresh PBS, in order to collect mononuclear cells (MNC). Patients were classified in 3 groups according to diagnosis of Ph-neg MPN and grade of BM fibrosis (WHO criteria): low fibrosis (0-1) no MF (LF-NOMF), low fibrosis MF (LF-MF) and high fibrosis (2-3) MF (HF-MF), respectively formed by 11, 4, and 8 patients. Mutations of JAK2, CALR and MPL genes were present respectively in 65%, 22% and 9% of cases. A triple negativity was documented only in one case (4%).
Results: The median age at the time of cell collection was 54 years (range: 38-77) for the patients and 50.5 years (44-58) for the HD group. A remarkably different number of MNC was harvested from biopsies digestion in different patients groups with a median value of 0.70x10^{6}cells/mm (0.23-2.88) for HF-MF and 2.27x10^{6}cells/mm (1.75-3.18) for LF-MF (p 0.028). LF-NOMF cellularity was 1.44x10^{6}cells/mm (0.40-2.78). No difference was noted between the two group with LF (p 0.131) (Fig.1A). Median value of CFU per 10^2 plated cells was 10.70 (0-14.50) for HF-MF, significantly reduced compared to the other groups: LF-MF 31.50 (11.70-36.30; p 0.042), LF-NOMF 36.70 (9.30-59.70; p 0.0049) and HD 32.50 (25.30-38.00; p 0,0012) (Fig.1B). The proliferation capacity was investigated by the population doubling (PD) assay. The median cumulative PD value at passage 5 for HF-MF was 5.78 (0.43-8.14), significantly reduced compared to other groups: LF-MF 8.31 (5.66-8.42; p 0.05), LF-NOMF 7.83 (5.68-10.26; p 0.0097) and HD 8.71 (6.90-9.46; p 0.014) (Fig.1C). All patient's MSC lines express the typical markers of mesenchymal cells (CD90, CD105, CD73, CD146), similarly to HD. Regarding trilineage differentiation potential, Ph-neg MPN-MSC showed a normal differentiation capacity into adipogenic tissue and the up-regulation of key adipogenic differentiation genes (*FABP4, LPL, PPARG*), with a similar trend compared to HD. In the same patients, MSC were efficiently differentiated into osteogenic lineage with a comparable up-regulation of key osteogenic differentiation genes (*RUNX2, ALPL, COL1A2, SPP1, SPARC, BGLAP*) with respect to the HD group. Ph-neg MPN-MSC were also able to differentiate into cartilagineous pellets, showing an up-regulation of key differentiation genes (*COL2A1, COL10A1, SOX9, ACAN*) similar to HD. No evident differences were observed between the 3 groups of patients. Finally, MSCs were tested for the presence of the respective HSC mutations (JAK2, MPL, CALR) and no one harbored them.

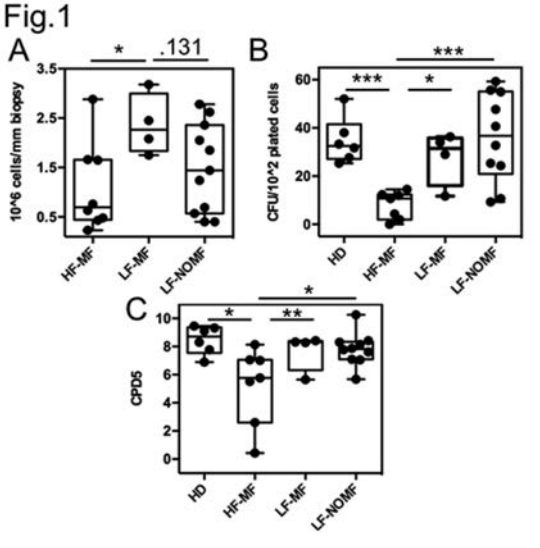

Figure 1.

Summary/Conclusions: Ph-neg MPN-MSC with HF showed a reduced clonogenicity and proliferation capacity compared to the LF groups, independently from the type of disease. These data suggest an alteration of MSC of Ph-neg MPN restricted to the MSC derived from patients who already have a HF in the BM.

P294

NEXT-GENERATION SEQUENCING IDENTIFIES MPL-TET2 MUTATED CLONES IN A SUBSET OF PATIENTS WITH JAK2V617F-POSITIVE MYELOFIBROSIS

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Background: Phenotype driver mutations (mut.) in *JAK2, CALR*, and *MPL* are present in ~90% of patients (pts) with myelofibrosis (MF). As these mut. are considered mutually exclusive, the diagnostic workup via PCR or melting analysis is limited to analyzing exon 14 of *JAK2*, exon 10 of *MPL*, and exon 9 of *CALR*.

Aims: Next-generation sequencing (NGS) is likely to enter clinical practice in near future. We compared an NGS-based approach to standard PCR techniques in detecting driver mut. in MF.
Methods: Genomic DNA was isolated according to standard procedures from whole-blood samples of 129 pts with MF (68 males; median age 60 y). *JAK2*, *MPL*, and *CALR* mut. analysis via PCR were performed as published. Next, NGS was performed: Coding regions and adjacent intronic sequences of 23 selected genes were amplified by multiplex PCR with a primer panel (692 primer pairs). PCR products were purified and ligated to adapters specific for NGS using the Ion AmpliSeq workflow (Thermo Fisher Scientific). DNA libraries were used for templating the sequencing particles. For sequencing, the semiconductor-sequencing technology of the Ion Torrent PGM sequencing platform (Thermo Fisher Scientific) was used. Sequencing reads were aligned to the human genome (hg19) with the Torrent Suite Software, variants were called using the Torrent Variant Caller, and variant annotation was done with the Ion Reporter Software (Thermo Fisher Scientific) according to the HGVS nomenclature.
Results: Standard PCR detected *JAK2*V617F, *MPL*W515L, and mutated *CALR* in 81 (63%), 1 (0.8%), and 25 (19.4%) pts respectively. One patient (0.8%) harboured both mutated *JAK2* and *CALR*. NGS identified *JAK2* mut. at positions R1063H [exon 24; (n=6)] and R893T [exon 20; (n=1)] in *JAK2*V617F+ pts and in *MPL* at positions W515L ± E335K, E259K, Y591D (n=3), and Y591D (n=2). *TET2* and *ASXL1* mut. were found in 27 (21%) and 25 (19%) pts respectively. None of the pts with *JAK2*R1063H or *JAK2*R893T mut. carried a *TET2* or *ASXL1* mut. *JAK2–TET2* double-mutant cells were present in 16 (12%) pts. Interestingly, 4 of 5 *MPL* mut. were found in the *JAK2–TET2* mutated group and non in the *CALR* mutated or triple negative cohorts. Patients with the *JAK2–MPL-TET2* mut. were older, had a higher *JAK2*V617F allele burden, larger spleens, and a more proliferative disease in terms of WBC and peripheral blasts compared to the *JAK2*V617F+ "only" cohort. Except for age and peripheral blasts, the clinical phenotype of *JAK2–MPL-TET2* and *JAK2–TET2* mutated pts was similar (table).

Table 1. Patients characteristics (n=129).

Variable	*JAK2*V617F+ "only" N=65	*JAK2 -TET2* double mutated N=12	*JAK2-MPL-TET2* triple mutated N=4
Median age (years)	60	64	69
Median *JAK2*V617F allele burden, (%)	77	93	86
Allele burden <50%, (%)	36	17	0
Palpable spleen >5cm, (%)	69	83	75
Median palpable spleen, (cm)	8	12	12
Median WBC x10^9/L	9.3	20	16.5
WBC >25x10^9/L, (%)	17	42	25
Median peripheral blasts, (%)	1	0.5	4
Hb < 100 g/L (%)	37	33	50
DIPSS (Int-2/high-risk), (%)	42/15	33/42	25/25

Summary/Conclusions: With NGS, additional activating mut. such as *MPL*Y591D (a gain of function mut. in exon 12 of *MPL*) could be detected through the inclusion of all coding exons of *JAK2* and *MPL*. Our data provide evidence that driver mutations are not always mutually exclusive as was the case in 4% of pts and imply that combination of mut. could have a specific effect on clinical phenotype. Analysis of larger cohorts will provide accurate estimates of the frequency of concomitant *JAK2* and *MPL* mut. Serial NGS permits the study of the chronology of mut. which might influence the biology of the disease and/or response to therapy.

P295

THE INNATE AND ADAPTIVE IMMUNE SYSTEM IS SIGNIFICANTLY DYSREGULATED IN MYELOFIBROSIS: EVIDENCE OF A CENTRAL ROLE OF MUTATIONAL STATUS

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Background: Myelofibrosis (MF) is a clonal neoplasia associated with myeloproliferation, extramedullary haematopoiesis and bone marrow fibrosis. MF patients have severely reduced life expectancy, with infectious complications constituting more than 10% of all causes of death. Besides molecular abnormalities (Janus Kinase 2 (JAK2), Calreticulin (CALR) and myeloproliferative leukemia protein (MPL) genes mutations), chronic inflammation has emerged as a key-player in MF pathogenesis and progression. However, thus far, few and incomplete studies focused on the contribution of the innate and adaptive immunity to MF pathogenesis.
Aims: To better understand MF pathogenesis and the mechanisms involved in infectious events, we studied key immune-cells and plasma cytokine levels in MF patients according to their mutational status. We evaluated circulating dendritic cells (DCs) and monocytes, focusing on their DCs differentiation capacity.

DCs promote T cell polarization into different subsets of T-helper cells (Th) responsible for the clearance of intra and extracellular pathogens. For these reasons, we analysed the percentages of Th1, Th2 and Th17 cells; in addition, the role of natural regulatory T cells (Tregs) was investigated. Due to the aberrant cytokine compartment in MF, we also evaluated Innate Lymphoid Cells (ILCs), a novel family of immune effector cells with a critical role in inflammation and immunosurveillance.
Methods: Mutations were monitored using RT-PCR (JAK2 and MPL genes) and exon 9 Next Generation Sequencing approach (CALR gene). Circulating Th1, Th2 and Th17 cells (identified as $CD3^+CD4^+CD45RO^+CXCR3^+CRTH2^-CCR6^-$, $CD3^+CD4^+CD45RO^+CXCR3^-CRTH2^+$ and $CD3^+CD4^+CD161^+CCR6^+$ cells, respectively), myeloid and plasmacytoid DCs (identified as $Lin^-HLA\text{-}DR^+/CD11c^+$ or $CD123^+$ cells, respectively) from 36 untreated patients and 10 healthy subjects were evaluated by flow cytometry. Percentages and function of total Tregs and ILCs (identified as $CD3^+CD4^+CD25^{high}CD127^{neg}$ and Lin^-CD127^+) plus their cognate subpopulations were analysed as well. After immunomagnetic isolation, we tested phenotype of circulating monocytes and their capacity to differentiate into DCs. Cytokines plasma levels were measured by ELISA.
Results: We demonstrated that in MF specific crucial subsets of the immune system show quantitative and/or qualitative abnormalities identifying a mutation-driven immunological signature. Th17, mDCs and Tregs Population II reduction, associated with an increase in ILC1 is specific of JAK2 mutated patients. Alternatively, CALR mutated patients are characterised by increased ILC3 population, diminished Th1 compartment together with reduced monocyte capacity to mature *in vitro* into DCs. Of note, Tregs from CALR mutated patients were less suppressive than the normal counterpart. This may be due to the increased *in vitro* proliferative capacity of responder T cells carrying CALR mutation. Nevertheless, irrespective of mutation status, ILC1 and 2 were hypofunctional. Finally, patients showed a reduced plasma levels of interleukin (IL) -4, -5 and Interferon-γ with concomitant increased levels of IL-1β, -6, -12, -13, -17, and Tumor Necrosis Factor-α.
Summary/Conclusions: This study shows that MF patients are characterized by a mutation-driven state of immunosuppression, with key cellular components of the immune system showing defective number/function. This state of immune deficiency may play a role in disease pathogenesis and explain, in part, the susceptibility of MF patients to infectious complications.
M.R., D.S. and S.T. equally contributed; F.P. and L.C. equally contributed. This research was supported by BolognAIL

LB296

THE H3K4 METHYLTRANSFERASE SETD1B IS ESSENTIAL FOR HAEMATOPOIETIC STEM CELL HOMEOSTASIS IN MICE

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Background: Lineage- and cell-specific transcription programs are established and maintained by a complex network of epigenetic modifications. Pertubations of epigenetic regulation are associated with many human diseases such as cancer. All expressed genes are trimethylated at histone 3 lysine 4 (H3K4me3) on promoter nucleosomes. The responsible enzymatic machinery belongs to the trithorax group of proteins and comprises six different enzymes: MLL1, MLL2, MLL3, MLL4, SETD1A and SETD1B. The founding member MLL1 has been identified due to frequent involvement in chromosomal translocations that are commonly found in human 'mixed-lineage leukaemias'. On the other hand, hardly anything is known about the specific roles of the other H3K4 methyltransferases in haematopoiesis.
Aims: The present study specifically addresses the function of Setd1b in the haematopoietic system of the adult mouse. Since the constitutive deletion of Setd1b turned out to be embryonic lethal before E11.5, we generated a conditional knockout mouse strain that relies on the tamoxifen-inducible Rosa26-CreER(T2) technology.
Results: Upon deletion of Setd1b mice progressively weaken, develop splenomegaly and do not survive beyond 30 weeks after tamoxifen treatment. Blood count analysis reveals general cytopenias in knockout mice, however both neutrophil granulocytes and monocytes are significantly increased in peripheral blood. Cytospin preparations and histological stainings further confirm an increased abundance of more immature myeloid cells that infiltrate both bone marrow and splenic tissue. Subsequent flow cytometric analysis of haematopoietic stem and progenitor cells (HSPCs) reveals an increased LSK fraction (Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$) and an abnormal ratio among individual stem cell types. One very prominent observation relates to an accumulation of short-term HSCs ($CD34^+$ $Flt3^-$) that are more myeloid-biased based on higher expression of key myeloid transcription factors such as Pu-1. Both *in vivo* - by BrdU incorporation - and *in vitro* - by methylcellulose replating assays - we were able to demonstrate an enhanced self-renewal capacity in Setd1b-deficient HSPCs. Finally, to rule out any putative effects derived from the stem cell niche, we performed bone marrow transplantations and additionally bred our mice to the haematopoietic-specific Vav-Cre deleter. In either case a comparable neoplastic phenotype was generated highlighting the intrinsic requirement of Setd1b in the haematopoietic compartment.
Summary/Conclusions: In conclusion, these findings strongly support an essential function of Setd1b to maintain haematopoietic homeostasis and provide a new model to study epigenetic changes that accompany myeloid malignancies. Given the known role of Mll1 in haematopoiesis, this study further highlights the importance of epigenetic regulation in both steady-state and malignant haematopoiesis. Future investigations will focus on the specific molecular pathways that depend on Setd1b function. In this respect, expression profiling data was recently generated and will be of great use.

LB297

STAT2 DEFICIENCY ENHANCES THE JAK2 V617F-INDUCED MPN THROMBOCYTOSIS PHENOTYPE: A NEW STAT PLAYER IN MYELOPRO LIFERATIVE NEOPLASMS AND RELEVANCE TO INTERFERON TREATMENT

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Background: STAT5 is a recognized driver of myeloproliferative neoplasms (MPNs), STAT1 is promoting megakaryopoiesis and STAT3 plays dual roles, reducing megakaryopoiesis, but promoting inflammation. No information exists for STAT2 role in MPN.
Aims: To determine the role of STAT2 in MPNs knowing that STAT2 is key for interferon action and is activated also by Tpo.
Methods: We used a combination of *in vivo* models and biochemical studies to determine what is the impact of STAT2 on MPN phenotype induced by JAK2V617F, and on gene expression induced via TpoR.
Results: Here we show that ablation of STAT2 enhances the thrombocytosis phenotype in a knock-in model of JAK2 V617F MPN. Furthermore, we show that STAT2 exerts a negative effects on thrombopoiesis. In addition to STAT5/1/3, Tpo can activate in also STAT2, resulting both STAT1/STAT2/IRF9 complexes, which are hallmarks of type I interferon signaling for anti-proliferative and anti-viral activities and other STAT2-containing complexes. We show that STAT2 activation by Tpo is globally defective in MPN patient platelets. Tpo activated STAT2 induces both common genes with type I interferon via STAT1/STAT2/IRF9, as well as distinct genes that are regulated by STAT2. The gene expression response varies with the amount of expressed JAK2 and STAT2.
Summary/Conclusions: Our results identify a novel regulator of the MPN phenotype and indicate that the cross-talk between Tpo and interferon pathways might be essential for the MPN phenotype. Given than type I interferon is effective in MPNs, we suggest that basal TpoR-mediated induction of STAT2 activity primes stem and progenitor cells for type I interferon action.

Myeloproliferative neoplasms - Clinical 1

P296

SAFETY AND EFFICACY OF RUXOLITINIB IN PATIENTS WITH DIPSS INTERMEDIATE-1-RISK MYELOFIBROSIS (MF) FROM JUMP: AN OPEN LABEL, MULTICENTER, SINGLE-ARM, EXPANDED-ACCESS STUDY

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Background: Ruxolitinib (RUX) is a potent JAK1/JAK2 inhibitor that has shown improvements in splenomegaly and symptoms and prolonged overall survival (OS) in patients (pts) with intermediate (Int)-2– and high-risk MF by the International Prognostic Scoring System (IPSS) in the phase 3 COMFORT studies. JUMP is a phase 3b, expanded-access trial in countries with no access to RUX outside a clinical trial and includes pts with IPSS Int-1 MF. Unlike the IPSS, which is used at diagnosis, the Dynamic IPSS (DIPSS) has been validated for use over the disease course and allows for comparisons with other MF populations.
Aims: To assess the safety and efficacy of RUX in pts with DIPSS Int-1 MF (N=700).
Methods: Pts with IPSS high- or Int-2–risk MF, or Int-1 MF and a palpable spleen (≥5 cm) were eligible for JUMP. Starting dose was based on baseline platelet (PLT) count (5 mg bid [≥50 to <100×10^9/L], 15 mg bid [100-200×10^9/L], or 20 mg bid [>200×10^9/L]) and was titrated during treatment. The primary endpoint was safety. OS data were investigated along with Int-1 pts in the DIPSS database who received standard therapy (N=74; Passamonti *Blood* 2010).

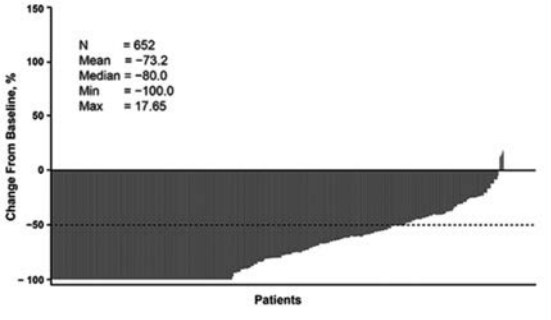

Figure 1. Best percent change from baseline in palpable spleen length at any time by week 72.

Results: JUMP includes 700 Int-1 pts (primary MF, 57%) who started treatment ≥1 y before data cutoff (01 Jan 2015). At baseline, median age was 64 y (range, 18-88); mean spleen length, 13 cm (SD, 6.6); time since diagnosis, 50 mo (SD, 61.4). DIPSS parameters (% of pts): >65 y, 43%; Hb <10 g/dL, 3%; ≥1% peripheral blasts, 19%; constitutional symptoms, 82%; WBC >25×10^9/L, 8%. The majority of pts started at 20 mg bid (n=468) or 15 mg bid (n=190); median exposure, 15.4 mo (range, 0.2-37.5). At time of data cutoff, most pts either remained on treatment (46%) or completed per protocol (27%). Main reasons for treatment discontinuation included adverse events (AEs; 14%) and disease progression (6%). The most common hematologic grade 3/4 AEs were anemia (22%) and thrombocytopenia (10%), which led to discontinuation of treatment in 1% and 2% of pts. Rates of nonhematologic grade 3/4 AEs were low (<2%). Infections (≥5%) included urinary tract infection (all grade, 6% [grade 3/4, 0.7%]), herpes zoster (6% [0.4%]), and nasopharyngitis (5% [0%]). At wk 24, 62% (339/547) of pts had a ≥50% reduction from baseline in spleen length, and 21% (117/547) had 25-50% reductions; rates were similar at wk 48 (68% [276/407] and 19% [78/407]), respectively. Best response in spleen length by wk 72 is shown in the **Figure 1**; 78.5% of pts achieved ≥50% reductions, including 192 (29%) with complete resolution of splenomegaly. Median time to response was 4.7 wk (0.1-75.0), and the estimated probability of maintaining a response was 0.92 (95% CI, 0.89-0.94) at 48 wk. From wk 4 to 48, 40-50% of pts achieved a clinically meaningful response on the FACT-Lym TS (wk 4, 46% [295/641]; wk 48, 44% [171/388]) and FACIT-Fatigue (wk 4, 51% [327/644]; wk 48, 43% [164/382]). The Kaplan-Meier estimated OS probabilities at wk 48 were similar between JUMP (median follow-up, 65 wk) and the DIPSS database (0.98 [95% CI, 0.97-0.99] *vs* 0.987 [0.96-1.00]), as expected in Int-1 pts given the short time period.
Summary/Conclusions: JUMP is the largest study of pts with MF treated with RUX and includes the largest cohort of DIPSS Int-1–risk pts, a risk group not included in the COMFORT studies. Pts with DIPSS Int-1–risk MF achieved spleen size reduction and symptom improvement similar to those seen in pts with Int-1 MF by IPSS and the overall JUMP population. The safety and efficacy of RUX in Int-1 pts in JUMP are consistent with that in the phase 3 COMFORT studies.

P297

PHASE 3 TRIAL TEAM-ET IN 106 HIGH-RISK ESSENTIAL THROMBOCYTHEMIA PATIENTS, DEMONSTRATING NON-INFERIORITY OF ANATHROMB, A NOVEL, EXTENDED-RELEASE ANAGRELIDE FORMULATION, TO THE LICENSED COMPARATOR

H Gisslinger[1,*], A Radinoff[2], E Karyagina[3], S Kyrcz-Krzemień[4], K Abdulkadyrov[5], R Gerbutavicius[6], A Melikyan[7], S Burgstaller[8], M Hus[9], J Kłoczko[10], V Yablokova[11], N Tzvetkov[12], M Całbecka[13], T Shneyder[14], K Warzocha[15], M Jurgutis[16], K Kaplanov[17], J Hodisch[1], C Klade[18], V Buxhofer-Ausch[19]

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Background: Anathromb (AR) is a novel, extended-release formulation of anagrelide hydrochloride (ana), a well-known compound used to selectively normalize platelet counts (plc) by inhibiting megakaryocyte development and maturation in high-risk patients suffering from the myeloproliferative neoplasm Essential Thrombocythemia (ET). Common side-effects of licensed formulations may be largely due to ana and its 3OH-metabolite peak plasma concentrations, whereas efficacy is proportional to AUC. Moreover, licensed formulations need to be dosed twice daily.
Aims: The aim of this study was to demonstrate the non-inferiority of Anathromb in comparison to the licensed comparator in a phase 3, randomized, active-control, double-blind, parallel group non-inferiority setting.
Methods: AR was compared to a commercially available ana formulation, Thromboreductin (TR) in a phase 3, randomized, active-control, double-blind, parallel group non-inferiority trial in high-risk ET patients, either ana-naïve or ana-experienced. After a 6 to 12 weeks titration period, the primary endpoint was the mean plc in the maintenance phase (3 consecutive measurements, centrally assessed, each 2 weeks apart).
Results: 106 patients (91 ana-naïve) were randomized and treated. All patients fulfilled the WHO 2008 diagnostic criteria, the median age was 61, roughly two-thirds were female, and both treatment arms were well balanced. The mean plc at screening was 821 for AR and 797 for TR groups, during the maintenance phase mean plc was 283 for AR and 316 for TR. Thus, both treatments were highly effective in normalizing plc, and the primary endpoint was met formally demonstrating non-inferiority of AR to the licensed TR (p<0,0001). Time from randomization to maintenance phase and number of responders were similar in both treatment arms. Results were consistent between ana-naïve and –experienced patients. Importantly, plc normalization was achieved in three-quarters of patients with a dosing corresponding to 1-2 tablets AR once daily, whereas >90% of the patients required 3 or more capsules TR per day divided in two doses morning and evening. Treatment was well-tolerated, in line with the known side effect profile of ana, the most frequent adverse events were cardiac, CNS and GI disorders. There was no statistically significant difference between the two treatment arms, numerically cardiac disorders occurred less frequently in the AR arm, whereas the opposite trend was observed for CNS and GI. Evaluation of potential differences between AR and TR regarding tolerability requires additional studies with more patients and longer exposure.
Summary/Conclusions: In summary, the novel extended-release formulation AR was well tolerated and equally effective as the licensed comparator TR in

normalizing plc. AR provides a more convenient once-daily dosing schedule and may offer an alternative to licensed immediate-release ana formulations in particular for patients not well tolerating ana side effects.

P298

CLINICAL IMPACT OF ONCOGENETIC PROFILES IN SYSTEMIC MASTOCYTOSIS WITH AN ASSOCIATED HEMATOLOGICAL NON-MAST CELL DISEASE

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Background: In a substantial fraction of systemic mastocytosis (SM) patients, SM coexists with an associated clonal hematological non-mast cell (MC) lineage disease (AHNMD). Most SM-AHNMD patients carry *KIT* mutations and AHNMD-associated genetic alterations; however, there is limited information about the frequency and clinical impact of the coexistence of both types of genetic/molecular alterations in distinct bone marrow (BM) cell compartments.
Aims: we report on the clinic-biological, immunophenotypic, genetic and prognostic features of 65 SM-AHNMD patients classified into three different ontogenetic groups based on the pattern of involvement of BM MC, AHNMD tumor cells and other residual BM cells, by both the KIT mutation and AHNMD-associated cytogenetic/molecular alterations
Methods: Here we studied 65 SM-AHNMD patients grouped into SM-AHNMD cases with: i) unrelated genetic alterations; ii) shared KIT mutation in BM MC and AHNMD tumor cells, in the absence of AHNMD-associated genetic alterations in BM MC, and; iii) shared AHNMD-associated genetic alterations. Cell Purification. Purification of specific BM cell populations was performed using a FACSAria flow cytometer (BD). Interphase fluorescence *in situ* hybridization (iFISH) and human androgen receptor assay (HUMARA) studies. iFISH studies aimed at detection of t(9;22), t(8;21), inv(16), 11q abnormalities, -5/del(5q), -7/del(7q), del(20q), trisomy 8, nulisomy Y, trisomy 12, del(17p13.1), del(13q14), t(14q32), t(18q21), t(11;14), t(3q27) and del(6q21) were performed on interphase nuclei from different FACS-purified and methanol/acetic fixed 3/1 (v/v) cell populations. KIT mutational analysis. The KIT D816V mutation was assessed in genomic DNA from FACS-purified cell populations, using a previously described polymerase chain reaction and peptide nucleic acid-clamping technique.

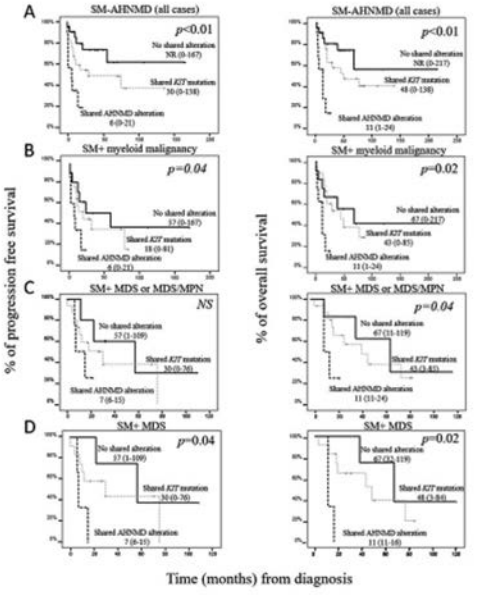

Figure 1.

Results: Overall, patients with shared AHNMD-associated genetic alterations showed a significantly poorer progression-free survival (PFS) and overall survival (OS) *vs* the other two groups (p<0.01). In addition, the pattern of involvement of BM cell compartments other than MC by the *KIT* mutation and the subtypes of SM and AHNMD, were also relevant prognostic factors in the univariate analysis (p<0.01). Multivariate analysis confirmed that the best combination of independent prognostic factors for OS and PFS were the pattern of involvement of BM cells by the *KIT* mutation (p<0.001 and p<0.01, respectively) and the oncogenetic subgroup of AHNMD (p=0.02 and p<0.01, respectively) together or not with the type of AHNMD (HR, 27.9; p<0.001), respectively.

Summary/Conclusions: Overall, patients with shared AHNMD-associated genetic alterations showed a significantly poorer progression-free survival (PFS) and overall survival (OS) *vs* the other two groups (p<0.01). In addition, the pattern of involvement of BM cell compartments other than MC by the KIT mutation and the subtypes of SM and AHNMD, were also relevant prognostic factors in the univariate analysis (p<0.01). Multivariate analysis confirmed that the best combination of independent prognostic factors for OS and PFS were the pattern of involvement of BM cells by the KIT mutation (p<0.001 and p<0.01, respectively) and the oncogenetic subgroup of AHNMD (p=0.02 and p<0.01, respectively) together or not with the type of AHNMD (HR, 27.9; p<0.001), respectively. SM-AHNMD patients show different oncogenetic profiles with an impact on disease outcome. Coexistence of the KIT mutation and AHNMD-associated genetic markers in BM MC andAHNMD cells is an adverse prognostic factor in SM-ANMD.

P299

MUTATIONAL STUDY OF GENES INVOLVED IN MYELOFIBROSIS BY MASSIVE SEQUENCING

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Background: Myelofibrosis (MF) is a clonal myeloproliferative neoplasm characterized by the presence of *JAK2 V617F* and *MPL* mutation in 65% and 10% of cases, respectively. *CALR* mutations have been described recently in most cases of *JAK2*-/*MPL*- myelofibrosis. In the last years other gene mutations have been identified, some of them with a probably prognostic value.
Aims: Investigate the frequency and clinical significance of mutations determined by massive sequencing (NGS) in patients with MF.
Methods: We retrospectively studied 155 patients diagnosed with primary MF (PMF) (n=98, 63%) or secundary MF: post-ET MF (n=29, 19%) or post-PV MF (n=28, 18%), according to the WHO 2008 (see table "clinical characteristics"). Sequencing was performed on a MiSeq NGS (Illumina) system using a panel of 99 genes previously related to myeloid malignancies. Sequencing results were analyzed by using the VariantStudio software package (Illumina), the threshold for mutation calling was set to 5%.

Table 1.

CLINICAL CHARACTERISTICS (N=155)	N (%)	Median (ratio)
Median age at diagnosis (years)		71 (30-87)
Sex:		
- Male	103 (66.5%)	
- Female	52 (33.5%)	
Diagnosis:		
- PMF	98 (63.2%)	
- post-ET MF	29 (18.7%)	
- post-PV MF	28 (18.1%)	
Median time between ET/PV and MF development (years)		10.6 (1.2-31.2)
IPSS:		
- Low/Int-1	60 (38.7%)	
- Int-2/High	95 (61.3%)	
DIPSS-plus:		
- Low/Int-1	54 (34.8%)	
- Int-2/High	101 (65.2%)	
Cytogenetic abnormalities	29 (18.7%)	
AlloHCT	10 (6.5%)	
AML transformation	22 (14.2%)	
Median time between AML and MF diagnosis (months)		30 (3-154)
Death	79 (51%)	
Causes of death (N=77):		
- MF progression	22 (28.6%)	
- AML transformation	20 (26%)	
- Infection	15 (19.5%)	
- Haemorrhage	4 (5.2%)	
- Thromboembolic events	1 (1.3%)	
- Others	15 (19.5%)	

Results: We found mutations in 153 patients (98.7%). A total of 465 gene variations were detected in 64 different genes. The median number of mutated genes by case was 3 (1-10). *JAK2 V617F* mutation was present in 98 patients (63.2%), *CALR* in 25 (16.1%) and *MPL* in 13 (8.4%), with 19 triple negative cases (12.3%). The most prevalent mutated genes were: *JAK2*, *ASXL1* (n=38, 24.5%), *TET2* (n=35, 22.6%), *CALR*, *SRSF2* (n=20, 12.9%), *RUNX1* and *U2AF1* (n=15, 9.7%), *MPL*, *DNMT3A* (n=11, 7.1%), *TP53* (n=10, 6.5%), *KMT2D*, *PTPN11* and *EZH2* (n=9, 5.8%), and *SETBP1* (n=8, 5.2%). Only 5 cases (3.2%) showed *IDH1* mutations while *IDH2* was mutated in 3 patients

(1.9%). The mutation profile was similar in PMF and post-ET/PV MF. We observed relationship between LMA transformation and mutation in *EZH2* (p=0.007), *IDH1* (p=0.003), *SF3B1* (p=0.026), *TET2* (p=0.026) and *CDH13* (p=0.010). In the multivariate analysis, only *EZH2* (p=0.008 [1.72-37.44]) and *IDH1* mutations (p=0.008 [2.01-114.93]) remained statistically significant. The 3-years overall survival (OS) was 68.5% with a median follow-up of 37 months (1.6-219.4). The presence of int-2/high risk IPPS was associated with a shorter OS (p=0.000). Moreover, ≥4 mutated genes by case was also related to a worse outcome (median OS: 39 *vs* 83 months) (p=0.005). Mutations associated to a bad prognosis such as *ASXL1*, *SRSF2*, *EZH2*, *IDH1* and *IDH2* (n=58, 37.4%) showed a shorter OS (p=0.000). In contrast, *CALR* mutated cases had a better outcome (median OS: 147 *vs* 52 months) (p=0.001). In addition, a shorter OS was observed in patients showing mutations in *U2AF1* (p=0.004), *TP53* (p=0.029), *SETBP1* (p=0.000), *PHF6* (p=0.015) and *ETV6* (p=0.000). However, the presence of *DNMT3A* mutations showed a trend of a long OS (p=0.049). In the multivariate analysis, the variables showing a worse prognosis in terms of OS were: int-2/high risk IPSS (p=0.000, HR 3.53 [2.02-6.16]), mutations in *ASXL1*, *SRSF2*, *EZH2*, *IDH1* and *IDH2* (p=0.003, HR 2.12 [1.28-3.49]), *TP53* mutation (p=0.022, HR 3.1 [1.18-8.19]), and *SETBP1* mutation (p=0.014, HR 3.34 [1.27-8.72]); while the presence of *CALR* (p=0.015, HR 0.33 [0.13-0.81]) or *DNMT3A* mutations (p=0.019, HR 0.22 [0.06-0.77]) were associated to a better outcome.

Summary/Conclusions: The presence of mutations in *ASXL1*, *SRSF2*, *EZH2*, *IDH1, IDH2, TP53* and *SETBP1* is associated to a bad prognosis in MF. By contrast, *CALR* and *DNMT3A* mutated cases have a better outcome. There is a relationship between LMA transformation and mutation in *EZH2* and *IDH1*. The use of NGS should be explored in addition to characterization of MF prognosis.

P300

PHASE II STUDY OF LCL161, A SMAC MIMETIC, IN PATIENTS WITH PRIMARY MYELOFIBROSIS (PMF), POST-POLYCYTHEMIA VERA MYELOFIBROSIS (POST-PV MF) OR POST-ESSENTIAL THROMBOCYTOSIS MYELOFIBROSIS (POST-ET MF

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Background: There is no standard therapy for patients (pts) with intermediate (int)-2 or high risk myelofibrosis (MF) who have failed or are intolerant to Janus kinase (JAK) inhibitors such as ruxolitinib. Second mitochondria-derived activator of caspases (Smac) mimetics, also known as, inhibitors of apoptosis (IAP) antagonists, lead to increased apoptotic cancer cell death, especially in high TNFα-expressing tumor models.
Aims: Primary objective: to determine efficacy (IWG-MRT, 2013) of LCL161 as monotherapy for pts with MF. Secondary objectives: to determine safety, durability of response, and change in symptom burden [Myeloproliferative Neoplasm (MPN)-Total Symptom Score (TSS)]. Exploratory objectives: to assess *JAK2*V617F and *CALR* allele burden; 28-gene panel for molecular mutations via next-generation sequencing; and LCL161 target inhibition.
Methods: We conducted an investigator-initiated, single-center, phase II study of LCL161 for pts with MF in a Simon's Optimal two-stage design. Pts age ≥18, PS=0-2, int-2 to high risk MF, who were intolerant to, ineligible for, or relapsed/refractory to JAK inhibitors were eligible. There was no threshold requirement for spleen size or platelet (plt) count, and pts with prior allogeneic stem cell transplant (SCT) were eligible. LCL161, an oral (po) drug, was given at starting dose 1500mg po once weekly. Each cycle=28 days. After 3 cycles, bone marrow exam and objective response assessments were performed.

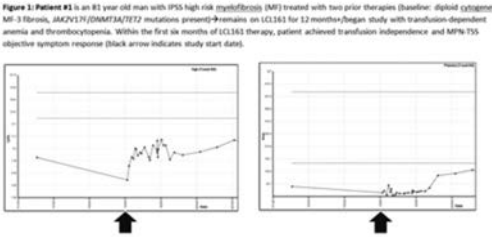

Figure 1.

Results: From January 2015 to January 2016, 13 pts have been enrolled. Baseline pt characteristics are listed in Table 1. *JAK2*V617F mutations were present in 7(54%), *CALR* mutations in 3(23%), and *MPLW*515L mutation in 1(8%) pt. Additionally, the most common other molecular mutations were: *DNMT3A*(n=2); *TET2*(n=2); *RAS*(n=2); *EZH2*(n=1). 10(77%) had ≥2 prior therapies. 2 pts had prior SCT. By IPSS, 11(85%) were high risk MF; 2(15%) were int-2. Median number of cycles received=5[1-13], median treatment duration=4.4 months (mos)[1-11.8]. All 13 pts are alive, with median follow-up of 4.7 mos[1-11.8]. 4 pts had 5 objective responses (1 pt: 2 separate IWG-MRT responses, Figure 1): Clinical improvement (CI) (anemia) in 2 pts; CI (Symptom) in 2 pts; CI (Spleen) in 1 pt. Grade 3/4 non-hematologic adverse events: syncope, n=2. No pts had cytokine release syndrome. Most common grade 1/2 non-hematologic toxicities: fatigue (n=7), nausea (n=6), dizziness/vertigo (n=3). Dose reductions: 6 pts, all to dose -1 level (1200 mg po once weekly); most common reason: grade 2 fatigue (n=5). 7 pts are now off study [n=3 pt request; n=2 progression of MF; n=1 progression to extramedullary AML (74 year old pt with high risk MF, 4 prior therapies, and prior SCT); n=1 proceeded to SCT]. Preliminary, ongoing correlative studies demonstrate significant on-target inhibition (by Western blot) of CIAP1 in 3/3 responding pts with available samples.

Table 1.

Table 1: Baseline Patient Characteristics

Patient Characteristic (N=13)	[Range] (%)
Age, median	74 [58-81]
ECOG Performance Status (PS)	
PS=1	11(85)
PS=2	2(15)
Median Baseline CBC	
Hemoglobin (g/dL)	9[6.3-11.4]
WBC (K/uL)	5[1.1-19]
Platelets (K/uL)	36[6-1365]
Spleen size (n=5), cm	12 [4-20]
Molecular mutations	
JAK2V617F	7 (54)
CALR	3 (23)
MPLW515L	1(8)
Prior treatment history	
Prior treatment, ≥2	10(77)
Prior JAK inhibitor	8 (62)
Prior IMID (Thalidomide, Lenalidomide, or Pomalidomide)	4(31)
Prior allogeneic stem cell transplant (SCT)	2(15)
IPSS (baseline)	
Intermediate-2	2 (15)
High risk	11 (85)

Summary/Conclusions: In this study, in an older group of pts with MF, 85% IPSS high risk, with median plt count of 36 at study entry, in whom 77% had received ≥2 prior therapies, we observed 5 objective responses in 4 pts among the first 13 pts enrolled. LCL161 has a convenient (po, weekly) dosing schedule, represents a novel target for pts with MPNs, and is able to be administered to pts who have failed or intolerant/ineligible for JAK inhibitor therapy. This study has met criteria for the pre-planned analysis for efficacy (Simon Stage 1) and therefore is able to proceed to Simon Stage 2. This clinical trial is registered at www.clinicaltrials.gov/ct2/show/NCT02098161.

P301

PREVENTION OF THROMBOSIS IN PATIENTS WITH POLYCYTHEMIA VERA TREATED WITH HYDROXUYUREA PLUS PHLEBOTOMIES OR HYDROXYUREA ALONE

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Background: Hematocrit control below 45% is associated with a lower rate of thrombosis in polycythemia vera (PV).
Aims: To assess if patients treated with hydroxyurea plus phlebotomies have the same risk of thrombosis than those controlled with hydroxyurea alone.
Methods: The Spanish Registry of Polycythemia Vera is a live ambispective study including, by February 2016, 1353 patients in which baseline characteristics, therapies and complications during follow-up are periodically updated. From this cohort, a total of 533 patients treated with hydroxyurea were selected for the present study. Data from the first 60 months of therapy with hydroxyurea were retrospectively recovered including Hematocrit, WBC, platelet count and number of phlebotomies. Hematocrit response was defined as a hematocrit <45% (with or without phlebotomy requirement) and complete hematological response (CHR) as the presence of hematocrit <45%, WBC <10x10^9/L and platelet count <400x10^9/L. Hematocrit response and CHR were assessed at months 6, 12, 18, 24, 36, 48 and 60. Sustained response was defined as a response lasting more than 50% of follow-up. Intermittent response was defined as a response lasting less than 50% of follow-up. Phlebotomy requirement during the time of study was recorded. Patients requiring ≥4 phlebotomies per year while they received therapy with Hu were categorized as phlebotomy dependent.
Results: Median follow-up was 36 months (range: 6-60). One or more phlebotomies were performed in 304 (57%) patients, 24% of patients were phlebotomy dependent. Hematocrit response and CHR was more frequently achieved in patients without phlebotomy dependency. Hydroxyurea dose was

similar among both groups of patients. A total of 36 thrombotic events (22 arterial, 14 venous) were recorded resulting in a 3 and 5-year probability of thrombosis of 6.9% and 11%, respectively. Phlebotomy dependent patients showed a higher rate of thrombosis than the reminder (19.3% *versus* 4.4% at 3 years, p<0.0001). Adjusting phlebotomy requirement by response in the hematocrit showed a higher risk of thrombosis for phlebotomy dependent patients (HR: 3.9, 95%CI: 1.9-8.1, p<0.0001) whereas sustained hematocrit response did not (HR: 0.98, 95%CI: 0.5-1.98, p=0.9). Multivariate analysis including sex, cardiovascular risk factors, thrombosis at PV diagnosis and need for phlebotomies showed a higher risk of thrombosis in phlebotomy dependent patients (HR: 3.5, 95%CI: 1.7-6.9, p<0.0001) whereas time in hematocrit response or in CHR were not associated with a lower risk of thrombosis. Twenty five bleeding events (6 major, 19 minor) were registered resulting in a 3- and 5-year probability of 4.4% and 7.2%, respectively. The 3-year probability of bleeding was higher in phlebotomy dependent patients than in the remainder (10% *versus* 3.2%, respectively, p=0.01). In multivariate analysis, dependency for phlebotomies showed a higher risk of bleeding (HR: 2.6 95%CI: 1.1-5.9, p=0.028) after adjusting for age and treatment with antiplatelet agents and oral anticoagulants.

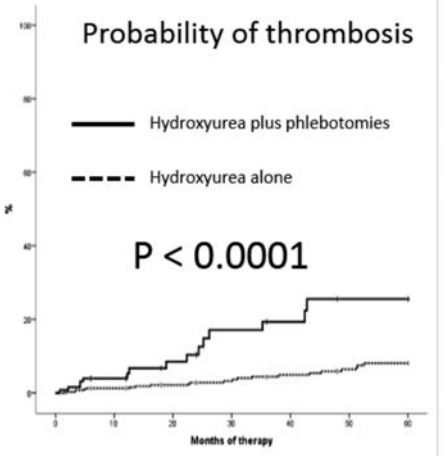

Figure 1.

Summary/Conclusions: Patients treated with Hu who remain dependent on phlebotomies have a higher risk of thrombosis and bleeding than those controlled with Hu alone.

P302

RATE OF MALIGNANT TRANSFORMATION IN HIGH RISK ET DURING 5 YEARS OF FOLLOW-UP OF CYTOREDUCTIVE THERAPY

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Background: First line therapy for essential thrombocythemia (ET), hydroxycarbamide (HC), has mutagenic properties and there is a concern for possible leukemogenicity. The issue has not been conclusively settled in previous studies.
Aims: To assess risk of acute leukemia and non-hematological malignancies in patients treated with HC or anagrelide (ANA) in the EXELS study.
Methods: EXELS, a prospective 5-year study, recruited 3649 high risk ET patients, on or needing cytoreductive therapy, given at the discretion of the treating physician. Previous exposure to ANA and HC was based on patient history. All subjects were followed until death or end of study. Risk of acute leukemia (AL) after study enrolment was estimated by the cumulative incidence. Disease progression may be confounding, influencing therapy choice, so a minimum exposure time of 6 months was used to account for this. The relative risk of AL transformation and non-hematologic malignancies was estimated as age-, gender- and country- standardized incidence ratios (SIRs) with 95% confidence intervals based on background cancer incidence in enrolling European countries. The relative risk ratio of AL for HC *vs* ANA was assessed using the ratio of SIRs. To estimate a 95% confidence interval around this, non-parametric bootstrapping with 10,000 samples was used.
Results: At registration, 481 patients had ANA treatment, 2305 had HC and 656 had been exposed to a combination of the two. Together these treatments included 94% of the patients. The median age in patients on ANA was 51 years, in the HC group 71 years. The SIR for all malignancies was close to 1 for all groups (table 1), indicating no increased risk for ET patients. In skin cancer, the SIR for patients on HC was higher than expected (1.14) and higher than for patients on ANA and patients with neither(1.15 *vs* 0.41 and 0.50). Due to the low number of events, the CIs were wide, and no statistically significant differences could be shown (table 1). A total of 67 cases of AL and 19 cases of myelodysplastic syndrome (MDS) were observed. With a minimum exposure time of 6 months, 62 cases of AL indicated a markedly increased risk for ET patients compared with a normal population, with high SIRs for all groups (table 1). Due to the low number of cases in the ANA group the CIs were wide and no statistically significant difference could be seen for HC *vs* ANA (table 1). Assessing the ratio of SIRs for HU and ANA did not reveal any significant differences; RR 0.91, 95% CI 0.52-3.27.

Table 1. Relative risks in each treatment category, as standardized incidence ratios (SIR) with 95% confidence intervals (CI), using a lag time of 180 days for exposure.

	Observed	Expected	Person-years	SIR	95% CI
Acute myeloid leukemia (ICD code C92.0)					
ET without hydroxycarbamide/anagrelide	3	0.16	1956	18.6	(3.74-54.4)
ET with hydroxycarbamide	42	0.88	8265	47.7	(34.4-64.5)
ET with anagrelide	5	0.10	2091	52.3	(16.9-122)
ET with hydroxycarbamide and then anagrelide	10	0.13	1711	77.2	(37-142)
ET with anagrelide and then hydroxycarbamide	2	0.03	582	58.2	(6.53-210)
Skin cancer (ICD code C43-44)					
ET without hydroxycarbamide/anagrelide	2	4.04	1956	0.50	(0.06-1.79)
ET with hydroxycarbamide	25	21.8	8265	1.15	(0.74-1.69)
ET with anagrelide	1	2.46	2091	0.41	(0.01-2.26)
ET with hydroxycarbamide and then anagrelide	3	3.20	1710	0.94	(0.19-2.74)
ET with anagrelide and then hydroxycarbamide	1	0.93	582	1.08	(0.01-5.99)
All cancers (ICD code C00-C96)					
ET without hydroxycarbamide/anagrelide	26	28.6	1943	0.91	(0.59-1.33)
ET with hydroxycarbamide	156	150	8301	1.04	(0.88-1.22)
ET with anagrelide	14	18.3	2095	0.76	(0.42-1.28)
ET with hydroxycarbamide and then anagrelide	24	22.9	1686	1.05	(0.67-1.56)
ET with anagrelide and then hydroxycarbamide	6	6.41	579	0.94	(0.34-2.04)

Summary/Conclusions: EXELS data enables a comparison of malignant transformation with treatments for ET. We previously showed that the AL event rate per 100 years of exposure for ANA was 0.07 compared with 0.28 for other cytoreductive treatment, and transformation to MDS only occurred in the HC group (event rate 0.12) Higher event rates for other malignancies were also shown (Birgegård et al). However, the age difference between treatment groups was large. This difference was accounted for in this study by estimating SIRs using country-specific cancer registry data. For non-hematologic malignancies the SIRs were close to 1, indicating no increased risk for any group. An increased risk of AL was seen for all patients. Most AL occurred in patients on HC, but likely due to a low event number there was no statistically significant difference between HC and ANA. The concern over risk of leukemia and skin cancer due to HC remains but could not be substantiated, probably due to the low number of events within the time frame of the study. There was no increased risk of other malignancies.

Reference

1. Birgegård G *et al.* Haematologica 2015;100:160-1.

P303

MIDOSTAURIN (PKC412) IN INDOLENT SYSTEMIC MASTOCYTOSIS: A PHASE 2 TRIAL

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Background: Indolent systemic mastocytosis (ISM) is characterized by mast cell infiltration of several organs, notably the skin and bone marrow, without evidence of organ dysfunction. Although ISM patients have a near normal life expectancy, in some the disease is associated with severe mediator-related symptoms such as anaphylaxis, fatigue, flushing, pruritus, osteoporosis and depression. The activating mutations (typically *KIT*D816V) in the cKIT receptor of mast cells hinder inhibition by most tyrosine kinase inhibitors. Midostaurin, a multiproteinase inhibitor that inhibits the tyrosine kinase activity of *KIT*D816V, showed in a large phase II trial impressive activity in patients with aggressive mastocytosis (Blood 2014;124, abstract 636). The drug appeared safe and showed rapid symptom reduction.
Aims: Because of this, we felt justified to investigate the potential therapeutic effect of Midostaurin on symptom severity, quality of life and mast cell burden in ISM patients.
Methods: In this investigator-initiated single center phase II study 20 patients with *KIT*D816V$^+$ ISM, elevated tryptase levels, and severe refractory symptoms were enrolled after written informed consent. They received oral Midostaurin 100 mg twice daily for 24 weeks with the option for continuation after a 2 month wash-out period. Midostaurin was supplied by Novartis Pharmaceutical Cor-

poration, which offered additional financial support. The primary endpoint was symptom response, defined as a reduction in the Mastocytosis Symptom Assessment Form (MSAF) sumscore at week 12. Secondary endpoints were improvements at week 12 and 24 of skin and bone marrow mast cell infiltration, serum tryptase levels, and disease-related quality of life. Study recruitment is closed; patient treatment and follow-up are ongoing. The study is registered with Clinical Trials.gov, NCT01920204.

Results: Three patients dropped out due to adverse events (all nausea) before week 12. Analysis is based on intention-to-treat. At week twelve 16 (80%) patients had significant median 35% (p=0.002; IQR: 16% - 56%%) reduction in symptom severity that further improved to an average 38% reduction at week 24 and was accompanied by a significant median 25% (p=0.001, IQR 12% - 47%) improvement of disease related quality of life. After 4 weeks all patients showed a statistically significant (P<0.000) reduction in tryptase levels (from 36.0 to 15.5 ug/l, P<0.001) that remained stably reduced for all but 1 patient throughout the study. At 24 weeks, 8 out of 16 histologically assessable bone marrow biopsies showed a reduction in mast cell infiltration, 2 an increase and 6 no change. Urticaria pigmentosa improved in 12 (80%) of the 15 patients with skin symptoms with the strongest reduction seen in the intensity of lesions. Grade 1-2 adverse events were reported by all patients; the most common being nausea (n=14), diarrhea (n=11) and headache (n=9). There was one SAE deemed to be probably not treatment related and 3 grade 3-4 adverse events (anaphylaxis, syncope and elevated AST). Notably, there were no hematological adverse events. After stopping of the drug at week 24 according to protocol, 16 patients showed a rapid relapse of symptoms objectified by an increase in tryptase levels. From them, 10 patients strongly favored restarting of Midostaurin.

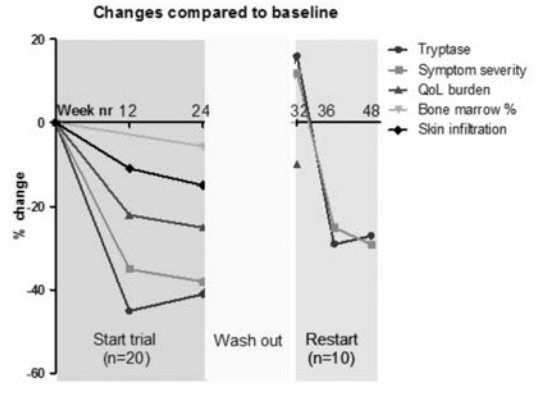

Figure 1.

Summary/Conclusions: In conclusion, Midostaurin is effective in symptom-reduction and even mast cell infiltration in patients with indolent mastocytosis. Despite side effects, most patients favor continuation of the drug.

LB304

RUXOLITINIB COMPARED WITH BEST AVAILABLE THERAPY FOR ESSENTIAL THROMBOCYTHAEMIA PATIENTS RESISTANT OR INTOLERANT TO HYDROXYCARBAMIDE IN MAJIC - AN INVESTIGATOR LEAD RANDOMIZED TRIAL

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Background: Ruxolitinib (RUX), a Janus kinase (JAK) 1 & 2 inhibitor has significant clinical benefits in myelofibrosis (MF) & those patients with polycythaemia vera whom are resistant or intolerant to Hydroxycarbamide (HC). We conducted a randomized, phase II, trial of RUX *vs* Best Available Therapy (BAT) in patients with essential thrombocythemia (ET) who were resistant or intolerant to HC.

Aims: To evaluate the activity & safety of RUX treatment of patients with ET who met modified European LeukaemiaNet (ELN) criteria for resistance or intolerance to HC. The primary end-point was rate of achievement of complete hematological response within 1 year (according to ELN guidelines); secondary endpoints included partial hematological response, safety, thrombosis, hemorrhage, transformation (adjudicated by central review), as well as symptom & quality of life assessment.

Methods: Patients with ET were recruited over 30 months (2012-2015), stratified by JAK2V617F status & randomized to receive 25mg bd of RUX or BAT. Post-ET MF was excluded at trial entry. Patients eligible for the modified intention to treat (mITT) analysis were those who commenced study treatment & received at least one response assessment. Patient reported outcome & quality of life were assessed using EQ5D, MDASI & MPN Symptom Assessment Form (MPN10). Symptom response was defined as ≥ 50% reduction in MPN10 total symptom score (TSS) & compared between arms using a linear mixed model of post-baseline scores through month 12 adjusting for baseline.

Results: Overall 116 patients were recruited, 110 were eligible for mITT analysis, 58 (52%) & 52 (48%) in RUX & BAT arms respectively, comprising 44 males, 66 females, mean age 64.2ys, who were resistant (24.5%), intolerant (51.8%) or both (22.7%) to HC. Baseline characteristics at randomization were balanced, (Table 1). The primary end point was achieved in 27 (46.6%) of the patients in the RUX arm *vs* 23 (44.2%) in the BAT arm (p= 0.81). Partial response occurred in 26 (44.8%) & 27 (51.9%) of those treated with RUX & BAT respectively. The median dose of RUX received was 15mg bd, & median follow-up at this analysis was 1.8 years. No new pattern of safety events for RUX was noted: grade 3 or 4 anemia occurred in 19% and 0% of RUX patients *vs* 0% in the BAT arm, grade 3 or 4 thrombocytopenia in 5.2% & 1.7% of RUX *vs* 0% of BAT patients respectively, & grade 3 and 4 infections occurred in 10.3% of patients in the RUX arm compared to 3.6% in the BAT arm. Overall 2 patients discontinued for anemia & none discontinued for thrombocytopenia. In RUX treated patients, thrombotic or hemorrhagic events were experienced by 9 patients (10 events) & 1 patient respectively *vs* 5 thrombotic and 5 hemorrhagic events in BAT patients (adjusted following central review). Transformations to post-ET MF occurred in 8 RUX *vs* 3 BAT treated patients, 1 RUX patient developed acute myeloid leukemia. There were 2 patient deaths in each arm, due to 1 each of multiple organ failure, cerebral hemorrhage in the BAT arm, bowel infarction due to adhesions, & ischemic cardiomyopathy in the RUX arm. Mean MPN-10 TSS for early satiety, itching, & weight loss during the first 12 months were all significantly lower for RUX *vs* BAT (all p<0.05).

Table 1. Baseline characteristics for ET patients.

Characteristics		BAT (52)	RUX (58)	Overall (110)
Age (years)	Median (SD)	65.6 (13.5)	62.9 (12.3)	64.2 (12.9)
	Range	37.2-85.4	34.5-90.5	34.5-90.5
Gender (%)	Female	30 (57.7)	36 (62.1)	66 (60)
	Male	22 (42.3)	22 (37.9)	44 (40)
JAK2V617F (%)	Negative	26 (50)	30 (51.7)	56 (50.9)
	Positive	26 (50)	28 (48.3)	54 (49.1)
Resistant*/Intolerant	Resistant	24 (46.2)	28 (48.3)	52 (47.3)
* Patients with both classed as resistant	Intolerant	57 (51.9)	30 (51.7)	57 (51.8)
	Missing	1 (1.9)	0 (0)	1 (0.9)
Time from diagnosis years	Mean (SD)	6.9 (5.9)	10.4 (6.7)	8.8 (6.5)
Prior thrombosis (%)	Yes	18 (34.6)	17 (29.3)	35 (31.8)
Prior hemorrhage (%)	Yes	4 (7.7)	2 (3.4)	6 (5.5)
Known diabetes (%)	Yes	4 (7.7)	6 (10.3)	10 (9.1)
Known hypertension (%)	Yes	19 (36.5)	23 (39.7)	42 (38.2)
Known angina (%)	Yes	3 (5.8)	0 (0)	3 (2.7)
Blood count				
Hemoglobin (g/L)	Mean (SD) Range	126 (17) 90-160	119 (17) 87-152	122 (17) 87-160
Hematocrit	Mean (SD) Range	.38 (.051) .27-.49	.36 (.051) .27-.46	.37 (.051) .27-.49
White blood cell count (x 10^9/L)	Mean (SD) Range	6.8 (2.7) 2.8-15	7.5 (4.8) 1.7-30	7.3 (3.9) 1.7-30
Platelet count (x 10^9/L)	Mean (SD) Range	572 (227) 166-1406	545 (215) 89-1139	558 (220) 89-1406
Splenomegaly	Yes	9 (17.3)	14 (24.1)	23 (20.9)
	No	37 (71.2)	37 (63.8)	74 (67.3)
	Missing	1 (1.9)	1 (1.7)	2 (1.8)

Summary/Conclusion: We report primary results from the ET arm of the MAJIC study, the first randomized study of RUX in ET, 110 patients were eligible for the mITT analysis. The results suggest that there is no significant difference in complete hematological response rates between RUX & BAT therapy. Partial hematological responses, transformation, thrombosis & hemorrhage rates appear similar. However symptom scores for early satiety, itching & weight loss were significantly improved by RUX. The side effect profile of RUX was as previously reported.

Hodgkin lymphoma - Clinical

P304

RISK FACTORS (RF) FOR RELAPSE IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA (RRHL) AFTER AUTOLOGOUS STEM CELL TRANSPLANT (ASCT): A REAL-WORLD ANALYSIS IN GERMANY AND THE UNITED KINGDOM (UK)

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Background: With the advance of novel therapeutic options, RFs for post-ASCT outcomes in patients with RRHL are of immediate interest. Several risk factors for relapse have been previously documented; however, there are limited data with respect to the prevalence and distribution of RFs in post-ASCT RRHL patients who have and have not had a subsequent relapse.
Aims: We aimed to describe the prevalence and distribution of RFs after ASCT in patients with RRHL in a sample of real-world patients in Germany and the UK.
Methods: We included patients who were ≥18 years old at the time of HL diagnosis, received ASCT between 1 January 2008 and 30 June 2014, were not enrolled in an HL-related clinical trial and treated under real-world setting at 45 clinical sites in Germany and the UK. This analysis included randomly selected post-ASCT patients, and was augmented by patients who relapsed post-ASCT. Patient characteristics including RFs for relapse were analyzed descriptively. RFs of interest included patient age; sex; B symptoms, extranodal disease, bulkiness, clinical stage, and/or ECOG performance status at the time of ASCT; number of salvage regimens prior to ASCT; response to salvage treatment; and time to relapse.
Results: A total of 350, predominantly male patients, with a median follow-up of 2.2 years post-ASCT were included (196 in Germany, 154 in the UK). The mean age at diagnosis was 42 years. In total, 267 (76%) had a relapse during the follow-up period. A similar proportion of patients who did and did not relapse had B symptoms at the time of ASCT, extranodal disease at the time of ASCT, and less than a partial response to salvage chemotherapy (Table). A greater proportion of patients who relapsed were male and ≥45 years old. Among the patients who relapsed, 83% had ECOG ≥1 at the time of first relapse relapse, 31% had stage IV disease, and 16% had bulky disease. The median number (range) of RFs present was 2 (0-7) in patients who relapsed and 0 (0-1) in patients who did not relapse.

Table 1. Prevalence of risk factors in patients who did and did not relapse after ASCT.

Risk factor, N (%)	Relapsed N=267	Did not relapse N=83
Male	153 (57.3)	48 (57.8)
Age ≥45 years	133 (49.8)	19 (22.9)
B symptoms at the time of ASCT	29 (10.9)	7 (8.4)
Extranodal disease at the time of ASCT	43 (16.1)	18 (21.7)
≥2 salvage regimens prior to ASCT	13 (4.9)	0 (0)
Non-response to salvage therapy (stable or progressive disease)	13 (4.9)	1 (1.2)
Bulkiness (>5 cm) at relapse	43 (16.1)	N/A
Clinical stage at relapse		N/A
I	8 (3.1)	
II	91 (35.1)	
III	79 (30.5)	
IV	81 (31.3)	
ECOG status ≥1 at relapse	221 (83.7)	N/A
Time to relapse <3 months	10 (3.7)	N/A

Summary/Conclusions: This real-world study demonstrates the prevalence and distribution of a myriad of RFs in patients post-ASCT RRHL. RF profiles are different in patients who did and did not have a subsequent relapse, and, as such, clinical evaluation of RFs is critical to identify patients at increased risk of subsequent relapse.

P305

BRENTUXIMAB VEDOTIN (BV) IN PATIENTS WHO ARE INELIGIBLE FOR AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA (RRHL): A UK AND GERMANY RETROSPECTIVE STUDY

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Background: BV is an anti-CD30 antibody-drug conjugate indicated for the treatment of CD30+ RRHL following ASCT or following at least two prior therapies in patients who are ASCT-ineligible. Clinical outcomes in the ASCT-ineligible population have not yet been evaluated in a real-world study.
Aims: We aimed to describe outcomes in real-world ASCT-ineligible patients with RRHL in two countries known to have different practice patterns in RRHL.
Methods: This was a retrospective medical chart review study that enrolled patients at 45 clinical sites representative of routine clinical practice in Germany and the UK. The study included patients ≥18 years old at the time of HL diagnosis, who progressed after ≥2 multi-drug chemotherapy regimens between 1 January 2008 and 30 June 2014 and were not ASCT candidates as identified by their clinicians, were subsequently treated with BV, and were not enrolled in an HL-related clinical trial. Patient demographics (eg, age, gender), clinical characteristics (disease stage, type, ECOG status, reason for ASCT ineligibility), and treatment characteristics (dose and duration of treatment) were described. Clinical outcomes included best response to treatment, progression-free survival (PFS), and overall survival (OS). All outcomes were descriptive, and reported in the full study population and by country.
Results: A total of 125 patients were included in this analysis (69 in Germany and 56 in the UK). The median age of study patients at HL diagnosis was 70 years, and 60% were male. Nearly all patients had had classical HL (96%), and over half had non-bulky (<5 cm) disease (55%). The most common reasons for ASCT ineligibility were comorbidities (74%), age (57%), and disease progression (11%). Coronary artery disease (41%), diabetes (25%), and chronic pulmonary disease (14%) were the most common comorbidities among study patients. Eighty-six percent of patients received at least 2 lines of treatment prior to initiating BV. At the time of BV initiation, 24% of patients had stage IV disease and 22% had extranodal involvement. The median duration of follow-up was 3.1 years from the time of HL diagnosis. Nearly three fourths of patients had a partial or complete response (Table). Other outcomes of interest included leukopenia (12%; of which 47% were serious), neuropathy (10%; all serious), and anemia (8%; 40% serious).

Table 1. Treatment, Response, and Survival in ASCT-Ineligible Patients Receiving Brentuximab Vedotin.

Outcome	ASCT-Ineligible Patients Receiving BV N=125
Median cycles administered (range)	8 (1-16)
Response	
Complete response	34%
Partial response	38%
Stable disease	15%
Progressive disease	12%
PFS from start of BV (months), median (95% CI)	12.3 (8.8-18.9)
OS from start of BV (months), median (95% CI)	15.6 (13.7-24.4)

Summary/Conclusions: Progression-free and overall survival in ASCT-ineligible patients receiving brentuximab vedotin were 12.3 and 15.6 months, respectively, after initiation of treatment.

P306

REAL-WORLD EFFECTIVENESS OF BRENTUXIMAB VEDOTIN (BV) *VS* OTHER TREATMENTS IN PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA (RRHL) POST AUTOLOGOUS STEM-CELL TRANSPLANTATION (ASCT)

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Background: BV is an anti-CD30 antibody-drug conjugate indicated for the treatment of CD30+ RRHL following ASCT or following at least two prior therapies in patients who are ASCT-ineligible. While no randomized trials have been conducted in the post-ASCT RRHL setting, patients receiving BV have been shown to have higher complete response rates and longer overall survival (OS) compared to historical control groups.
Aims: We aimed to compare effectiveness in post-ASCT patients with RRHL receiving BV to those receiving other treatments or no treatment in clinical settings in Germany and the UK.
Methods: A retrospective medical chart review study was conducted that enrolled patients at 45 clinical sites in Germany and the UK. The study included patients ≥18 years old at the time of HL diagnosis, who received ASCT between

1 January 2008 and 30 June 2014, had at least 12 months of available clinical data from the initiation of post-ASCT treatment, and were not enrolled in an HL-related clinical trial. This analysis included randomly selected post-ASCT patients who subsequently relapsed, and an augmented sample of patients who relapsed post-ASCT and were treated with BV or another treatment regimen after relapse. Patients were grouped according to the first line of therapy received after post-ASCT relapse (BV, salvage chemotherapy, or no treatment). Patient demographics (eg, age, gender), clinical characteristics (disease stage, type, ECOG status), and treatment characteristics (dose and duration of treatment) were described for BV and non-BV patients. Clinical outcomes included progression-free survival (PFS), OS, and best response. Median PFS and OS were calculated using Kaplan-Meier methods; all other outcomes were summarized using descriptive statistics, by treatment (BV and non-BV) and country.

Results: A total of 267 patients were included in the study (131 Germany, 136 UK). Of these, 163 received BV (88 Germany, 75 UK), 87 received other treatments, (39 Germany, 48 UK), and 17 received no treatment for post-ASCT RRHL (4 Germany, 13 UK). The groups were similar in median age at diagnosis (42, 46, and 50 years, respectively), gender (56%, 59%, and 65% male), and type of HL (>96% classical HL in all groups). The most common salvage chemotherapy regimens in the non-BV group were gemcitabine-based regimens (41% and 42% in Germany and UK) and CHOP (13% in both countries). Patients in the BV group received a median of 7 of the potential 16 cycles of treatment (Table). The median duration of follow-up was approximately 2 years in all groups. Median PFS was 8.6 months longer in patients receiving BV compared to those receiving salvage chemotherapy. Median OS could not be estimated because <50% of patients died during the follow-up period. Other events of interest included anemia (12%) and leukopenia (12%) in the BV group and leukopenia (12%) and thrombocytopenia (6%) in the salvage chemotherapy group.

Table 1. Treatment and Survival Outcomes in Patients with RRHL after ASCT relapse.

Outcome	Brentuximab vedotin	Salvage chemotherapy
Median cycles administered (range)	7 (1-16)	4 (1-8)
Best response to therapy		
Complete response	45%	41%
Partial response	34%	22%
Stable disease	12%	6%
Progressive disease	8%	29%
PFS from start of post-relapse therapy (months), median (95% CI)	22.0 (19.3-NE)	13.4 (6.7-28.0)

NE, not estimable

Summary/Conclusions: In this observational, real-world study, patients receiving BV as the first post-relapse line of therapy after RRHL had longer PFS than patients receiving salvage chemotherapy; however, confidence intervals were wide.

P307

ABDOMEN/PELVIS COMPUTED TOMOGRAPHY IN STAGING OF PEDIATRIC HODGKIN LYMPHOMA: IS IT ALWAYS NECESSARY?

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Background: Cancer incidence and use of computed tomography (CT) and positron emission tomography (PET) in childhood have been increasing over time and in some studies an association between pediatric CT and cancer risk was found. The risk linked to overuse of diagnostic radiation has recently inspired 2 different international campaigns, "Image Gently" and "Eurosafe," both aimed at understanding what are the most careful practices and in 2013 the American Society of Hematology, included "*surveillance CT scans in asymptomatic patients after curative-intent treatment for aggressive lymphoma*" among tests not well supported by evidence.

Aims: Based on the evidence that the majority of HL presentation is in the mediastinum and/or in a superficial lymphadenopathy (abdomen/pelvis disease is present in 39.0% in the present series) we decided to determine if abdomen/pelvis CT can be safety omitted in the staging of a subgroup of children with Hodgkin Lymphoma (HL). Since it is well known that the measurement accuracy of US is inferior to CT, a randomized trial would be addressed as not ethical, and so we organized a complex study with a centralized revision of CT images.

Methods: Every participating center sent *local* staging reports of PET and abdominal ultrasound (US) along with digital images of staging CT to the investigation center where the CT scans were evaluated by an experienced pediatric radiologist. The *local* radiologist who performed the US was unaware of local CT and PET reports (both carried out after US), and the *reviewer* radiologist examining the CT images was unaware of local US, PET and CT reports. A *Final Staging* (FS) of 123 patients performed on the basis of *local* US and PET reports and *centralized* CT report was compared to a *Test Staging* (TS) based on *local* US and PET. US/PET evaluation of lymphonodes (LNs), spleen and liver (no other site was affected) was based on criteria adopted in the Euronet-PHL-C1 trial and could be *positive* (involved), *negative* (not involved) or *doubtful* (table 1 and 2). Also the TS (table 3) could result *positive*, *negative* or *doubtful* (cases where CT was mandatory to assess the involvement).

Results: There were 71/123 patients with a negative TS: no new lesion was diagnosed by CT. In 27/123 patients the TS was positive: no site was added or withdrawn by CT, and specifically in the group of isolated US positivity (or concordant US/PET positivity) in spleen + concordant US/PET negativity in LNs no additional information was provided by CT. In 25/123 patients the TS was doubtful: in 21 (84%) the FS was positive, and in 4 (16%) negative. According to the study methods, in doubtful TS cases at least one technique (US and/or PET) was doubtful or positive: if we consider these cases positive, the analysis in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) shows these results: 1)Sensitivity: 1.00 (CI: 0.89-1.00); 2)Specificity: 0.95 (CI: 0.87-0.99); 3)PPV: 0.92 (CI: 0.81-0.98); 3)NPV: 1.00 (CI: 0.93-1.00).

Table.

Table 1 - US/PET evaluation of lymph nodes

Lymph nodes		
US	PET	US/PET evaluation
Negative	Negative	Negative
Positive	Positive	Positive
Positive	Negative	Doubtful
Negative	Positive	Doubtful
Doubtful	Positive or Doubtful or Negative	Doubtful
Positive or Negative	Doubtful	Doubtful

Table 2 - US/PET evaluation of spleen/liver

Spleen or Liver		
US	PET	US/PET evaluation
Negative	Negative	Negative
Negative	Positive or Doubtful	Doubtful
Doubtful or Positive	Positive or Doubtful or Negative	Positive

Table 3 – Test staging

US/PET evaluation	Test staging
Negative in lymph nodes and spleen/liver	Negative
Positive in at least one site	Positive
Doubtful in at least one site (and no positive site)	Doubtful

Summary/Conclusions: We think CT remains useful in whatever type of LN positivity in PET and/or US since CT can better assist the choice as to radiotherapy planning. At the same time, we think that, with the strictest approach, it is possible to omit CT at least in PET/US negative patients (57.7%) even though it seems that CT could be safely omitted also in cases of isolated US positivity (or concordant US/PET positivity) in spleen + concordant US/PET negativity in LNs (9.7%). Avoiding abdomen/pelvis CT would have some positive effects in patients in whom it is deemed possible: 1)Reducing radiation exposure. 2)Cutting possible side effects and adverse/allergic reactions of contrast materials and of general anesthesia (in the minority of patients needing it).3)Cutting the costs. All these advantages would be present even compared to an initial staging performed through combined PET/CT (where usually a CT scan with iodine contrast media is performed after PET) or, apart from the radiation risk, through chest CT and abdomen/pelvis MRI, which is a much more complicated approach.

P308

INCREASED CORONARY ARTERY CALCIUM SCORE AFTER RADIOTHERAPY IN HODGKIN LYMPHOMA SURVIVORS

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Background: Coronary artery disease (CAD) is a well know complication of mediastinal radiotherapy (MRT). Long term survivors of Hodgkin Lymphoma have an increased risk of myocardial infarction compared to the general population. Pre-clinical CAD, detected using modern imaging techniques, has been rarely described in these patients.
Aims: The aim of this study is to compare computer tomography (CT) detected CAC-scores in HLS treated with and without MRT. Furthermore we tried to identify risk factors (RFs) predisposing HLS to develop coronary artery calcifications.
Methods: In this cross-sectional study we describe 82 Hodgkin Lymphoma survivors (HLS) with a mean current age of 47.8 years and mean follow-up time from diagnosis of 13.4 years. Fifty patients were treated with MRT with or without chemotherapy. Seventy-five patients underwent coronary artery calcium (CAC) score measurements.
Results: Significantly increased CAC-scores (*i.e.* CAC-score >75th percentile for age and gender) were more frequently observed in HLS treated with MRT 41.3%, than in HLS treated without MRT 10.3% (p=.004). In multivariable analysis, HLS treated with MRT ≥10 years ago had an odds ratio of 12.1 of having a CAC-score >75th percentile compared to HLS treated without MRT. HLS irradiated <10 years ago had a similar incidence of significantly increased CAC-scores compared to the no-MRT group. HLS treated with MRT and CAC-score >75th percentile had more traditional cardiovascular risk factors (median 3) than HLS treated with MRT with a CAC-score <75th percentile (median 2), p=.036. Five HLS treated with MRT and one treated without MRT had already developed symptomatic CAD before screening. During four years follow-up after screening, two more patients developed CAD for which revascularisation therapy was needed.

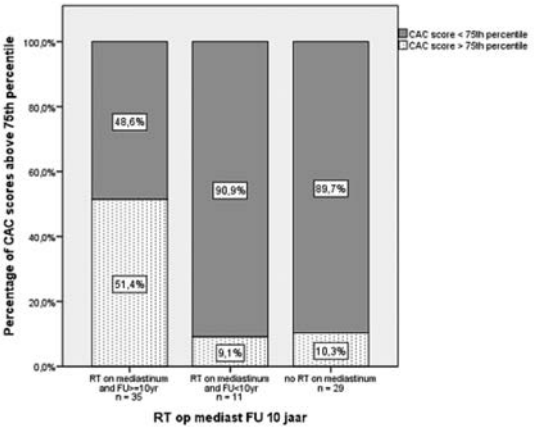

Figure 1.

Summary/Conclusions: We therefore recommend that screening HLS by CAC-score measurements should begin 10 years after MRT and that traditional cardiovascular risk factors should be addressed in this high risk population.

P309

THE LYMPHOMA ASSOCIATED MACROPHAGES-HODGKIN/REED-STERNBERG CELLS RATIO IS A POOR PROGNOSTIC FACTOR IN CLASSIC HODGKIN LYMPHOMA PATIENTS

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Background: Classical Hodgkin lymphoma (cHL) is the most common lymphoid malignancy under the age of 30. Despite the relatively high curability, about 20-30% of patients relapse after front-line therapy. Recent years have brought novel information about close Hodgkin/Reed-Sternberg (HRS): lymphoma associated macrophages (LAM) cross-talk promoting tumor growth and resistance to therapy.
Aims: To assess the prognostic role of LAM to HRS ratio in lymph node biopsies using a novel automated system for scanning large tumor sample areas in cHL patients.
Methods: High-quality tissue samples obtained from 38 patients at time of diagnosis were analyzed. The median age at diagnosis was 35 (17.5-93) years; the male-to-female ratio was 0.66:1. The lymphoma subtypes were nodular sclerosis (NS) in 25 (66%) and mixed cellularity (MC) in 13 (34%) patients. German Hodgkin Study Group stages were: limited in 4 (12%), intermediate in 8 (23%) and advanced in 23 (66%) patients. Chemotherapy was given to 35 patients (92%): BEACOPP in 20 (57.1%), ABVD in 6 (17.1%), Stanford V in 4 (11.4%) and other (COPP, COPP/ABV) in 5 (14.3%) cases. Involved-field radiotherapy was applied in 11 (29%) cases. Tissue array analyses were performed using the TissueFAXS (TissueGnostics, Austria). Data were analyzed with TissueQuest software. Paraffin-embedded biopsies were prepared from diagnostic samples stained with anti-CD30 and anti-CD68. Analyzed areas were reviewed by pathologist and fibrotic (≥10%), necrotic or residual lymphatic structures were excluded.
Results: After treatment, 31 (82%) patients achieved complete remission, three partial remission, one stable disease and three patients progressed. After a median follow-up of 64.3 months, 9 (24%) patients relapsed or progressed and 7 (18%) died. Five-year overall survival (5-y OS) reached 81.0%; 5-year progression survival (PFS) was 68.3%. The mean sample scanned area covered 27.2±13.5 mm^2 with the mean total number of cells 461,504±286,491. The means (medians) of HRS and LAM densities were 279±232 (181) and 1724±1115 (1317) cells per mm^2, respectively. Mean and median number LAM per HRS cell (LAM to HRS ratio, LHR) achieved 13.1 and 7.48, respectively. LHR did not correlate with sex (p=0.44), age (p=0.19), systemic symptoms (p=0.68), tumor bulk (p=0.35) or Ann Arbor stage (p=0.59). High LHR (>7.48) correlates with lower probability of CR achievement (63% *vs* 95%, p=0.02). High LHR was associated with inferior 5-y PFS (41.1% *vs* 94.7%, p<0.001) and OS (62.2% *vs* 100%, p=0.004). Multivariate Cox regression identified high LHR as an unfavorable prognostic factor for PFS (p=0.01, HR=13.3) independent of age, sex, disease stage and lymphoma subtype.

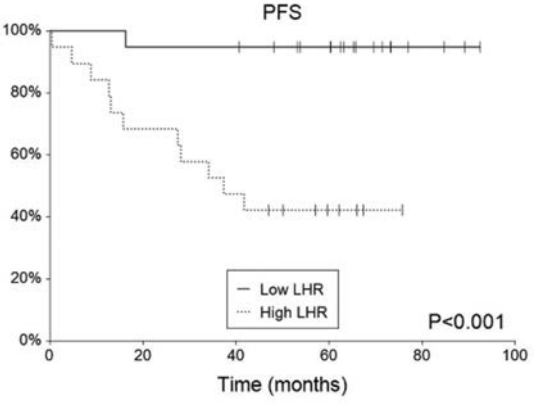

Figure 1.

Summary/Conclusions: This is the first evidence about the possible predictive role of LAM to HRS ratio in cHL patients. High LHR at diagnosis is associated lower probability of CR and higher risk of lymphoma progression or death. Automated image analysis is a new tool overcoming technical limitations caused by small (microarray) samples in lymphomas with high intra-tumor heterogeneity. Further analyses will define the role of HRS and LAM density analysis in cHL in the context of risk-adapted and CD30-targeted therapies.
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P310

THE PROGNOSTIC ROLE OF BCL-2 EXPRESSION IN PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA ASSESSED FOR CD68+ CELL COUNT

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Background: Risk stratification in classical Hodgkin Lymphoma (cHL) is largely based on clinical, radiological and laboratory findings. In the last years, interim PET-CT has added important prognostic information for therapy stratification. Among risk factors that reflect tumor biology, Bcl2 expression in Hodgkin/Reed-Sternberg cells (HRS) has been reported to be an adverse prognostic factor in HL. More recently, studies using genome-wide expression data have identified the number of tumor-infiltrating macrophages (TAMs) as an important tissue biomarker. Several studies have shown that patients with TAMs over 5% identified by immunohistochemical staining for the CD68 antigen had an inferior outcome.
Aims: The aim of this study was to evaluate Bcl-2 expression in HRS cells, analyze for associations of Bcl-2 expression with clinical characteristics, interim PET-CT and in particular, the CD68+ cell count and clinical outcome.

Methods: We studied 122 patients with cHL (58 females, 64 males, median age 38 years), diagnosed at our Institution between 2004 and 2016, and treated with ABVD (95 patients), BEACOPP (21 patients) or COPP (6 patients). Tissue sections of patients were stained for Bcl-2, and cases were considered positive if any HRS cell expressed Bcl-2 (Rassidakis, Blood 2002; 100;3935). CD68+ cells were assessed by staining with the PGM-1 antibody. PET was performed after 2 cycles of chemotherapy in 95 patients and evaluated according to the 5-point Deauville scale (5p-DS). Primary endpoint was event-free survival (EFS), defined as time from date of diagnosis to date of first relapse, disease progression, or death from any cause.
Results: Bcl-2 was expressed in HRS cells of 60/122 (49%) cases. CD68 count was >5% in 66/122 (54%) cases. We found a significant association between Bcl-2 expression and the CD68+ count >5% (p=0.04). Both Bcl-2-positivity and CD68+ cell count >5% were associated to elevated levels of LDH (p=0.006; p=0.02 respectively) and the nodular sclerosis type 2 according to BNLI criteria (p=0.002; p=0.005 respectively). Using a score of >3 as cut to define a positive interim PET-CT, 83 patients had a negative interim PET, 12 patients were PET-positive. No association was found between Bcl-2-positivity and the PET result; instead we observed a direct correlation between CD68+ count and 5p-DS (p<0.002). Bcl-2 expression was associated only with a trend for poor EFS (p=0.07), while the CD68+ count >5% was a strong predictor for reduced EFS (p<0.001). In a multivariate analysis adjusted for the type of chemotherapy, CD68+ count and interim PET retained their independent prognostic significance (p<0.001, and p<0.001, respectively).
Summary/Conclusions: Our study suggests that BCL-2 expression in HRS cells does not provide prognostic information in addition to the CD68+ cell count and interim PET-CT that remain strong and independent outcome predictors in cHL.

P311

AUTOLOGOUS STEM CELL TRANSPLANTATION (AUTO-SCT) IS HIGHLY EFFECTIVE IN RELAPSED NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA (NLPHL): A RETROSPECTIVE STUDY BY THE EBMT LYMPHOMA WORKING PARTY

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Background: NLPHL is a rare CD20-positive subtype of Hodgkin lymphoma (HL). Although long term survival is better than in classical HL, frequent relapses are common and progression / transformation to aggressive non-Hodgkin lymphoma (NHL) may happen. Whilst high-dose therapy (HDT) with auto-SCT is considered as standard treatment for relapsed / refractory classical HL, information on auto-SCT in relapsed / refractory NLPHL is sparse.
Aims: We report a registry study of auto-SCT for NLPHL using the EBMT database, representing the largest sample analyzed to date.
Methods: Eligible for this study were patients 18 years or above with NLPHL who underwent auto-SCT between 2003 to 2013 and were reported to the EBMT. Baseline patient, disease, and transplant data were collected from EBMT MED-A standard forms. Centers with potentially eligible patients were contacted to provide additional treatment and follow-up information including a written histopathology report for central review. *Statistical analysis* was descriptive and employed log rank comparisons for univariate assessment of the impact of baseline characteristics on survival endpoints.
Results: Altogether, 92 patients met the inclusion criteria and had a full data set including written diagnostic report available. Of these, 36 patients had to be excluded after histopathology report review (17 classical HL, 2 NHL, 17 no sufficient information), leaving 56 patients in the final study sample. There was a predominance of male patients (88%), and the median age was 36 (interquartile range (IQR) 29-50) years. Prior to auto-SCT, 71% had 2, 20% had 3, and the remainder more than 3 lines of treatment (median 2 lines), containing rituximab in 62% of the patients. Median time from diagnosis to auto-SCT was 21 (IQR 14-51) months. At auto-SCT, 54% of the patients were in complete remission (CR), and 43% in partial remission (PR). BEAM was used as HDT in the vast majority of patients (84%), with additional rituximab in 13%. With a median follow-up of survivors of 5.0 (IQR 3.6-6.6) years, the 5-year progression-free and overall survival were 67% (95%CI 55-82), and 86% (95%CI 77%>96%). 5-year incidence of relapse was 32% (95%CI 20-46). There were no transplant-related deaths. Univariate comparisons considering age, time from diagnosis to transplant, number of pretreatment lines, disease status at auto-SCT and rituximab use during induction, salvage and/or HDT failed to identify significant predictors of any survival endpoint.
Summary/Conclusions: Patients with relapsed NLPHL undergoing auto-SCT have an excellent outcome, even after failing many lines, comparing favorably with historical data on auto-SCT for relapsed classical HL. In this retrospective analysis, a significant effect of rituximab treatment prior to and/or during HDT did not emerge.

Non-Hodgkin & Hodgkin lymphoma - Novel agents

P312

BIOLOGICALLY MEANINGFUL CHANGES IN CYTOKINE AND CHEMOKINE PRODUCTION FOLLOWING IBRUTINIB THERAPY IN WALDENSTROM'S MACROGLOBULINEMIA

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Background: Waldenström's macroglobulinemia (WM) is characterized by bone marrow (BM) infiltration of lymphoplasmacytic lymphoma along with a serum IgM monoclonal protein. Recently, MYD88 and CXCR4 activating somatic mutations were identified in approximately 90% and 35% of WM patients, respectively. CXCR4 mutations occur almost exclusively in MYD88 mutated patients. Ibrutinib is an oral BTK inhibitor and the only drug specifically approved for WM by the European Medical Association. Ibrutinib is highly active in previously treated WM patients with the best responses in MYD88 mutated CXCR4 wild-type (WT) patients. The serum cytokine profile for WM based on MYD88 and CXCR4 genotype has yet to be defined and the serum cytokine response to ibrutinib based therapy has not been previously studied in WM.
Aims: The aim of this study is to define cytokines that correlate with WM clinical characteristics, MYD88 and CXCR4 mutation status, and response to ibrutinib therapy.
Methods: A total of 137 peripheral blood derived plasma samples were tested, obtained from 52 previously untreated WM patients and 34 previously treated and symptomatic WM patients who received ibrutinib therapy as part of a prospective clinical trial (Treon et al, NEJM 2015). The untreated group included 23 patients that were MYD88 mutated and CXCR4 wild type, 20 patients that were both MYD88 and CXCR4 mutated, and 9 patients that were MYD88 and CXCR4 wild type. The patients participating in the ibrutinib trial were tested just before and one year after initiation of ibrutinib therapy. Twenty age and gender matched healthy controls were included for comparison. Cytokines were tested using magnetic multiplex enzyme- linked immunosorbent assays (R&D Systems Inc., Minneapolis, MN). The following 27 cytokines were tested: TNF, IL6, IFNG, CXCL10, IL10, CCL2, IL8, IL1b, IL7, IL1RA, CCL3, CCL4, CCL5, IL17A, IL4, IL5, IL2, GMCSF, IL2RA, CXCL9, IL12, IL15, IL13, CCL11, CXCL12, CXCL13 and CD27. Statistical analysis was performed with R statistical computing software. Clinical parameters and cytokine levels were analyzed following log transformation. Correlation between variables was tested using Spearman's rho and when appropriate Holm-Bonferroni multiple hypothesis correction was applied. Adjusted p-values <0.05 were deemed significant for this analysis.

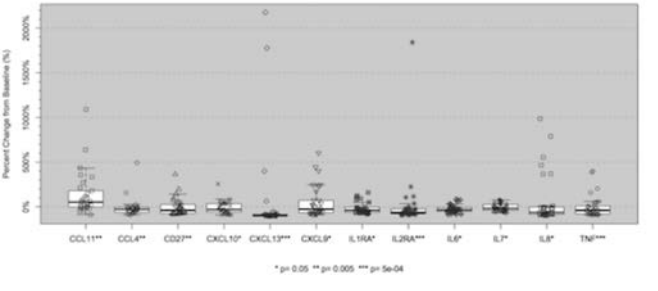

Figure 1.

Results: CXL10, IL10, IL2RA, CXCL13 and CD27 were significantly higher between WM patients *versus* controls. At baseline CXCL13, IL2RA and CD27 strongly correlated with HGB levels (p<0.001 for all) with correlation coefficients of -0.55, -0.55, and -0.47, respectively. CXCL13 (rho=0.56; p<0.001) and CD27 (rho=0.46; p<0.001) correlated with BM infiltration. None of the cytokines showed significant correlated with IgM levels. Based on CXCR4 mutation status within MYD88 mutated patients, IL2RA (p=0.025), IL1RA (p=0.003), CXCL10 (p=0.026) and CD27 (p=0.016) were higher in CXCR4 WT patients. No significant difference in cytokine levels based on MYD88 mutation status was noted though this was due to MYD88 WT cytokine levels being similar to those found in CXCR4 mutated patients. Twelve cytokines were significantly changed after 1 year on ibrutinib (Figure 1). Only the change in CXCL13 was significantly correlated to achieving at least at least a partial response (PR; >50% decrease in serum IgM; p=0.019). Baseline CXCL13 levels were significantly higher in patients that subsequently reached at least a partial response (PR; >50% decrease in serum IgM) after 1 year (p=0.048). This was also true using logistic regression to account for age, gender, MYD88/CXCR4 mutation status, and baseline BM involvement (p=0.035).
Summary/Conclusions: CXCL13 is a strong biomarker of BM tumor involvement in WM and baseline levels are predictive of achieving a major response

to ibrutinib. These data suggest a role of CXCL13 in WM tumor biology and warrants further study.

P313

A PHASE III STUDY OF OFATUMUMAB VS RITUXIMAB IN INDOLENT B-CELL NON-HODGKIN LYMPHOMA RELAPSED AFTER RITUXIMAB CONTAINING THERAPY (HOMER): RESULTS OF THE INTERIM ANALYSIS

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Background: Rituximab, (R) as a single agent or in combination, is standard therapy for untreated or relapsed indolent B-cell non-Hodgkin lymphoma (iNHL). Due to the ubiquitous use of R in the treatment of iNHL and the invariable relapse of patients with iNHL, there remains an unmet need for novel therapies in relapsed iNHL. Ofatumumab (OFA) is an anti-CD20 human monoclonal antibody (mAb) that binds to a membrane-proximal epitope of the CD20 molecule.
Aims: The study aimed to evaluate the efficacy and safety of treatment with OFA compared with R in iNHL patients who had relapsed following R-containing treatment. Here we report the results of the interim futility analysis.
Methods: In this Phase III, multicentre, open-label study, patients (aged ≥18 years) who had relapsed following R-based therapy were randomized to receive either 1000 mg OFA or 375 mg/m^2 R every week for 4 weeks, followed by every 2 months for four additional doses. All patients provided informed consent and were R-sensitive: a complete or partial response to their last prior R-containing therapy lasting at least 6 months beyond the end of R treatment was required. Randomization was stratified by FLIPI score (0–2 *vs* 3–5), disease type (FL *vs* non-FL), and prior R (monotherapy *vs* combination). The primary endpoint was progression-free survival (PFS), as assessed by an independent radiology reviewer (IRR). An interim futility analysis was reviewed by an independent data monitoring committee (IDMC) when 50% of the 373 planned events for the primary endpoint analysis had been reported. A recommendation to stop the study would occur if the IRR-assessed PFS had a conditional power ≤30%.
Results: A total of 409 patients (205 in the OFA arm and 204 in the R arm) were enrolled and evaluated in the interim analysis. The majority of patients (98%) had follicular lymphoma. Patient and disease characteristics were similar between the treatment arms. Just under a third of patients (31% OFA, 27% R) discontinued from study treatment, with the most common reason being disease progression (21%). The IRR-assessed median PFS was 16.2 months in the OFA arm and 21.2 months in the R arm (Table 1). IRR-assessed response rates were 50% and 63% in the OFA and R arms, respectively. Investigator (INV)-assessed median PFS was 16.1 months and 17.9 months in the OFA and R arms, respectively. INV-assessed response rates were 47% for OFA and 58% for R. Median overall survival and median time to next therapy appeared to be similar between arms. Infusion-related adverse events appeared higher in the OFA arm *vs* R arm (any grade: 87% *vs* 58%; grade 3/4: 22% *vs* 5%), and mostly affected the first infusion administered. Incidences of gastrointestinal disorders appeared higher in the OFA arm *vs* R arm (38% *vs* 27%), whereas neutropenia (OFA: 3% *vs* R: 7%) and pyrexia (OFA: 4% *vs* R: 10%) appeared to be more frequent in the R arm.

Table 1. Efficacy endpoints of the HOMER study.

	R (N=204)	OFA (N=205)	HR (95% CI)
Median IRR-assessed PFS, mo (95% CI)	21.2 (16.3–25.2)	16.2 (15.7–21.5)	1.32 (0.99–1.76)
Median INV-assessed PFS, mo (95% CI)	17.9 (16.1–21.6)	16.1 (15.5–18.5)	1.14 (0.89–1.48)
Median overall survival, mo (95% CI)	47.1 (45.5–NA)	45.9 (39.2–NA)	1.18 (0.61–2.30)
Median time to next therapy, mo (95% CI)	31.8 (25.8–35.5)	28.6 (24.2–33.7)	1.05 (0.77–1.44)
IRR-assessed response rate, n (%)			
CR+PR (95% CI)	128 (63) (55.7–69.4)	103 (50) (43.2–57.3)	
INV-assessed response rate, n (%)			
CR+PR (95% CI)	119 (58) (51.2–65.2)	96 (47) (39.8–53.9)	

CI, confidence interval; CR, complete response; HR, hazard ratio; INV, investigator; IRR, independent radiology reviewer; mo, months; NA, not available; OFA, ofatumumab; PFS, progression-free survival; PR, partial response; R, rituximab.

Summary/Conclusions: Based on the reported observations of efficacy, the IDMC recommended stopping the study for futility, as there was little likelihood of OFA demonstrating superiority over R, and accrual was terminated.

P314

A PHASE IB STUDY EVALUATING THE SAFETY AND CLINICAL ACTIVITY OF ATEZOLIZUMAB COMBINED WITH OBINUTUZUMAB IN PATIENTS WITH RELAPSED OR REFRACTORY NON-HODGKIN LYMPHOMA

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Background: Even with modern chemo-immunotherapy regimens, outcomes for patients (pts) with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) or follicular lymphoma (FL) remain poor. Atezolizumab (atezo), a humanized engineered IgG1 monoclonal antibody (mAb) against programmed death-ligand 1 (PD-L1), blocks the interaction between PD-L1 and its receptors PD-1 and B7.1, thereby preventing inhibition of T-cell activity. Atezo monotherapy has demonstrated clinical activity in multiple types of cancers, including non-Hodgkin lymphoma (NHL). Increased PD-L1/PD-1 expression has been reported in DLBCL and FL on cancer cells, stromal cells and tumor-infiltrating immune cells and may represent escape from immune surveillance. Obinutuzumab (obi) is a next-generation anti-CD20 mAb with enhanced ADCC.
Aims: To determine whether the combination of atezo and obi can activate both innate and adaptive immunity to enhance anti-tumor responses in lymphoma.
Methods: This multicenter, open-label Phase Ib study (NCT02220842) is assessing atezo combined with obi in pts with relapsed or refractory DLBCL or FL. Primary endpoints are safety and tolerability; secondary endpoints are pharmacokinetics and clinical activity. Key eligibility criteria were measurable disease and treatment with ≥1 prior chemo-immunotherapy regimen. Prior autologous stem cell transplant was allowed but not allogeneic stem cell transplant. In cycle 1, pts received obi intravenously (IV) alone on days 1 (100 mg), 2 (900 mg), 8 and 15 (1000 mg). From cycles 2–8, atezo (1200 mg) and obi (1000 mg) were administered IV on day 1 q3w. Atezo consolidation followed (1200 mg q3w) for an additional 6 mo. Objective response rate (ORR) was assessed by IWG NHL criteria. Pretreatment biopsies and on-treatment samples were collected to determine PD-L1 expression levels and examine other biomarkers of response and resistance.
Results: As of Feb 5, 2016, 31 pts (17 DLBCL; 14 FL) were evaluable for safety. Median age was 60 yrs (range 26–90) and 58% of pts were male. Median disease burden at baseline was 3027.9 mm^2 (range 598.0–31400.0). Median duration of therapy was 43 days (range 2–387) for DLBCL and 117 days (range 2–211) for FL. 1 potentially atezo-related adverse event (AE) (Grade 2 myalgia) led to treatment discontinuation. AEs led to study drug interruptions in 7 pts. 26 pts experienced ≥1 treatment emergent (TE) AE (Gr 1–4). The most common Gr 3–4 TEAEs were neutropenia (12.9%) and abdominal pain (6.5%). 4 deaths (3 due to disease progression; 1 unknown cause) were reported. 20 pts with ≥1 imaging assessment were evaluable for efficacy and had a median of 3 prior therapies (range, 2–7). At the first response assessment (after cycle 4), the ORR was 15%; 2 FL pts achieved a complete response, and 1 FL pt had a partial response. 6 pts had stable disease (1 with DLBCL; 5 with FL). PD-L1 expression was detected in DLBCL and FL; corresponding measurements of biomarkers associated with immune activity including infiltration by CD8+ T cells were seen. Updated safety, efficacy and biomarker data will be presented.
Summary/Conclusions: Preliminary results indicate that atezo combined with obi is well tolerated with evidence of clinical activity in pts with heavily pretreated relapsed or refractory DLBCL and FL.

P315

LONG-TERM FOLLOW-UP OF THE NEXT GENERATION PI3K-DELTA INHIBITOR TGR-1202 DEMONSTRATES SAFETY AND HIGH RESPONSE RATES IN NHL: INTEGRATED-ANALYSIS OF TGR-1202 MONOTHERAPY AND COMBINED WITH UBLITUXIMAB

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Background: TGR-1202 is a novel, once-daily PI3Kδ inhibitor with a differentiated safety profile from other PI3Kδ inhibitors, and proven activity in patients (pts) with advanced hematologic malignancies. TGR-1202 is in clinical development as monotherapy and in combination with the glycoengineered CD20 mAb, ublituximab (UTX).
Aims: An integrated-analysis was conducted of pts dosed with TGR-1202 monotherapy or combined with ublituximab with a focus on patients with indolent and aggressive NHL.
Methods: In both studies, there were no limits on prior therapies (Tx) and TGR-1202 was administered once-daily and escalated in 3 + 3 design with expansion cohorts explored. In the combo study, UTX was administered at a fixed dose of 900 mg per infusion. The primary endpoint was safety and efficacy was a secondary endpoint.
Results: Across both studies, a total of 152 pts (81 monotherapy/71 combined with UTX) were exposed to at least one dose of TGR-1202, including 40 CLL/SLL and 112 lymphoma patients. Among patients with lymphoma, histologies included 41 Follicular (FL), 38 DLBCL, 11 Marginal Zone (MZL), 9 Hodgkins (HL), 8 Mantle Cell (MCL) and 5 other. Median age 65 yrs (22-86); 100 M/52 F; median # prior Tx=3 (1-14); 53% refractory to immediate prior Tx. Most frequent reported AE's (all grades; Gr 3/4): nausea (44%; 1%), diarrhea (42%; 2%), fatigue (36%; 3%), vomiting (23%; 0%) and neutropenia (19%; 16%). AST/ALT increase was 6% (3% Gr 3/4), pneumonia 6% (5% Gr 3/4) and pneumonitis 1% (<1% Gr 3/4). 64 pts received TGR-1202 for ≥6 mos, 33 for ≥1 year, with longest on >30 mos. Discontinuations due to AE's occurred in 8% of pts. Across both studies, 74 NHL pts received the therapeutic targeted dose and were evaluable for efficacy. Amongst 37 patients with indolent NHL (FL and MZL), the ORR was 49% (11% CR), and amongst 37 patients with aggressive NHL (DLBCL, MCL, and Richter's), the ORR was 24% (8% CR). Notably, greater activity was observed in patients treated with the combination of TGR-1202 + UTX, with an ORR of 71% (24% CR) in indolent NHL and an ORR of 32% (16% CR) in aggressive NHL patients treated with the combination. The majority of DLBCL responses were observed with the combination of TGR-1202 + UTX, including 3 CR's and were mostly of GCB subtype. The median PFS for TGR-1202 monotherapy in indolent NHL was 27 mo, and has not been reached for patients treated with the TGR+UTX combo.
Summary/Conclusions: TGR-1202 has exhibited a markedly differentiated safety profile from other PI3Kδ inhibitors to date with few discontinuations due to AE's and limited G 3/4 events. In particular we highlight the minimal rates of transaminitis / pneumonitis / colitis events across a large CLL/NHL population. A global Phase 3 trial evaluating TGR-1202 in combination with UTX is ongoing in patients with treatment naïve and relapsed CLL. Registration directed Phase 2 and Phase 3 studies in patients with DLBCL and indolent NHL are planned.

P316

THE COMBINATION OF IBRUTINIB AND VENETOCLAX (ABT-199) RAPIDLY ACHIEVES COMPLETE REMISSIONS IN PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA: PRELIMINARY RESULTS OF THE PHASE II AIM STUDY

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Background: The targeted agents ibrutinib & venetoclax (ABT-199) individually achieve response rates of 68-75% in patients (pts) with relapsed/refractory (R/R) mantle cell lymphoma (MCL). However, individually both drugs are associated with complete remissions (CR) in <25%, and median remissions <18 months. Multiple preclinical models showed synergy between ibrutinib and venetoclax.
Aims: We report the first-in-human experience with the combination of ibrutinib and venetoclax in the ongoing phase II, investigator-initiated AIM study.
Methods: Pts gave written consent, have R/R MCL, ECOG ≤2, and adequate marrow and organ function. The treatment plan comprises of 4 weeks ibrutinib induction at 560mg daily, followed by a 4 week ramp-up of venetoclax (to a target dose 400mg daily) with tumour lysis syndrome (TLS) monitoring, and continuation of both drugs thereafter. At full recruitment (n=24), the AIM study is powered to show improvement in CR rate after 4 months (primary endpoint) from a historic 9% for ibrutinib alone, to ≥30%. Pts are restaged using FDG PET/CT scans & minimal residual disease (MRD) by flow cytometry, ASO-PCR and circulating tumor DNA.
Results: As of 29-Jan-2016, 8 pts were enrolled: median follow-up was 38 days (range, 1-188), median age was 72 years (53–77), median prior therapies was 2 (1–7); 63% of pts had high MIPI scores. The most common adverse events (AE, all grade 1-2) were nausea (n=4), diarrhea (n=2) and oral candidiasis (n=2). Six serious AE have occurred: none were related to study drugs. Response to 4 weeks of ibrutinib induction were 2 partial responses (PR), 2 stable disease and 1 progressive disease; 3 pts were still receiving induction. The full dose of 400mg was reached in all 4 pts entering venetoclax ramp-up, without occurrence of laboratory or clinical TLS. Three pts have completed primary staging at 4 months (1 month of ibrutinib and 3 months of ibrutinib & venetoclax): 2 subjects were in CR with normalization of PET±endoscopy, and complete clearance of previous marrow involvement, including flow cytometry at $>10^{-4}$ sensitivity (Figure). The remaining pt had uninvolved marrow and non-FDG avid, small volume disease following ibrutinib induction that was inadequately visualized on the 4-month PET/CT scan, and was thus classified as PR. Updated safety, efficacy & MRD data (available in at least 8 pts) will be presented at the meeting.

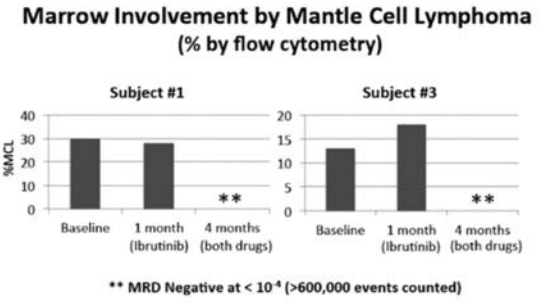

Figure 1.

Summary/Conclusions: The early experience with ibrutinib and venetoclax in combination shows no unexpected safety signals, and indicates promising efficacy.

P317

IDELALISIB MONOTHERAPY IN RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA (FL): EXPERIENCE THROUGH AN EARLY ACCESS PROGRAM IN EUROPE AND AUSTRALIA

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Background: Idelalisib (IDELA) is an oral first-in-class selective PI3Kδ inhibitor approved in EU for use as a monotherapy in patients (pts) with FL who are refractory to two prior lines of treatment. Pts with FL of any grade (grade 1, 2, or 3a) who were refractory to two prior lines of treatment were eligible for this post-authorization, pre-national reimbursement early access program (EAP). Refractoriness required 2 out of the following criteria to be confirmed: (i) refractory to rituximab with chemotherapy; (ii) refractory to alkylating agent (administered with or without rituximab) or (iii) refractory to rituximab without chemotherapy. IDELA data outside clinical trials and in the real-world is limited.
Aims: The objective of this analysis was to characterize the baseline characteristics of refractory FL pts treated with IDELA monotherapy, in a real-world setting. The serious adverse events (SAE) reported are also presented.

Table 1.

Baseline Demography	Total (n=66)
FL Grade, n (%)	
1	9 (14.1)
2	32 (50)
3a	23 (35.9)
Documented	64
Missing data	2
Age, years	
Median, (range)*	66 (40-86)
Male*, n (%)	32 (48.5)
Ann Arbor stage at enrollment, n (%)	
I	2 (3.0)
II	4 (6.1)
III	19 (28.8)
IV	41 (62.1)
High risk FLIPI-2 score at enrollment, n (%)	39 (63.9)
Documented	61
Missing data	5
ECOG, n (%)	
0	24 (36.4)
1	32 (48.5)
2	10 (15.2)
3	0
Number of prior regimens, median (range)	4 (2-13)

**Indicates mandatory information collected upon enrolment*

Methods: This analysis only included pts with refractory FL treated with IDELA monotherapy within the EAP, in Australia, Belgium, Greece and Spain. This

program began enrollment from March 2015 and the data cut off was January 15, 2016. Available data were collected from de-identified pt registration data, follow-up resupply data and SAEs reports. This analysis summarizes the baseline pt characteristics reported which were either mandatory or optional on the pt enrolment form. SAEs were reported whilst the pts were on treatment. The proportion of missing data where information was unreported or unrecoverable is also shown in the table. Of 66 pts with refractory FL who had documented prior treatment regimens (60 pts with specific treatment documented and 6 with number of prior regimens only), the most common prior agents included rituximab (98.4%), cyclophosphamide (96.7%), prednisolone (95%), vincristine (95%), doxorubicin (76.7%) and etoposide (46.7%). A total of 12 pts (20%) had received a prior autologous stem cell transplant. With a median follow up of 109 days (range: 3-309), IDELA monotherapy was well-tolerated with 6/66 pts (9.1%) reporting an SAE. Reported SAEs, regardless of causality, include 1 (1.5%) each of febrile neutropenia, neutropenia, diarrhea, gastrointestinal inflammatory disorder, pancytopenia, progressive disease, liver enzyme elevation, hypotension and colon adenocarcinoma.

Results: Of 66 pts with refractory FL who had documented prior treatment regimens (60 pts with specific treatment documented and 6 with number of prior regimens only), the most common prior agents included rituximab (98.4%), cyclophosphamide (96.7%), prednisolone (95%), vincristine (95%), doxorubicin (76.7%) and etoposide (46.7%). A total of 12 pts (20%) had received a prior autologous stem cell transplant. With a median follow up of 109 days (range: 3-309), IDELA monotherapy was well-tolerated with 6/66 pts (9.1%) reporting an SAE. Reported SAEs, regardless of causality, include 1 (1.5%) each of febrile neutropenia, neutropenia, diarrhea, gastrointestinal inflammatory disorder, pancytopenia, progressive disease, liver enzyme elevation, hypotension and colon adenocarcinoma.

Summary/Conclusions: The results of this large real-world cohort of pts with FL who are refractory to 2 prior lines of therapy treated by IDELA monotherapy outside of the clinical trial setting confirm the acceptable tolerability profile of IDELA.

P318

PHASE 2A STUDY OF THE PHOSPHATIDYLINOSITOL-3-KINASE (PI3K) INHIBITOR COPANLISIB IN PATIENTS WITH RELAPSED/REFRACTORY, INDOLENT OR AGGRESSIVE LYMPHOMA

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Background: Copanlisib (BAY 80-6946) is a potent and selective pan-class I PI3K inhibitor with preferential activity against the δ- and α-isoforms. In an expansion cohort of a phase I study, promising activity was seen in patients with non-Hodgkin lymphoma (NHL), with 6 of 6 responses in follicular lymphoma (FL) patients [2 of which were durable (>3 years) complete responses per post-hoc radiologic review (Patnaik et al, submitted)] and 1 of 3 diffuse large B-cell lymphoma (DLBCL) patients achieving a partial response (PR).

Aims: We therefore investigated the efficacy and safety of copanlisib in subjects with relapsed/refractory indolent or aggressive NHL or chronic lymphocytic leukemia (CLL). (NCT01660451)

Methods: Patients with histologically confirmed indolent or aggressive lymphoma or CLL, relapsed or refractory to ≥2 prior lines of treatment were enrolled. Copanlisib (0.8 mg/kg) was administered intravenously on days 1, 8 and 15 of a 28-day cycle until disease progression or unacceptable toxicity. The primary endpoint was objective response rate (ORR) as assessed by independent radiology review according to the response criteria for lymphoma (Cheson et al, JCO 1999) or the guidelines for diagnosis and treatment of CLL (Hallek et al, Blood 2008). Secondary endpoints included progression-free survival (PFS) and duration of response (DOR), safety and tolerability.

Results: A total of 20 patients with indolent NHL (iNHL; 16 FL, 3 marginal zone lymphoma, 1 small lymphocytic lymphoma), 13 CLL and 48 with aggressive NHL [15 DLBCL, 14 peripheral T-cell lymphoma, 11 mantle cell lymphoma (MCL), 6 transformed, 1 mediastinal, and 1 Grade 3b FL] were treated; median age 67 years; 53% male; median 3 (range 1-10) number of previous lines of treatment and prior rituximab in 80% of patients. At the time of analysis, the ORR was 47% (90% CI 27-68) and stable disease (SD) was 47% (CI 27-68) in patients with iNHL, 38% (CI 17-65) and 46% (CI 22-71) in CLL, and 26% (CI 16-39) and 17% (CI 9-29) in aggressive NHL, respectively. For patients with FL, the ORR was 40% (CI 19-64), with 1 CR, 2 uCRs, and 3 PRs; SD in 53% (CI 30-76). The ORR in the MCL patients was 64% (CI 35-86; 2 uCR and 5 PRs). The median DOR was 390 days in the indolent group and 166 days in the aggressive group. The most common treatment-related adverse events (AEs) of all grades (G) were hyperglycemia (59%), hypertension (54%), diarrhea (33%), and fatigue (28%). G3-4 treatment-related AEs occurring in >10% of patients included hypertension (39%) and hyperglycemia (25%). Treatment-related AEs leading to dose reduction were observed in 4%, interruption in 36%, interruption and reduction in 7%, or permanent discontinuation in 17% of patients, respectively. There were 4 treatment-related grade 5 events: meningitis, respiratory failure and 2 lung infections.

Summary/Conclusions: Copanlisib is active as a single agent in heavily pretreated patients with a variety of relapsed/refractory indolent or aggressive lymphoma subtypes, with promising CR activity in FL and MCL. Copanlisib exhibited a manageable toxicity profile. Final results of survival rates will be presented. Based on these encouraging results, phase II studies in patients with FL, MCL, and DLBCL are ongoing.

P319

PRELIMINARY SAFETY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF DUVELISIB PLUS RITUXIMAB OR OBINUTUZUMAB IN PATIENTS WITH PREVIOUSLY UNTREATED CD20+ FOLLICULAR LYMPHOMA

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Background: Duvelisib (IPI-145), a dual PI3K-δ,γ inhibitor, is being evaluated as an oral therapeutic for hematologic malignancies as a monotherapy or in combination with anti-CD20 antibodies.

Aims: We report herein results for the CONTEMPO (NCT02391545) study, an ongoing 2-part, 2-arm, Phase 1b/2 study that evaluates the safety, pharmacokinetics, pharmacodynamics and efficacy of duvelisib combined with the anti-CD20 antibodies rituximab (DR) or obinutuzumab (DO) in patients with previously untreated CD20+ follicular lymphoma.

Methods: Duvelisib is administered at 25 mg BID continuously in 28-day treatment cycles combined either with rituximab (375 mg/m^2 for 4 weekly doses, then 1 dose every 2 cycles) or obinutuzumab (1000 mg for 4 weekly doses, then 1 dose every 2 cycles). We report results from Part 1 which evaluated the safety of the combinations by monitoring adverse events (AEs) and dose limiting toxicities (DLTs).

Results: Twelve patients were treated in Part 1 of the study (6 DR and 6 DO). At the time of data cut-off, patients had received duvelisib for a median of 2.5 (DR) and 1.9 (DO) months. No DLT occurred with DR. One DLT of treatment-related Grade 3 elevated lipase occurred with DO (Cycle 1). No serious AEs occurred on either arm, and no patient discontinued treatment. Four patients (1 DR; 3 DO) had ≥Grade 3 AEs (DR: amylase increased, lipase increased; DO: neutropenia, ALT increased, lipase increased). Duvelisib was rapidly absorbed (t_{max}~1 hour; C_{max}~910 ng/mL; single dose); after multiple doses, plasma steady state levels were ~500-540 ng/mL and approximately half of the duvelisib was transformed into its metabolite IPI-656. Postdose serum cytokine levels were decreased by Cycle 1 Day 8 compared to baseline. Preliminary activity will be reported at the time of the meeting.

Summary/Conclusions: Combination of duvelisib 25 mg BID with rituximab or obinutuzumab was well tolerated in these patients, with one DLT observed. Both DR and DO arms were continued to Part 2 of the study, which is ongoing.

P320

SAFETY AND EFFICACY PROFILES OF CLARITHROMYCIN MONOTHERAPY IN 55 PATIENTS WITH EXTRANODAL MARGINAL ZONE LYMPHOMA (EMZL)

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Background: Macrolides have been proposed as new anticancer agents. In particular, clarithromycin displays different immunomodulatory effects on several pathways, and is active in different tumor models. Two phase II trials have demonstrated that clarithromycin monotherapy is safe and active in patients (pts) with relapsed/refractory EMZL (Govi *et al.* BJH 2010; Ferreri *et al.* Ann Oncol 2015), but the best administration schedule of this antibiotic as antineoplastic agent remains to be defined.

Aims: To address tolerability, activity and efficacy of clarithromycin monotherapy in a large retrospective series of pts with EMZL. Results with three different administration schedules were analyzed.

Methods: 55 pts with EMZL and at least one measurable/parametrable lesion treated with single-agent clarithromycin between 2003 and 2015 were analyzed. Clarithromycin was used with three different schedules: a 6-month regimen at 500 mg twice a day, every day (n=13); three courses of 500 mg twice a day, days 1-21, every 35 days (n=19); or four courses of 2.000 mg/d, days 1-14, every 21 days (n=23).
Results: Median age of analyzed pts was 65 years (range 30-88), with a M:F ratio of 0.57. EMZL affected a single organ in 40 pts, and was multifocal in 15: the most commonly involved organs were ocular adnexae (n=30), stomach (n=9) and lung (n=7). International Prognostic Index (IPI) score was 0-1 in 40 pts and 2-4 in 15; the IELSG risk score (age, LDH and stage) was 0 in 25 pts, 1 in 23 and >1 in 7. A prior history of chronic infection was recorded in 20 pts: HBV/HCV, *H. pylori* and *C. psittaci* in 5 pts each, with multiple combinations of these micro-organisms in 5. Clarithromycin was the first treatment line in 8 pts, the second in 24, the third in 15, and the 4^{th}-6^{th} in 8. Tolerability was excellent: only 2 pts had grade-3 toxicity (nausea); the main side effects were grade 1-2 nausea (17 pts), dysgeusia (7), dizziness (4), headache (3), arthralgia (2), and rash (2). Five pts interrupted treatment due to nausea (3), rash or dysgeusia. There were no differences in feasibility and tolerability among different treatment schedules, but nausea was significantly more common when a daily dose of 2.000 mg was used (52% *vs* 25%; Fisher exact, p=0.03). Objective response after clarithromycin treatment was complete in 13 (24%) pts and partial in 13, with an overall response rate (ORR) of 47% (95% CI= 34-60). Responses were more common among pts with gastric MALT lymphoma (7/9 *vs* 19/46; p=0.04). ORR was not associated with prior treatment, IPI, IELSG score, and clarithromycin dose. At a median follow-up of 33 months (range 7-137), 29 pts remain progression-free, with a 3-year PFS of 52±7%. Pts with lymphoma refractory to prior treatment and pts with IPI score ≥2 had a significantly and independently poorer PFS. Conversely, clarithromycin dose was not associated with outcome, with a 3-year PFS of 60±9% and 42±10% (log-rank test; p=0.47) for pts treated with a daily dose of 1 g and 2 g, respectively. Fifty-two pts are alive, with a 3-year OS of 96±3%; causes of death were liver failure, stroke and lung cancer.
Summary/Conclusions: Clarithromycin monotherapy is active in pts with EMZL, and exhibits an excellent safety profile when used at a dose of up to 2 g/d for 4-6 months. The recommended daily dose of clarithromycin in pts with EMZL is 1 g as it is associated with a lower incidence of nausea and similar efficacy than 2 g. Further investigation of clarithromycin, alone or associated with immunomodulators, in lymphoma pts is warranted.

P321

A PHASE I STUDY IN T CELL LYMPHOMA PATIENTS TREATED WITH ANTI-CD70 SIMPLE ANTIBODY™ ARGX-110

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Background: CD70 is a cell surface marker transiently expressed on activated B, T and dendritic cells. The interaction via its receptor CD27 results in survival, proliferation and lymphocyte differentiation. In a large number of solid tumours types CD70 is overexpressed, but without receptor co-expression. The CD70/CD27 axis appears critical for proliferation and survival of malignant cells in T-cell lymphoma. Indeed, overexpression of CD70 is paralleled by high expression of its receptor CD27 in TCL.
Aims: ARGX-110 is a novel glycoengineered monoclonal antibody targeting CD70 and blocking CD27 signaling, leading to a direct mode of action lysing $CD70^+$ tumor cells (via CDC, ADCP and enhanced ADCC) and indirect anti-tumor restoring immune surveillance. (NCT01813539) A phase I trial was initiated with ARGX-110, dose escalated to investigate safety, clinical pharmacology and determine the RP2D, in refractory or relapsed solid tumors and hematological malignancies.
Methods: Patients with >10% $CD70^+$ expression on the tumor in immunohistochemistry were included in the study. ARGX-110 was given to 65 patients at doses from 0.1 to 10 mg/kg intravenously every 3W. In TCL patient's biomarkers were measured during the course of treatment.
Results: Immunohistochemistry on TCL patient samples confirmed overexpression of CD70 (>10% CD70+ tumor cells) in 40/73 PTCL (55%) and 15/21 CTCL (71%) samples. In 68% of these samples CD70 and CD27 were simultaneously expressed. Serum soluble CD27 levels were elevated in TCL patients compared to healthy subjects (670±1096 *vs* 192±44 IU/ml, respectively, p=0.0062). Nine patients TCL patients were included in the study (3 Cutaneous TCL (CTCL) and 6 Peripheral TCL (PTCL)). ARGX-110 was well tolerated with no grade >3 adverse events related to the study drug. A clinical and/or biological response was observed in 4/9 of T-cell Lymphoma cohort patients; CTCL (n=3) and PTCL-AITL (n=1) patients. The activity in 2 patients with Sezary syndrome (treated at 0.1 and 10 mg/kg, respectively) resulted in a >90% reduction in the circulating malignant clone and a clinical partial response in the skin in one patient. The patient with CTCL T_{FH} lymphoma (T_{FH}L; 5 mg/kg) achieved a PR. One patient with AITL reached a PR (5 mg/kg dose) and had a resolution of lymphoma associated auto-immune hemolytic anemia.
Summary/Conclusions: In the TCL patients treated with ARGX-110, we observed a clinical and/or biological response in 4/9 patients. These preliminary results support further investigation of ARGX-110 in TCL.

Stem cell transplantation - Experimental

P322

TO SCREEN BIOLOGICAL MARKER GENE ASSOCIATED WITH B CELL IN CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD) PATIENTS

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a widely used therapy for a lot of malignant and nonmalignant hematologic diseases. Graft-*versus*-host disease (GVHD) remains one of the major barriers to a more widespread and successful application of allo-HSCT. Despite immune-suppressive prophylaxis, most survivors develop chronic GVHD (cGVHD). Identification of biologic markers of the syndrome could facilitate significant advances in understanding to help guide prevention and treatment approaches. Data in humans support a role of both T- and B-cells in a highly complex network leading to cGVHD. However, there was no report about biological marker gene associated with B cell in cGVHD patients.
Aims: So we screened biological marker gene associated with B cell in cGVHD patients. Microarrays were used to detect expressed gene profiles of peripheral blood mononuclear cells total RNA from subjects in the mild-moderate cGVHD, severe cGVHD, non-cGVHD, immune tolerance and healthy donors groups.
Methods: Microarrays were used to detect expressed gene profiles of peripheral blood mononuclear cells total RNA from subjects in the mild-moderate cGVHD, severe cGVHD, non-cGVHD, immune tolerance and healthy donors groups. Found out the genes associated with B cell in cGVHD patients reached statistical significance after a false discovery rate (FDR) correction was applied to the data. Real-time quantitative RT-PCR was used to confirm the B cell genes.
Results: Found out the genes associated with B cell in cGVHD patients reached statistical significance after a false discovery rate (FDR) correction was applied to the data. Real-time quantitative RT-PCR was used to confirm the B cell genes. 284 genes were found by microarrays between cGVHD patients and patients without cGVHD, 5 genes (CDKN2A, SOX4, ZBTB32, CD70, IL21R) associated with B cell. 800 genes were found by microarrays between immune tolerance patients and patients without cGVHD, 3 genes (NFAM1, CD27, BST1) associated with B cell. Real-time quantitative RT-PCR confirmed significantly lower expression of CDKN2A in cGVHD relative to without cGVHD patients (P=0.001). Significantly higher expression of CD27 and lower expression of BST1 in immune tolerance relative to without cGVHD patients (P=0.038, P=0.022).
Summary/Conclusions: The preliminary results indicated that CDKN2A gene had significantly lower expression in cGVHD relative to without cGVHD patients, CD27 gene had significantly higher expression and BST1 gene had significantly lower expression lower expression in immune tolerance patients relative to without cGVHD patients. The changes of these genes may be potential biomarkers for cGVHD and immune tolerance.

P323

MITOCHONDRIAL INHIBITION BY NICOTINAMIDE RIBOSIDE POTENTLY STIMULATES HEMATOPOIESIS AFTER TRANSPLANTATION VIA THE MITOCHONDRIAL UNFOLDED PROTEIN RESPONSE

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Background: The slow nature of blood regeneration from hematopoietic stem cells (HSCs) severely compromises the survival of patients upon primary bone marrow failure, intensive ablative chemotherapy or HSC transplantation. Current strategies to accelerate HSC regeneration and shorten the time of bone marrow aplasia, including treatment with granulocyte colony stimulating factor (G-CSF), are insufficient to avoid severe and often lethal infections during the time of aplasia. Indeed, HSCs are predominantly quiescent during homeostasis. The proliferative stress imposed on HSCs to reconstitute the full hematopoietic system in the 4-6 weeks following HSC transplantation entails activation of oxidative phosphorylation within mitochondria, interfering with pyruvate dehydrogenase kinase (PDK) and hypoxia-inducible factor (HIFa)-driven metabolic checkpoints of HSC self-renewal and thus induces premature HSC aging. In turn, activation of the mitochondrial unfolded protein response (UPR^{mt}) enhances mitochondrial health and prolongs lifespan from *C. elegans* to mice, mostly due to the activation of the Sirtuins and their convergence into DAF-16/FOXO3 signaling, also shown to regulate HSCs maintenance.
Aims: We set to test whether mitochondrial inhibition and/or activation of UPRmt in HSCs would lead to accelerated recovery from aplasia after bone marrow transplantation in mice.
Methods: Wildtype C57BL/6 mice or NOD-SCID mice reconstituted with human CD34+ cells were treated with Nicotinamide Riboside for 7 days during homeostasis, and from day -7 to 35 in the context of HSC transplantation.
Results: Here we show that distinct hematopoietic progenitor compartments show specific increases in mitochondrial activity during commitment, and that mitochondrial activity is a functional predictor of HSC engraftment both *in vivo* and *in vitro*. We have shown that modulating mitochondrial potential through the use of uncouplers can displace the long-term (LT)/short-term (ST) -HSC equilibrium *in vivo* by maintaining LT-HSCs while increasing the ST-HSC pool. Dietary supplementation in NAD^+-boosting agent Nicotinamide Riboside (NR) inhibits mitochondrial activity via UPRmt and activation of autophagy within long-term HSCs, resulting in the expansion of functional short-term HSCs without HSC exhaustion in mice. Indeed, NR treatment dramatically improves survival and accelerates blood recovery after murine HSC transplantation, also increasing the production of human blood cells in humanized mice.
Summary/Conclusions: Our work establishes a novel link between UPRmt and autophagy in self-renewing HSCs, demonstrates for the first time an effect of NAD+ boosting strategies at the level of the most primitive blood stem cells, and unveils the potential of NR to improve the outcome of patients suffering from bone marrow insufficiency.

P324

MESENCHYMAL STROMAL CELLS STIMULATE THE PROLIFERATION AND IL-22 PRODUCTION BY TYPE 3 INNATE LYMPHOID CELLS

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Background: Graft-*versus*-host disease (GvHD) remains a challenging complication after allogeneic hematopoietic stem cell transplantation (HSCT). Mesenchymal stromal cells (MSCs) are increasingly applied to treat steroid-refractory GvHD. The therapeutic effect of MSCs is mainly ascribed to their ability to suppress the proliferation of (alloreactive) lymphocytes and to enhance the tissue repair activity of endothelial cells, fibroblasts, and epithelial cells. However, only about half of the GvHD patients actually respond to MSC therapy, and which determinants are related to MSC responsiveness remains to be elucidated. We recently found an inverse association between the proportion of circulating, activated type 3 innate lymphoid cells (ILC3s) before allogeneic HSCT and susceptibility to GvHD development, which may be related to the ability of ILC3s to produce tissue-protective interleukin 22 (IL-22).
Aims: To investigate the potential interaction between MSCs and ILC3s *in vitro*. In particular we aimed to test whether MSCs can induce ILC3s to produce the tissue protective cytokine IL-22.
Methods: ILC3s isolated from human tonsils were CellTrace-labeled and co-cultured with bone-marrow derived MSCs for 5 days in the presence of IL-2. Following co-culture, we determined ILC3 proliferation and cytokine production, and analyzed surface-marker expression on MSCs by flow cytometry.
Results: Co-culture with MSCs significantly enhanced the proliferation of ILC3s and resulted in an increased production of IL-22. ILC3s proliferated slightly better in the presence of MSC culture supernatant compared to IL-2 only, and addition of IL-7 blocking antibodies to MSC-ILC3 co-cultures had an inhibitory effect on ILC3 proliferation in MSC-ILC3 co-cultures. These findings suggest that the MSC-driven stimulation was at least partly mediated via IL-7. In transwell experiments, however, proliferation of ILC3s was significantly reduced when ILC3s and MSCs were cultured in separate compartments, showing that cell-cell proximity or cell-cell contact is important for the stimulation of ILC3s by MSCs. Reciprocally, ILC3s also influenced the MSCs, as ICAM1 and VCAM1 expression was upregulated in co-cultures. Addition of blocking antibodies against ICAM1, VCAM1, or the integrin subunits these adhesion molecules bind to, did not affect the proliferation of ILC3s.
Summary/Conclusions: We here show that MSCs can interact with ILC3s *in vitro*, resulting in enhanced proliferation and cytokine production of ILC3s. These results suggest that MSCs may have a dual mechanism by which they contribute to the control of GvHD: (1) by inhibiting the proliferation of (alloreactive) T lymphocytes and NK cells, and (2) by promoting expansion and IL-22 production of tissue-protective ILC3s.

P325

GRANULOCYTE COLONY-STIMULATING FACTOR MOBILIZATION INCREASES THE PROPORTION OF REGULATORY GAMMA DELT T CELLS

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Background: The immune modulatory effect of granulocyte colony-stimulating factor (G-CSF) on T cells resulted in an unexpected low incidence of graft-*versus*-host disease (GVHD) in allogeneic peripheral blood stem cell transplantation. Our previous studies demonstrated that G-CSF mobilization influenced

the distribution and clonality of TRGV and TRDV repertoire (T cell receptors of γδ T cells). Regulatory γδ T cells (γδ Tregs), which express Foxp3 and primarily belong to $CD27^{+}CD25^{high}$ phenotype, are a novel subset of cells with immunosuppressive function. However, whether G-CSF mobilization could influence the expression and immunosuppressive function of γδ Tregs remains unknown.
Aims: To explore the effect of G-CSF mobilization on γδ Tregs, the expression and immunosuppressive function of γδ Tregs before and after G-CSF mobilization were compared.
Methods: Peripheral blood $γδ^{+}$ T cells were sorted by magnetic activated cell-sorting (MACS) from 10 donors before and after G-CSF mobilization, respectively. MACS-sorted γδ T cells were used as effector cells in the carboxylfluorescein diacetate succinimidyl ester (CFSE) assays and cell immunophenotyping was analyzed by flow cytometry. Autologous $CD4^{+}$ T cells were purified by MACS, labeled with CFSE, used as responder cells, and finally co-cultured with effector cells. After 5 d incubation, cells were harvested and analyzed for flow cytometry by gating on the CFSE-labeled cells. The percentages of divided responder T cells before and after G-CSF mobilization were compared.
Results: Compared with that before mobilization, the proportions of $Foxp3^{+}γδ$ T, $Foxp3^{+}$ Vδ1 T and $Foxp3^{+}$ Vδ2 T cells were significantly increased in sorted γδ T cells after G-CSF mobilization (P=0.035, P=0.017 and P=0.004). The proportions of $CD27^{+}γδ$ T, $CD27^{+}$Vδ1 T and $CD27^{+}$Vδ2 T cells were also significantly increased in sorted γδ T cells after G-CSF mobilization (P=0.035, P=0.026, P=0.037). The proportions of $CD25^{+}γδ$ T, $CD25^{+}$ Vδ1 T and $CD25^{+}$Vδ2 T cells were similar in sorted γδ T cells before and after mobilization (P=0.252, P=0.126, P=0.237). We then compared the inhibitory effect of sorted γδ T cells before and after G-CSF mobilization on the proliferation of autologous $CD4^{+}$ T cells. We found that MACS-sorted γδ T cells before and after G-CSF mobilization both exerted certain suppression on proliferation, whereas γδ T cells after G-CSF mobilization had a more significant suppressive effect on $CD4^{+}$ T cells than γδ T cells before mobilization (P=0.002).
Summary/Conclusions: G-CSF mobilization could increase the proportions of regulatory T cells in sorted γδ T cells, thus having a higher suppressive effect on $CD4^{+}$ T cells.

P326

PREACTIVATED MESENCHYMAL STROMAL CELLS IMPROVE T-CELL REGENERATION AFTER THEIR COTRANSPLANTATION WITH HEMATOPOIETIC STEM CELLS

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Background: Mesenchymal stromal cells (MSCs) are known to support hematopoiesis both *in vitro* and *in vivo*. However, there is scant evidence on the effect of preactivated MSCs with inflammatory cytokines on the engraftment of hematopoietic stem/progenitor cells.
Aims: The aim of this study was therefore to investigate whether preactivation ("licensing") of subsets of MSCs with inflammatory cytokines (IFN-gamma, TNF-alpha) may affect regeneration of T-cells after hematopoietic cell transplantation.
Methods: MSCs were generated from positively selected $CD271^{+}$ bone marrow mononuclear cells (CD271-MSCs) by using the MSC Research Tool Box–CD271 (LNGFR)-APC (Miltenyi Biotec GmbH), according to manufacturer's instructions. $CD34^{+}$ hematopoietic stem/progenitor cells (HSCs) were cotransplanted with either resting or preactivated ("licensed") CD271-MSCs with IFN-γ or TNF-α in immunodeficient NSG-mice.
Results: Flow cytometric analysis of peripheral blood (PB) of NSG-mice 4 weeks after cotransplantation of $CD34^{+}$ cells with CD271-MSCs demonstrated that $CD34^{+}$ HSCs differentiated in B and NK-cells, but not in T-cells. However, FACS analysis of PB at week 14, showed a 6.6-fold increase of T-cells in the cotransplanted group *versus* the group transplanted with CD34+ HSCs only (control group). Moreover, cotransplanted mice with "licensed" CD271-MSCs with TNF-α or IFN-γ showed a significantly increased number of T-cells (23- or 30-fold, respectively) compared to control group. All regenerated T-cells (helper and cytotoxic) had a naïve (CD3+CD4+CD45RA+CCR7+ or CD3+CD8+ CD45RA+CCR7+) but not an effector phenotype. Both, resting and preactivated CD271-MSCs normalized the CD4/CD8 ratio from 0.8 (control group) to 1.3. TREC-analysis of the thymus cells revealed no difference between cotransplanted groups and control. However, Vβ spectratyping (23 Vβ-subgroups) of spleen cells showed an increased T cell repertoire complexity (Vß2, Vß3, Vß4, Vß9, Vß17 und Vß21) in the group cotransplanted with IFN-γ preactivated CD271-MSCs and $CD34^{+}$HSCs. T-cell regeneration was also confirmed by immunohistochemistry of the spleen.
Summary/Conclusions: Cotransplantation of preactivated CD271-MSCs with IFN-γ appears to be a novel strategy to improve T-cell regeneration and development of T-cell receptor repertoire after hematopoietic transplantation.

P327

EVIDENCE OF COMPLEMENT DYSREGULATION IN TRANSPLANT ASSOCIATED THROMBOTIC MICROANGIOPATHY

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Background: Transplant-associated thrombotic microangiopathy (taTMA) is a devastating complication occurring in 10-39% of patients undergoing hematopoietic cell transplantation (HCT) with a high mortality rate. However, our understanding of the pathophysiology which is strongly related to diagnosis and treatment options is limited. Recent evidence has linked taTMA with atypical hemolytic uremic syndrome (aHUS), a disease of excessive activation of the alternative pathway of complement (APC) that is successfully treated by terminal complement inhibition with eculizumab. We have recently developed the modified Ham test as a functional assay that can reliably distinguish APC activation observed in aHUS from other TMAs.
Aims: We aimed firstly to detect evidence of increased APC activation by the modified Ham test in taTMA and secondly to test the *in vitro* ability of eculizumab to inhibit APC activation in taTMA.
Methods: Patients were enrolled in a prospective fashion from December 2014 to April 2015 as all patients admitted to our service with taTMA according to clinical judgement, in keeping with the current diagnostic criteria (BMT-CTN or IWG). Patients with graft-*versus*-host-disease / GVHD and transplanted patients with neither complication / controls were enrolled during the same timeframe. APC activation was detected in the modified Ham test, a cell proliferation assay that measures complement-mediated cell killing caused by patient serum, and was compared to a previously described marker of complement activation, serum C5b-9 levels. Eculizumab containing serum (ECU) was collected from a patient with paroxysmal nocturnal hemoglobinuria (PNH) within 60 minutes after the infusion and used to test complement blockade by eculizumab in the modified Ham test.
Results: We studied 5 patients with taTMA, 4 patients with GVHD and 6 transplanted patients with neither complication. We first measured C5b-9 levels as a biomarker of terminal complement activation. There was no difference in serum C5b-9 levels among patients with taTMA, patients with GVHD and controls (57.8±28.1, 49.2±25.5 and 53.4±31.9 ng/ml respectively, p=0.913). We next utilized the modified Ham test, which detected increased APC activation in taTMA. Serum from patients with taTMA caused significantly increased complement-mediated killing compared to patients with GVHD and controls (Figure 1). As previously described, increased complement activation was also found in taTMA patients while on eculizumab (grey symbols in Figure 1). In the ROC curve analysis, a cutoff of 23.5% identified taTMA with 100% sensitivity and specificity in this small cohort. Then, we evaluated the efficacy of complement inhibition by eculizumab in the modified Ham test. Mixing eculizumab serum (ECU) with taTMA sera demonstrated a dose-killing relationship that was consistent across the three patients.
Summary/Conclusions: Our results confirm recent suggestions that complement dysregulation is involved in the pathophysiology of taTMA. If confirmed in larger series, the modified Ham test may also form the basis for therapeutic trials based upon complement inhibition.

P328

FAMILY-DIRECTED CORD BLOOD BANKING FOR SICKLE CELL DISEASE: A 20-YEAR EXPERIENCE, ON BEHALF OF EUROCORD-MONACORD AND THE INTERNATIONAL SICKLE CELL DISEASE OBSERVATORY

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Background: Cord blood transplantation (CBT) from a related family member is an effective therapy for patients with Sickle Cell Disease (SCD) resulting in encouraging outcomes with similar or superior survival to adult donor transplant. Efforts to implement family-directed umbilical cord blood (UCB) banking have been developed in the past two decades for siblings requiring stem cell transplantation (SCT).
Aims: Public umbilical cord blood banks are faced with the challenge regarding the units to be stored or to be discarded or used for other endeavors such as research. To this end, we report our 20-year experience of family-directed UCB banking for SCD and review the characteristics of the UCB units collected between 1995 and 2014.

Methods: Families were eligible if they had a child with SCD, and expecting the birth of a sibling. Participation was voluntary and free of charge. All mothers underwent a panel of serologic blood donor screening assays. UCB units were collected in remote sites, cryopreserved and stored in a single bank. UCB testing included viral serology, bacterial cultures and cell counts. HLA typing on the UCB were not routinely performed unless requested by the transplant physician.
Results: 189 families from 27 different centers were enrolled. 17 (10%) families had >1 UCB unit stored, due to multiple pregnancies. Potential recipients had a median age of 6 years (range 11 months - 12 years) at time of UCB harvest. 14 families had >1 affected child. A total of 210 UCB units were collected from 189 mothers. All UCBs were negative for HIV and 64 UCBs (30%) had positive anti-HBs and/or anti-HBc with negative HBsAg. Median UCB volume at cryopreservation was 92.5 mL (range 33-194).Median total nucleated cell (TNC) count at cryopreservation was 9.2 $x10^8$ (range 1-75.3). The median collected CD34+ and CFU-GM cell counts were 4.5 $x10^6$ (range 0.18-61) and 4.5 $x10^5$ (range 0.14-67) respectively. The hemoglobin status of the UCB units was assessed through the neonatal screening. Data were available for 179 UCBs (85%) including 64 (30%) hemoglobin AA, 85 (40%) hemoglobin AS, 25 (14%) hemoglobin SS, 3 (<1%) hemoglobin AC, 1 S-beta+-thal and 1 beta-thal. Eight (4%) out of the 210 banked UCB units were released for CBT with a median TNC count of 7.0$x10^8$ (3.0$x10^8$ - 21.8$x10^8$). Five patients were transplanted using a single UCB and 3 patients with the sibling's bone marrow and UCB. Post-transplant data were available for 6 patients: all of them had stable engraftment of donor cells and are alive, free of SCD.
Summary/Conclusions: Our data showed that family-directed UCB banking is feasible and yields good quality cord blood units for sibling transplantation. However, the number of CBT performed is disappointing despite the good results of sibling transplantation in SCD. Therefore, we must think about the cost-effectiveness of this approach when HLA identical sibling donor is available.

Stem cell transplantation - Clinical 1

P329

CONSOLIDATION BY ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN CR1 AGED 40-69 YEARS: A COMPARISON OF MYELOABLATIVE AND REDUCED INTENSITY CONDITIONING

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Background: While survival in ALL patients<40 years has improved with the introduction of pediatric regimens, outcome in patients ≥40 years is still disappointing. Complete remission (CR1) rates upon induction therapy estimate 70-80%, but inferior survival is caused by both enhanced relapse and a higher incidence of treatment-related mortality (TRM). Reduced intensity conditioning (RIC) allogeneic stem cell transplantation (alloSCT) was developed as a less toxic but effective type of consolidation therapy and applied as from 2000 in several centers in the Netherlands, while some centers continued to apply myeloablative (MAB) alloSCT.
Aims: We set out to retrospectively compare RIC alloSCT and MAB alloSCT as consolidation therapies in patients with ALL aged 40-69 years in CR1.
Methods: 151 patients (age: 40-67 years) with ALL in CR1 receiving consolidation by alloSCT between 2001 and 2013 were included. Data were provided by the Dutch Stem Cell Transplantation Working Group of HOVON. The median follow up was 68 months for MAB alloSCT (n=62) and 56 months for RIC alloSCT recipients (n=89). MAB was predominantly applied by the combination of total body irradiation (TBI) and cyclophosphamide, while RIC by the Seattle regimen (fludarabin and 2 Gy TBI). Univariable and multivariable Cox-regression analysis were performed with endpoints overall survival (OS), relapse free survival (RFS), TRM and relapse. Cox-regression was adjusted for age, white blood cell count at diagnosis, cytogenetic risk, interval from diagnosis to alloSCT, year of treatment, donor type.
Results: Statistically significant differences between RIC and MAB recipients were found with respect to age (median 55 *vs* 47 years, p<0.001) and year of transplantation (median 2009 *vs* 2006, p=0.001). Although not statistically significant, RIC alloSCT patients had higher EBMT scores (score 3-4: 62% *vs* 45%, p=0.105) and more alternative donors were used in RIC alloSCT patients (48% *vs* 35%, p=0.071). Underlying ALL risk parameters did not differ between either type of alloSCT. The relapse rate at 5 years was significantly higher after RIC alloSCT as compared to MAB alloSCT (47% *vs* 20%, p=0.001, Figure). TRM was predominantly affected by age and interval from diagnosis to transplantation. By univariate analysis, improved OS was found in recipients of MAB alloSCT as compared to RIC alloSCT, which appeared significantly affected by age and type of conditioning. Multivariable analysis showed again that RIC alloSCT was associated with a significantly higher relapse rate (HR: 3.47 [95% CI: 1.70-7.11] p=0.001) and significant reduction of TRM (HR: 0.42 [95% CI: 0.18-0.97] p=0.043). These opposing effects, however, resulted in a non-significant difference between RIC and MAB as regards RFS (HR: 1.47 [95% CI: 0.88-2.47] p=0.144) and OS (HR: 1.27 [95% CI: 0.74-2.19] p=0.392).

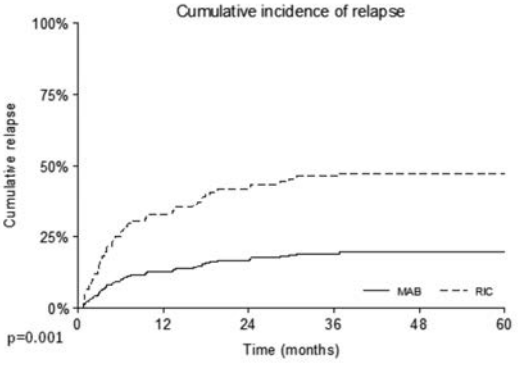

Figure 1.

Summary/Conclusions: Recipients of RIC and MAB conditioning appeared to differ for a number of variables, which hampered an unbiased comparison. Nevertheless, RIC alloSCT was associated with a significantly higher relapse rate, while these patients did not exhibit more high-risk ALL features. Due to less TRM, that difference did not translate into a significant difference in RFS and OS. Collectively, these results suggest that MAB alloSCT may provide a more effective type of consolidation therapy for reduction of relapse and that RIC alloSCT may especially be applied in patients at higher risk for TRM because of age, EBMT score and/or comorbidities.

P330

RELAPSED/REFRACTORY HIGH GRADE B CELL NON-HODGKIN LYMPHOMA: SHOULD ONGOING PET POSITIVITY PRECLUDE AUTOLOGOUS TRANSPLANTATION IN PATIENTS ATTAINING A CT RESPONSE TO SALVAGE CHEMOTHERAPY?

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Background: High dose chemotherapy and autologous stem cell transplantation (ASCT) have been shown to increase overall (OS) and event-free survival (EFS) in patients with chemo-sensitive relapsed or refractory high grade B cell Non-Hodgkin lymphoma (NHL) as assessed by computerized tomography (CT). Positron Emission Tomography (PET) scanning, when compared to CT, has been shown to upstage disease both at diagnosis and assessment of treatment response and can help to predict outcome. However, the positive predictive value for end of treatment PET scans varies widely, especially for patients treated with rituximab. It is therefore unclear whether CT responders who have residual PET positive lesions will benefit from ASCT since this has not been evaluated in a randomized controlled trial. This question is important since historically (prior to PET) these patients will do poorly when treated with conventional dose chemotherapy alone. We therefore assessed whether ongoing PET positivity adversely affected outcome in chemo-sensitive patients with high grade B cell NHL who received ASCT by the South Wales Blood and Marrow Transplant (SWBMT) Programme.
Aims: To compare outcome in patients who had ASCT for chemo-sensitive relapsed/refractory high grade B cell NHL (as defined by CT) according to the presence or absence of residual PET positive lesions.
Methods: The prospectively maintained database of the SWBMT programme which serves 77% of the Welsh population (mid-2014 census estimate) was interrogated to identify patients with high grade B cell NHL who received ASCT between January 1999 and December 2014 to allow a minimum of 12 months follow up. All patients with a pre-ASCT PET were assessed by the Deauville criteria to define their response with patients being considered to have achieved a complete metabolic response (CMR) if lesions demonstrated uptake of grade 3 or less. The data was analyzed according to patients achieving CMR, less than CMR or no prior PET.

Table 1.

Parameter	No PET	PET Negative	PET Positive	TOTAL
Number	61	22	18	101
Male, N (%)	35 (57%)	13 (59%)	15 (83%)	63 (62%)
Age group:				
16-30	7	1	3	11
31-50	23	4	8	35
51-60	17	5	3	25
>60	14	12	4	30
Subtype:				
DLBCL	45	18	14	77
T cell rich DLBCL	5	2	2	9
Mediastinal B cell	3	1	1	5
Plasmablastic	1	0	0	1
High grade	7	1	1	9
Ann Arbor stage:				
I	2	0	1	3
II	7	4	3	14
III	10	3	6	19
IV	26	8	5	39
Unknown	16	7	3	26
Indication for ASCT:				
Primary refractory	34	17	16	67
Relapsed	27	5	2	34
Prior rituximab, N (%)	42 (69%)	20 (91%)	17 (94%)	77 (76%)
Lines of therapy:				
1	4	1	0	5
2	25	11	6	42
3	18	5	8	31
4+	14	5	4	23
Status at follow-up (N, %):				
Relapsed	17 (28%)	05 (23%)	05 (28%)	27 (27%)
Dead	29 (48%)	06 (27%)	04 (22%)	39 (39%)
Alive	32 (52%)	16 (73%)	14 (78%)	62 (61%)
Follow up:				
Years, median (range)	7.8 (1.5-15.1)	4.2 (2.1-11.8)	3.8 (1.8-15.2)	5.0 (1.5-15.2)

Results: A total of 101 patients received ASCT for chemo-sensitive refractory or relapsed high grade B cell NHL between 1999 and 2014. Patient characteristics are summarised in the Table. The median age was 54 (range 17-69) years and there was an overall male predominance (62%). The most common subtype was DLBCL (76%) and the majority had stage IV disease. Two-thirds of patients had primary refractory disease and a median of 3 (range 1-7) lines of therapy were administered prior to ASCT. Forty-two of 61 (69%) patients with no PET received rituximab compared with 37/40 (93%) of the PET group. Of the 40 patients with a pre-ASCT PET, 22 had achieved a CMR (PET NEG) and 18 had residual lesions (PET POS). Relapse occurred in 28% of the No PET and PET POS groups compared to 23% in the PET NEG group. With a median follow-up of 5.0 (1.5-15.2) years 62 (61%) of patients are alive: No PET 52% v PET NEG 73% v PET POS 78%.
Summary/Conclusions: This study with relatively long follow-up beyond the typical relapse interval of 2 years, shows that residual PET positivity did not adversely affect outcome in chemo-sensitive relapsed/refractory patients undergoing ASCT for high grade B cell NHL. This may be explained by the fact that the majority of the PET group (93%) received rituximab, a known association with false PET positivity. In addition, patients with residual PET lesions did not routinely undergo biopsy to evaluate the significance of the lesions. The older No PET group which used CT to assess response, experienced survival similar to the pivotal Parma Trial (Philips et al, NEJM 1995) with 52% being alive at 7.8 years compared to 53% 5 year survival. In the more recently treated PET POS group, which is the current equivalent of the No PET group, survival had improved to 78% at 3.8 years follow-up. Given the similarity in relapse rate, this probably reflects improved supportive care. However, given that survival was not inferior to the contemporaneous PET NEG group it is probably premature to exclude these patients from ASCT solely on the basis of residual PET lesions. We suggest that patients with chemo-sensitive relapsed/refractory high grade NHL should not be denied ASCT on the basis of residual PET lesions and that randomized trials are needed to further address this question.

P331

MARAVIROC CONCENTRATIONS ARE HIGHLY PREDICTIVE OF ACUTE GRAFT-VERSUS-HOST DISEASE IN REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: We previously published favorable results with the use of maraviroc (MVC), a CCR5 antagonist, as a prevention strategy against acute graft-*versus*-host disease (GVHD) (Reshef *et al.* NEJM 2012). Various genetic and physiologic factors affect the pharmacokinetic (PK) profile of MVC, which leads to significant interpatient variability in MVC exposure. Whether variability in MVC PK affects the protective activity of MVC against GVHD is unknown.
Aims: We sought to identify whether a relationship exists between MVC trough concentrations and clinical outcomes in hematopoietic stem cell transplantation (HCT) recipients.
Methods: We evaluated the associations between MVC trough concentrations and clinical outcomes in a *post hoc* analysis combining data from two prospective clinical trials that tested the addition of MVC to tacrolimus/methotrexate in 67 patients (pts) undergoing their first allogeneic HCT at the University of Pennsylvania between 2009 and 2015. All pts received a uniform reduced-intensity conditioning (RIC) regimen consisting of fludarabine (120mg/m^2) and busulfan (6.4mg/kg), followed by infusion of peripheral-blood stem cells. Most pts were at high-risk for HCT-related complications given their age (median 62, range 21-74 years), donor source (matched unrelated 72%; single-antigen mismatch unrelated 16%; matched related 12%), and HCT-comorbidity index (low 30%, intermediate 34%, high 36%). The most common underlying diseases were acute myeloid leukemia (55%) and myelodysplastic syndromes (18%). All pts included in this PK analysis received MVC 300mg twice daily (BID) except for 4 pts in the first trial that received MVC 150mg BID as part of a dose-finding phase. The primary endpoint was cumulative incidence (CI) of acute grade 2-4 GVHD by day 180. Univariate analyses were performed using cumulative incidence and Cox regression models. Multivariate models were constructed using the backward elimination method.
Results: The median MVC trough concentrations (range) at days 0, 7, and 14 were 64.9 (11.7-315.7), 72.7 (7.9-555.0), and 142.0 (10.9-1064.0) ng/mL, respectively. The day-180 CI (±SE) of acute grade 2-4 GVHD and acute grade 3-4 GVHD were 24.7±5.1% and 11.3±3.8%, respectively. Pts with a day 0 MVC trough concentration ≤ median had a higher incidence of acute grade 2-4 GVHD compared with pts > median (38.7±8.6% *vs* 6.1±4.2%; *P*=.001). In multivariate analysis, day 0 MVC concentration remained an independent predictor of acute grade 2-4 GVHD (HR 0.35; 0.13-0.95; *P*=.039), as shown in Figure 1. We also observed a nonsignificant trend towards a higher incidence of acute grade 3-4 GVHD in pts with day 0 MVC trough concentrations ≤ median (12.2±5.9% *vs* 3±3%; *P*=.20). In addition, no pt (n=20) with day 0 MVC trough concentration >100ng/mL developed GVHD by day 180. The CI of relapse at 1 year was 42.5±5.9%. Interestingly, pts with a day 0 MVC trough concentration ≤ median had a higher relapse incidence (62.4±8.7% *vs* 21.7±7.4%; *P*=.001). Day 0 MVC concentrations were not significant predictors of chronic GVHD or survival. No

associations between clinical outcomes and MVC concentrations on day 7 or 14 were noted. We repeated the same analysis including only pts who received MVC 300mg BID and observed similar results.

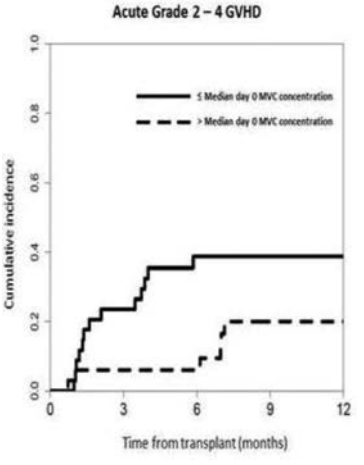

Figure 1.

Summary/Conclusions: Higher MVC concentrations on day 0 of RIC HCT were associated with significantly reduced risk of both acute grade 2-4 GVHD and relapse. These data highlight the importance of optimizing initial dosing of MVC in RIC HCT recipients.

P332

HYPERACUTE GRAFT *VERSUS* HOST DISEASE: RISK FACTORS, OUTCOMES AND IMPACT OF TACROLIMUS/SIROLIMUS PROPHYLAXIS

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Background: Hyperacute graft *versus* host disease (haGVHD) is associated with poor prognosis in the context of acute GVHD due to its severity and to its worse response to steroids. Most reports include majority patients receiving calcineurin inhibitors/MTX prophylaxis.
Aims: Analysis of patients with haGVHD *vs* patients who developed other acute GVHD comparing clinical characteristics, outcomes, response to treatment and risk factors in both groups, specially in those with tacrolimus/sirolimus prophylaxis in our Unit.
Methods: We prospectively analysed 627 patients consecutively transplanted in our Unit between 1995-2014 excluding paediatrics and 2ndallo-HSCT. HaGVDH was defined as that occurring within 14 days after HSCT.

Table 1.

	All patients	Value n (%/range) II-IV haGVHD	II-IV oaGVHD
	n = 627	n = 70	n = 264
Male sex	365 / 58%	50 / 71%	143 / 54%
• Sex mismatch	137 / 22%	27 / 38%	51 / 19%
Median age patient	49 (16-69)	49 (17-65)	50 (16-69)
Donor			
• Sibling	412 / 66%	26 / 38%	161 / 62%
• Matched Unrelated	109 / 17%	25 / 36%	44 / 18%
• Unrelated with 1 or 2 mismatch	77 / 13%	18 / 26%	39 / 16%
• Haploidentical	23 / 4%	0 / 0%	10 / 4%
Related or unrelated with mismatch	92 / 15%	20 / 29%	47 / 18%
Diagnosis			
• AML/MDS/MPS Ph-	293 / 47%	18 / 26%	127 / 48%
• ALL	73 / 12%	15 / 22%	24 / 9%
• HL/NHL/CLL	151 / 24%	29 / 41%	66 / 25%
• CML	40 / 6%	3 / 4%	15 / 6%
• AA	16 / 3%	0 / 0%	4 / 2%
• MM	53 / 8%	5 / 7%	26 / 10%
EBMT status disease at HSCT			
• Early	249 / 43%	27 / 39%	112 / 43%
• Intermediate	246 / 40%	32 / 46%	99 / 38%
• Late	95 / 17%	11 / 15%	49 / 19%
Myeloablative	237 / 38%	28 / 40%	85 / 32%
Conditioning regimen			
• FluBu +/- Thiotepa	234 / 37%	8 / 11%	102 / 39%
• FluMel	179 / 29%	34 / 49%	80 / 30%
• FluMelThiotepa	6 / 1%	1 / 4%	3 / 1%
• BuCy+/- Thiotepa	78 / 13%	6 / 9%	32 / 12%
• CyTBI	68 / 11%	16 / 23%	28 / 11%
• Others	53 / 9%	3 / 4%	18 / 7%
PB cell source/ Median CD34x106/kg	526 (84%) / 5,3 (0,9-22)	58 (83%)/ 5,1 (1,2-9,6)	217 (82%) / 5,5 (1,3-16,3)
GVHD prophylaxis			
• CSA + MTX	329 / 53%	18 / 26%	130 / 49%
• Tacrolimus + MTX	76 / 12%	10 / 15%	35 / 14%
• Calcineurin inhibitor + MMF	64 / 10%	8 / 11%	28 / 11%
• Tacrolimus + Sirolimus	141 / 22%	32 / 45%	64 / 24%
• Others	17 / 3%	2 / 3%	5 / 2%
In vivo T cell depletion	69 / 11%	4 / 6%	21 / 8%

Results: 70 patients (11%) developed II-IV haGVHD, 264 (42%) other acute II-IV GVHD (oaGVHD). Median of days to haGVHD and oaGVHD were +10 (4-14) and +35 (12-362) respectively. Patient's characteristics are shown in table 1. Differences between haGVHD and oaGVHD include a higher skin involvement and a higher proportion skin III-IV grades in haGVHD: 75 *vs* 45%, p=0,00 and 44% *vs* 18%, p=0,03 respectively. In multivariate analysis, risk factors associated with haGVHD include sex mismatch (p=0,004; OR 2,1; IC95% 1,02-4,4), conditioning regimen other than Fludarabina-Busulfan (p=0,00), Tacrolimus-Sirolimus prophylaxis (p=0,001; OR 2,1; IC95% 1,4-3,4) and unrelated donor +/- mismatch (p=0,004, OR 3,2, IC95% 1,4-7,1), whereas only mismatch unrelated donor was associated with a higher risk of oaGVHD (p=0,003, OR 3,2, IC95% 1,4-7,1). Steroids resistance was similar in both groups (haGVHD 23% *vs* 17% in oaGVHD). Factors influencing higher risk of steroid resistance in the multivariate analysis were HLA mismatch (p=0,04; OR 5,9; IC95% 1,05-33,7) and grade III-IV (p=0,004, OR 11,7, IC95% 2,2-62) in haGVHD and grades III-IV (p=0,00, OR 21, IC95% 9,2-48) in the group of oaGVHD. With a median follow-up of 38 months, OS and TRM for the whole series is 57% and 19%. Comparing haGVHD *vs* oaGVHD, haGVHD hadn't impact neither in OS (56% *vs* 51%; p=0,5) and TRM (33% *vs* 25%; p=0,17). While Tacrolimus/sirolimus prophylaxis was associated with a higher risk of haGVHD, this scheme was also associated with a better OS in the global series (p=0,002, HR 0,38, IC95% 0,2-0,7). Although we investigated the role of serum immunosuppressive levels in this group, we failed to demonstrate that infratherapeutic levels had a role in the haGVHD development.
Summary/Conclusions: According to our centre experience, haGVHD is characterized for a higher skin preference; it was not associated with a poorer outcome neither with a higher steroid resistance as compared with oaGVHD; althought tacrolimus/sirolimus is one of the risk factors to develop haGVHD, this regimen is also associated with a better OS.

P333

REDUCED INTENSITY CONDITIONING TRANSPLANTATION (RIC) AS PART OF FIRST LINE THERAPY FOR MANTLE CELL LYMPHOMA: RESULTS FROM THE PHASE II MINI ALLO TRIAL (CRUK: C7627/A9080)

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Background: For young patients with MCL, standard therapy comprises high dose cytarabine based chemotherapy which is usually consolidated by autologous stem-cell transplantation (ASCT). Although the median overall survival with this approach is impressive (>10 years in some series) no plateau is observed, whereas at relapse, allogeneic transplantation can lead to long term disease-free survival in some individuals. Up front allogeneic stem cell transplantation is rarely used in this condition due to concerns over the associated mortality and morbidity, however it is the treatment of choice following relapse.
Aims: This was a prospective study evaluating reduced induced conditioning allogeneic transplantation (RIC allo) as part of first consolidation for patients responding following front line chemotherapy. The primary endpoint was a progression free survival (PFS) improvement from 45% to 70%, (15/25 patients) after 2 years.
Methods: This study evaluated the role of RIC-Allo in first remission for MCL. The induction chemotherapy adopted was at the physicians discretion but patients required at least a partial response to be able to receive the transplant. Eligible patients received a BEAM-C (BCNU, etoposide, Ara-C, melphalan and alemtuzumab) conditioned sibling or unrelated donor allograft in first remission. Donor lymphocyte infusions (DLI) were administered for persistent, mixed chimerism or at relapse.
Results: Twenty-five patients were recruited from 8 centres (January 2010 - September 2013). The median age was 54 (34-70) and 22 (88%) were male. Eleven (44%) were in CR and 14 (56%) PR at registration. Eleven (44%) donors were siblings and 15 (56%) were unrelated. All 25 patients engrafted by day 100. Toxicity was as expected: grade 3-4 adverse events in 96%; the treatment related mortality (TRM) rate was 8% (2/25). GvHD incidence was low, in keeping with T cell depletion: acute GvHD (12%); chronic GVHD (24%). Fifteen patients were alive and progression free at 2 years (with 2 more yet to reach this point). The 2 year PFS estimate was 68% (95% CI: 46.1 - 82.5%). Of those alive and in remission, 14/16 (88%) have reached full donor T-cell chimerism, 5 after DLI.
Summary/Conclusions: RIC-Allo is a feasible option for consolidation of first remission in MCL. In this study, the target endpoint for PFS was achieved and further exploration of RIC-Allo in patients identified to be at high risk of disease progression with ASCT is warranted.

P334

THE ROLL OF HYPOMETHYLATING AGENTS IN MYELOID RELAPSES AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANT

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Background: Allogenic hematopoietic stem cell transplant (HSCT) remains the only curative treatment for high-risk hematological malignancies and post-HSCT relapses confer a particularly bad prognosis. Optimal therapy in this situation keeps unclear; hypomethylating agents (HMA) such as 5-azacitidine (5Aza) or decitabine (Dec) can be feasible therapy in this set of patients.
Aims: Analyze the outcome of patients receiving HMA for acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS) relapsing after HSCT.
Methods: From 355 AML/MDS patients who underwent HSCT in our institution from November 1995 to December 2015, relapse occurred in 112 (32%), and 45 of them (40%) were treated with HMA. From the total of these patients, we selected 40 patients with a follow-up period longer than 30 days and we analyzed them in terms of response rate (RR) and overall survival (OS).
Results: Median age was 54 years (25-71) and 19 (47%) were male. Diagnoses were AML in 30 (75%) and MDS in 10 (25%), with high risk cytogenetic in 43%. Peripheral blood was the stem cell source in 36 patients (90%) and donor was related in 70%. Reduced-intensity conditioning was used in 75%. Mean number of previous therapies was 2 (1-5); 5-azacitidine had been used before HSCT in 15 (37%), with 87% of patients responding. Patients relapsed after a median of 98 days (20-524), 56% with <10% blast, 22% with 10-20% blast and 22% with >20% blast. 5Aza was the HMA used in 39 patients (97%) and Dec in 1. Median number of HMA courses was 3 (1-19). Together with this strategy, the immune effect was enhanced in 38 patients (95%), tapering immunosuppressive treatment in 30 (75%) and with donor lymphocyte infusion (DLI) in 8 (21%). Chronic or acute graft *versus* host disease (GVHD) occurred in 21 patients (52%). Overall response rate (ORR) was 60% (n=24), including 19 CR (47%) (17 of them with negative minimal residual disease (MRD) by flow cytometry). Median time to best response was 91 days (21-209) and it was reached after a median of 2 cycles (1-8). Thirteen out of 24 responding patients (54%) progressed or relapsed after a median of 214 days (84-729). With a median follow up of 174 days (34-1779) from time of relapse, estimated OS was 55% at 6 months and 37% at 1 year post-relapse. Variables with significant influence on OS in univariate Kaplan-Meier analysis were: response to HMA [6 months OS: 82.5% for those responding *vs* 14% for non-responders; *p*=0.000], blast percentage at time of relapse considering >10% [6m OS: 48% *vs* 64%; *p*=0.05] and >20% blast [29% *vs* 65%; *p*=0.0014], developing GVHD after relapse [6m OS: 71% *vs* 36%; *p*=0.015]. Early relapses considered as <120 days after HCST was also associated with a better OS [6m OS: 62% *vs* 52%; *p*=0.078] although did not achieved statistical significance in univariate analysis. In a Cox multivariate regression model, all these variables (response to HMA, tumor burden at relapse, development of GVHD and early *vs* late relapse) showed to be independent factors with influence in OS.
Summary/Conclusions: 5-Azacitidine is an effective treatment for AML/MDS patients who relapse after HCST, especially for those with lower tumor burden and late relapses. In addiction enhancement of graft *versus* tumor effect together with HMA is a capital strategy to achieve longer OS.

P335

IMPROVED CLINICAL OUTCOMES OF GBM/GPB MIXTURE HAPLOIDENTICAL TRANSPLANTATION WHEN COMPARED TO A PROPENSITY SCORE-MATCHED GPB HAPLOIDENTICAL TRANSPLANTATION: A MULTICENTER STUDY

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Background: The effects of haploidentical(H) rhG-CSF-mobilized blood(B) and marrow(M) transplantation (HBMT) on hematological malignances are well established. Previous prospective single-center studies have demonstrated better survival after HBMT *vs* haploidentical rhG-CSF-mobilized peripheral blood stem cells transplantation (HPBSCT) for acute leukemia (AL) not in remission (NR) or in more than second complete remission (>CR2).
Aims: The aim of this study was to test the hypothesis that HBMT would still be a superior method for patients with AL, multiple myeloma (MM), and Non-Hodgkin lymphomas(NHL) in CR1/CR2 and patients with chronic myeloid leukaemia in first and second chronic phase lacking a matched donor as compared to HPBSCT.
Methods: We designed a propensity score method based multicenter study.
Results: Hematopoietic recovery, acute graft-*versus*-host disease (aGVHD), chronic GVHD was comparable between HBMT group(n=168) and HPBSCT group (n=42). No significant differences were found in the non-relapse mortality (20.17%±3.58% and 27.24%±7.16%, P=0.18) rates and relapse rates (19.96%±3.72% and 28.49%±8.25%, P=0.32) between HBMT group and HPBSCT group. HBMT recipients had better overall survival (65.0%±4.2% and 54.2%±8.3%, P=0.037) and disease free survival (59.9%±4.6% and 44.3%±8.7%, P=0.051). Multivariate analysis showed that HPBSCT was associated with poorer DFS (HR(95%CI), 1.639(0.995-2.699), P=0.052).
Summary/Conclusions: Our comparisons showed that HBMT was superior compared to HPBSCT as postremission treatment for patients lacking an identical donor.

P336

TREATMENT OF CHRONIC GRAFT *VERSUS* HOST DISEASE WITH A COMBINATION OF B-CELL DEPLETION AND TYROSINE KINASE INHIBITION

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Background: Chronic Graft *Versus* Host Disease (cGVHD) still has a large impact on morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Affected patients require long term use of immunosuppressive drugs, mainly corticosteroids, which lead to development of severe side effects. Therapeutic approaches for cGVHD are limited. Generally recommended first line therapy consists of glucocorticoid therapy combined with a calcineurin inhibitor. Options for second line therapy are numerous but no consensus on the most favourable choice of agents has been reached.
Aims: Both monotherapy with rituximab and monotherapy with tyrosine kinase inhibitors have shown to be effective in reducing cGVHD symptoms. We aimed to test whether the sequential therapy of the anti CD20 antibody rituximab followed by a 6 month treatment period with the tyrosine kinase inhibitor nilotinib is a good treatment strategy for patients with sclerotic cGVHD (EudraCT nr 2008-004125-42).
Methods: We treated patients with sclerotic cGVHD with 4 infusions of rituximab (375mg/m2 i.v. once weekly) followed by six months of nilotinib (start dose 300mg b.i.d.). Patients were followed up monthly for 13 months.
Results: We included 29 patients, 4 patients went offstudy, 13 patients have currently completed the follow up. Of these 13 patients, 1 patient reached a complete response, 8 patients showed a partial response and 4 patients remained in stable disease. Moreover, 2 out of 5 patients who suffered from severe ulcerations at the start of the study had a complete resolution of ulcers at the end of the treatment period. There is a significant decrease in cGVHD affected body surface area (Figure 1). Patients with a (partial) response also show a decrease in self attributed severity of cGVHD and their immunosuppressive drugs could be tapered. Sixty percent of responding patients could taper >50% of prednisolone dose.

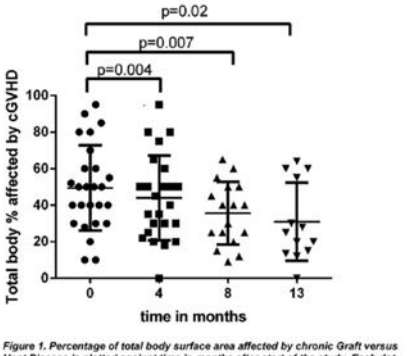

Figure 1. Percentage of total body surface area affected by chronic Graft versus Host Disease is plotted against time in months after start of the study. Each dot represents 1 patient. P-values are calculated by means of Wilcoxon matched-pairs signed rank test.

Figure 1.

Summary/Conclusions: The sequential therapy of B-cell depletion and tyrosine kinase inhibition provides a new and interesting alternative treatment option for this difficult and heavily pretreated patient category. Approximately 70% of patients achieve a (partial) response that results in a significant reduction in use of corticosteroids.

P337

FIRST-LINE EXTRACORPOREAL PHOTOCHEMOTHERAPY (ECP) FOR ACUTE GVHD AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Acute graft-*versus*-host disease (aGVHD) is a major complication of allogeneic hematopoietic cell transplantation (HCT), and glucocorticoids are typically used as first-line treatment.
Aims: The aim of our study was to evaluate the effect of ECP +/- steroid therapy in order to reduce the incidence of infections and toxicity.
Methods: From December 2010 to November 2015, 47 of 179 pts (26%), were diagnosed with aGVHD grade ≥2 following alloSCT and 33 (70%) pts were treated with ECP +/- steroid as first-line therapy. 8 (25%) pts were treated with ECP only, 24 (75%) pts with ECP + steroid 1 or 2 mg/kg/day, with a total of 32 pts evaluated for aGVHD response. Conditioning regimen was myeloablative (MAC) n=7, nonmyeloblative (NMA) n=14 or reduced-intensity (RIC) n=11. Acute GVHD diagnosis was performed using Keystone criteria and scored by Seattle and IBMTR criteria. ECP was performed using the offline technique. ECP was started as soon as possible with a treatment schedule consisted of 4 rounds of two procedures per week, 3 rounds of two procedures every other week and finally two procedures every month until clinical improvement and/or immunosuppressive therapy (IST) tapering.
Results: The median follow-up time was 20 (range: 5-60) months. The median age was 51 (20-71). 17 pts were affected by lymphoma, 8 by AML, 3 by MM, 2 by ALL, 1 by CLL and 1 by MDS. Donors were: haploidentical (n=21), HLA-identical (n=5), matched unrelated (n=5) and cord blood (n=1). Stem cell source was: peripheral blood (PBSC, n=17) and bone marrow (BM, n=14). aGVHD was diagnosed after a median time of 39 days (13-150) from alloSCT. aGVHD grade was 2 in 28 (88%), 3 in 2 (6%) and 4 in 2 (6%) pts. Visceral involvement was reported in 5 (15%) pts: gut (n=4) and liver (n=1). ECP was started after a median of 4 (0-30) days from aGVHD diagnosis. The median duration of steroid therapy was 29 days (3-104) and every patient underwent a median of 19 (2-83) ECP procedures, during a median time of 6 months. At 30 days from GVHD diagnosis, 21 (66%) pts achieved complete remission and 6 (19%) pts partial remission, with an overall response rate of 85%. At univariate analysis, we found a trend towards higher risk of GVHD progression for pts receiving PBSC and with visceral involvement (Table 1). Among responders, 6 (22%) pts had aGVHD relapse during steroid tapering or discontinuation. Overall, not responders patients were 11 (34%), and 10 pts received second-line therapy (1 pt not included for early death). From GVDH diagnosis, 17 (53%) had cytomegalovirus reactivation, 12 (38%) had a bacterial infection and 3 (9%) fungal pneumonias were reported. The cumulative incidence of chronic GVHD was 26.5% (95% CI: 12.2-43.3), the 1-yr cumulative incidence of relapse and non-relapse mortality were 16.4% (95% CI: 5.8-31.8) and 22.7% (95% CI: 9.8-38.9), respectively. The 1-yr overall survival and GVHD-free/relapse-free survival were 70.0% (95% CI: 53.5-86.5) and 34.7% (95% CI: 17.6-51.8), respectively.

Table 1. Univariate analysis of variables predicting lack of response to first-line therapy.

Variables	HR	95% CI	p-value
Haploidentical donor	1.67	0.17-16.16	0.66
PBSC source	3.30	0.34-32.01	0.30
aGVHD grade >2	1.61	0.17-15.51	0.68
Visceral involvement	3.32	0.46-23.71	0.23
ECP only	0.88	0.09-8.44	0.91

Summary/Conclusions: This observational study suggests that ECP +/- steroids is safe and effective first-line treatment for aGVHD after alloSCT. The response rate is high with a low cumulative dose of steroids. However, the duration and speed of steroid tapering needs to be further assessed, in order to maintain a sustained response, when patients received PBSC and had visceral GVHD involvement.

P338

EFFICACY OF HOST-DENDRITIC CELL VACCINATIONS WITH AND WITHOUT MINOR HISTOCOMPATIBILITY ANTIGEN LOADING, COMBINED WITH DONOR LYMPHOCYTE INFUSION IN MULTIPLE MYELOMA PATIENTS

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Background: Donor lymphocyte infusions (DLI) can induce durable remissions in multiple myeloma (MM) patients, but this occurs only in a fraction of the patients. The T cell-mediated graft-*versus*-tumor (GvT) effect of DLI depends on the presence of host dendritic cells (DC), but these are rapidly replaced by donor DC after allogeneic stem cell transplantation (alloSCT).
Aims: We tested in a prospective phase I/II trial whether the efficacy of DLI in MM patients could be improved by simultaneous vaccination with *ex vivo* generated host DCs. As the donor T cells involved in GVT are mainly directed at mismatched minor histocompatibility antigens (mHags), we also analyzed the possibility to further improve the GvT effect by loading the host-DCs with peptides of mismatched hematopoietic cell-specific mHags.
Methods: Eleven MM patients, not responding to a first DLI, were treated with a second equivalent dose DLI combined with DC vaccinations, generated from host-monocytes (moDC). In four of these patients the DCs were loaded with mHag peptides.
Results: Toxicity was limited and no acute graft-*versus*-host disease (GvHD) occurred. Most patients developed objective but temporary anti-host T cell responses however, with only modest clinical effects. In a patient vaccinated with mHag ACC-1 peptide-loaded DCs, a distinct ACC-1 specific T cell response accompanied a temporary clinical response.
Summary/Conclusions: These findings confirm that DLI combined with host-DC vaccination, either unloaded or loaded with mHag peptides, is feasible, safe and capable of inducing host-specific T-cell responses. The limited clinical effects observed in some patients may be improved by developing more immunogenic DC products or by combining this therapy with immune potentiating modalities like immune checkpoint inhibitors.

Stem cell transplantation - Clinical 2

P339

TRACING OF URINARY EXCRETION OF BKV AND JCV BY QUANTITATIVE PCR IN RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTS (ALLO-HSCT) TO ANTICIPATE THE DEVELOPMENT OF HEMORRHAGIC CYSTITIS (HC)

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Background: BK virus (BKV) and JC virus (JCV) are frequently identified in the urine of HSCT pts with hemorrhagic cystitis (HC). However, viruria is common even in asymptomatic pts, making difficult to establish a direct causative role of these viruses.
Aims: Many studies have identified an association between polyomaviruses and late HC and the studies have noted that viruria preceded or coincided with the onset of disease. Some of these studies indicate that some factors, such as conditioning regimen intensity, unrelated donor, etc contribute to the development of HC and others, like receiving prophylaxis with quinolones could protect for this disease.
Methods: We conducted a prospective monitorization of urinary BK and JC shedding by qPCR in 968 urine samples from 128 pts who underwent 135 allo-HSCT between January 2010 and December 2014. BKV and JCV quantitative polymerase chain reaction (qPCR) were performed in urine samples collected at admission for transplant and weekly until discharge. Seventy-seven were male (57%). Median age was 49 years old (range: 1-68). Most frecuent baseline disease was acute leukemia (n=53). Seventy-two (53.3%) received cells from unrelated donor, 58 (43%) from matched siblings and the remainder haploidentical-HSCT. Stem cell source were bone marrow (n=59), peripheral blood (n=27) and umbilical cord (n=9).

Table 1.

Median	HC(+)	HC(-)	*p* Value	Quinolones(+)	Quinolones(-)	*p* Value
Time for first BKV	3.5	8.5	NS	10	-1	NS
Duration of BKV excretion	77	16.5	0.001	14	31	NS
First BKV detection	3.07	2.69	NS	2.69	3.08	NS
Maximum BKV load	8.3	5.13	0.004	4.28	7.98	NS
log increase in BKV copies/mL	1.96	0.62	0.012	0.53	3.48	0.048
Time for first JCV	55	-6.5	NS	-6	-6	NS
Duration of JCV excretion	46.5	27.5	NS	28	27.5	NS
First JCV detection	3.05	4.64	NS	3.43	5.18	NS
Maximum JCV load	9	5.4	NS	5.86	5.79	NS
log increase in JCV copies/mL	2.5	0.00	NS	0.00	0.00	NS

Results: BK viruria was demonstrated in 85 pts (63%), JCV in 56 patients (41.5%) and both viruses in 36 pts (19.2%). BKV was excreted at day +5.5 as a median (range: -12 to +167) during 20 days (range: 1-554). There was a significant correlation between the first and maximum BKV load and intensity of hematuria (R=.242; p=.026 and R=0.408; p<.001). Also, we observed a direct correlation between the maximum BKV load and the number of lymphocytes (R=0.248; p=.022) and an inverse with the quantity of immunoglobulins (R=-0.28; p=.01). JCV was excreted in 56 pts (41.5%) at day -6 (range: -12 to +347) for 27.5 days (range: 1-535). At maximum viral load of JCV, there was an inverse correlation with the immunoglobulins and platelets (R=-0.378; p=.005 and R=-0.289; p=.033) and a direct correlation with the intensity of hematuria (R=.387; p=.004). Only one patient receiving stem cell source from umbilical cord blood had urinary JCV excretion. Eighty-four percent of pts who excreted JCV had received a fludarabine-based conditioning regimen (p=.046). Fifteen patients (11.1%) developed HC, 13 out of 15 excreted BKV (86.7%), 8 JCV (53.3%) and 6 (40%) both. Patients who excreted BKV had more risk to developed HC (p=.044). The development of HC was correlated to excretion of at least one polyomavirus in all cases (p=.018). Patients who had clinical HC showed higher BK viral load at maximum detection (log10 8.3 *vs* 5.13copies/mL; p=.004), more log copies/mL increase (1.96 *vs* 0.62, p=.012) and virus shedding for a longer time (77 *vs* 16.5 days; p=.001). JCV maximum load detection and days of excretion were higher in pts who develop HC but it is not significant. Eighty-seven pts (64.4%) received prophylaxis with quinolones and 10.3% of them developed HC. Further, 60.9% and 73.25% of them excreted BKV and JCV respectively, without differences with patients who did not get prophylaxis.
Summary/Conclusions: BK and JC excretion were frequent in our series of allo-HSCT recipients. High viral load is correlated with more hematuria. Higher increase in log BKV urine qPCR copies/mL, higher BKV load and longer shedding were associated with risk of HC. We could not demonstrate that prophylaxis with quinolones impact on duration of excretion, amount of polyomavirus shedding or protection of development of HC.

P340

OUTCOMES AFTER AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS WITH MYC/BCL2 CO-EXPRESSION, DOUBLE-HIT LYMPHOMA, OR MYC COPY GAIN

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Background: Double-hit lymphomas (DHL) - DLBCL with rearrangements of *MYC* and *BCL2* and/or *BCL6* - and double expressing lymphomas (DEL) - DLBCL with co-expression of MYC and BCL2 by immunohistochemistry (IHC) - are associated with poor outcomes after upfront therapy. Data are limited regarding outcomes of patients with rel/ref DHL who undergo SCT, and no study to date has examined SCT outcomes in patients with DEL.
Aims: Evaluate the prognostic impact of DHL and DEL status on SCT outcomes in patients with rel/ref DLBCL.
Methods: We studied patients with rel/ref DLBCL or transformed indolent lymphoma (TIL) who underwent autologous SCT (autoSCT) at Dana-Farber Cancer Institute (DFCI) or City of Hope between 1/2000 and 7/2013. We also studied patients with rel/ref DLBCL or TIL who underwent allogeneic SCT (alloSCT) at DFCI or Massachusetts General Hospital during the same period. In the autoSCT cohort, IHC for MYC, BCL2, and BCL6 were performed and cutoffs of ≥40% MYC-positive and ≥50% BCL2-positive cells were used to define DEL. Cell-of-origin (COO) classification was determined by Hans criteria. In both cohorts, FISH for *MYC* was performed using dual-color break-apart probes; cases with *MYC*-rearrangement or copy gain (CG) also had *BCL2* and *BCL6* FISH performed. Rearrangement was defined as ≥10% nuclei with break-apart signals, and CG was defined as ≥30% nuclei with extra signals. DHL had rearrangement of *MYC* and *BCL-2* and/or *BCL-6*, and "atypical DHL" had at least 3+ *MYC* CG and *BCL2* and/or *BCL6* CG or rearrangement.

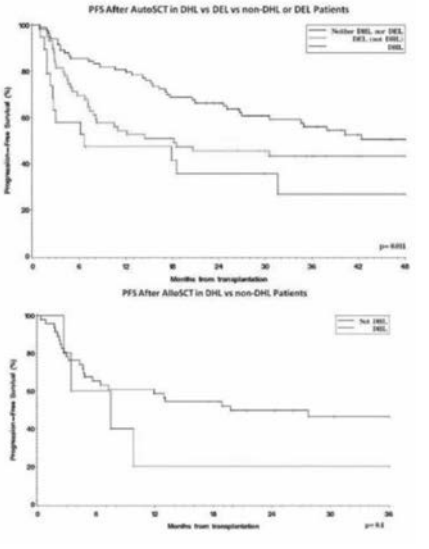

Figure 1.

Results: Cohort Characteristics AutoSCT cohort: 201 patients; median age 60y (range 30-77); 26% TIL; median 2 prior therapies; 99% prior rituximab; 53% primary refractory disease/relapse <6 mo; 59% CR at ASCT; median follow-up 53 months. AlloSCT cohort: 61 patients; median age 55y (range 28-69); 33% TIL; median 4 prior therapies; 64% prior autoSCT; 72% chemosensitive; 80% reduced intensity conditioning; 30% matched related, 54% matched unrelated, 6% mismatched unrelated, 5% haploidentical, 5% cord; median follow-up 41 months. Overall, 4y progression-free survival (PFS)/overall survival (OS) in the autoSCT were 44%/61%, and 3y PFS/OS in the alloSCT cohort were 42%/47%, respectively. Outcomes AutoSCT: Of 184 autoSCT patients with IHC data, 40% were DEL. 4y PFS in DEL v non-DEL patients was 37% v 52% (p=0.001), 4y OS was 52% v 69% (p=0.007). Of 167 autoSCT patients with FISH and IHC data, 13% were DHL, 38% had atypical DHL with 3+ *MYC* CG, and 8% had atypical DHL with 4+ *MYC* CG. 4y PFS in DHL v non-DHL patients was 24% v 48% (p=0.005), 4y OS was 31% v 62% (p=0.002) [Figure]. In a multivariable model, only DEL (PFS HR 2.1, p=0.0005; OS HR 1.8, p=0.022), DHL (HR 1.9, p=0.039; HR 2.4, p=0.012), and SD/PD at autoSCT (HR 3.1, p=0.02; HR 3.4, p=0.014) were associated with inferior PFS and OS. Neither PR *vs* CR status at autoSCT, isolated MYC, BCL2, or BCL6 expression, atypical DHL with 3+ or 4+ *MYC* CG, nor COO were associated with outcome

when DHL/DEL status was taken into account. AlloSCT: 51 alloSCT patients had complete FISH data – 10% had DHL, 29% had atypical DHL with 3+ *MYC* CG, and 10% had atypical DHL with 4+ *MYC* CG. 3y PFS/OS in DHL v non-DHL patients were 20% v 47% (p=0.4) and 20% and 53% (p=0.24) [Figure]. Atypical DHL was not associated with outcome.
Summary/Conclusions: Rel/ref DEL and DHL are associated with inferior PFS and OS after autoSCT, independent of other prognostic factors. Rel/ref DHL appears to also have a worse outcome after alloSCT, but the sample size is not large enough to draw definitive conclusions.

P341

BTLA GENOTYPE AND CLINICAL OUTCOME AFTER HLA-IDENTICAL SIBLING STEM CELL TRANSPLANTATION
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Background: The co-inhibitory receptor B and T lymphocyte attenuator (BTLA) is thought to negatively regulate the proliferation and differentiation of T-cells modifying immune response. The BTLA genotype has been associated with the development of some autoimmune diseases. However, various *in vivo* models have showed that BTLA could have dissimilar roles in controlling immune responses depending on the tissue examined. The BTLA gene is located on chromosome 3 in q13.2 and consists of five exons
Aims: Here, we focus on the relevance of the rs9288953 BTLA C>T polymorphism on the clinical outcome after allogeneic stem cell transplant (allo-HSCT).
Methods: We carried out a retrospective analysis of 1060 allo-HSCTs from HLA-identical sibling donors performed between 1991 and 2013. Patients who received a T-depleted graft HSCT were not included. BTLA genotyping was made by allelic discrimination assays and detection system Life Technologies on DNA samples obtained from peripheral blood of the donors. Written informed consent was obtained from patients and donors. Statistical cumulative incidence was estimated for aGVHD, relapse and transplant related mortality (TRM) according to BTLA donor's genotype. TRM was defined as death due other causes but relapse. The Kaplan Meier method was applied to analyze overall survival (OS) and relapse-free survival (RFS), comparing curves by the log-rank test. Multivariate analysis was performed using the Cox regression model.
Results: BTLA allele and genotype frequencies were similar to the previously described in Caucasian populations. The homozygous BTLA CC genotype was the predominant in 44.3% of cases. Homogeneity in clinical variables between genotype groups was confirmed. There were not statistical differences in cumulative incidence of aGVHD grades II-IV (p: 0.367) and the secondary clinical endpoints OS (p: 0.500), TRM (p: 0.597) or RFS (p: 0.143), but we detected higher cumulative incidence of relapse between BTLA CC *versus* TT/CT genotypes (p: 0.015). Multivariate analysis identified BTLA genotype, stage of the disease, and acute leukemia diagnosis as independent risk factors for relapse (p: 0.038; HR1.3; 95%CI: 1 to 1.7; p<0.001; HR2.5; 95%CI: 1,9 to 3.2 and p: 0.03; HR1.6; 95%CI: 1.2 to 2.3 respectively).
Summary/Conclusions: The BTLA genotype of the donor seems to be independent risk factor for relapse. There were no statistically differences in other clinical endpoints, suggesting that BTLA should be relevant in the prevention of relapses, but not in the initial process of allorecognition. These preliminary results should be confirmed by other studies. This work has been financed by grants PI11/01690 and PI14/01646 from the Instituto de Salud Carlos III, co-financed by FEDER (Fondo Europeo de desarrollo regional) and by grant MTV3 Fundació Marató TV3.

P342

HIGH-DOSE *VERSUS* STANDARD-DOSE RITUXIMAB WITH BEAM IN AUTOLOGOUS STEM CELL TRANSPLANTATION (SCT) FOR RELAPSED AGGRESSIVE B-CELL NON-HODGKIN'S LYMPHOMAS (NHL)
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Background: High-dose rituximab (HD-R) combined with carmustine, cytarabine, etoposide, and melphalan (BEAM) and SCT was effective and tolerable in a single-arm study for relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) and other aggressive B-cell NHLs. This prospective randomized phase 2 study compared the efficacy and safety of HD-R *vs* standard-dose rituximab (SD-R) combined with BEAM in R/R-NHL.
Aims: 1. To determine the disease-free survival (DFS) for patients with R/R aggressive B-cell lymphomas in the two study arms: HD-R *vs* SD-R plus BEAM. 2. To compare the safety of the two treatment arms.
Methods: Patients with R/R-NHL (44 DLBCL, 49 other aggressive B-cell lymphomas) were randomized to HD-R or SD-R using Bayesian adaptive algorithm. HD-R (1,000 mg/m^2) or SD-R (375 m^2) were administered on days +1 and +8 after stem cell infusion. Primary endpoint: DFS, defined as time from SCT to disease progression or death. Overall survival (OS) and safety analysis were secondary endpoints. Planned sample size was 100 patients; however, the trial was closed to new patient entry by the Data and Safety Monitoring Board after 93 patients were enrolled because it was considered extremely unlikely that either treatment arm would be found significantly superior. Intention-to-treat Kaplan-Meier estimator (DFS & OS) and Cox proportional hazards regression were used. An informed consent was granted from patients before study enrollment.
Results: 93 patients (29% females) with a median age of 63 years (6-75) were randomized to HD-R (n=42) or SD-R (n=51). No significant differences in patient demographic and clinical characteristics. With a median follow-up of 7.92 years, the 5-year DFS and OS were 42% and 48% for the whole study population, respectively. We found no statistically significant differences between the HD-R and SD-R arms in 5-year DFS (37% *vs* 47%; p=0.2) and OS (42% *vs* 52%; P=0.4) (Figure 1). In multivariate analyses, only disease status at the time of SCT [complete remission (CR) *vs* no CR] (HR 0.57, 95% CI: 0.35-0.95) and number of prior treatment lines (a line of treatment is defined as any active treatment before BEAM and transplant, using chemotherapy and/or chemoimmunotherapy as single agent or in combination), (≤2 *vs* >2) (HR 0.53, 95% CI: 0.32-0.89) were associated with worse DFS. Similarly, these were found to be significant poor prognostic factors for OS: HR 0.56 (95% CI 0.33-0.95, p=0.03) for CR at time of SCT, and HR 0.53 (95% CI 0.24-0.74, p=0.002) for ≤2 lines of treatment. Patients transplanted in CR had better 5-year DFS (50% *vs* 30%, P=0.026) and OS (57% *vs* 35%; P=0.02). Similarly, those with ≤2 prior lines of treatment had significantly better 5-year DFS (45% *vs* 30%, P=0.006) and OS (54% *vs* 30%, P=0.001). No differences in Grade 3-4 toxicities, infection rates or engraftment were seen between both arms.

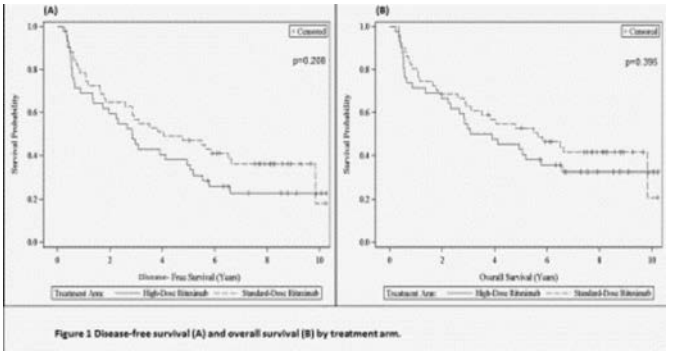

Figure 1.

Summary/Conclusions: SD-R is not significantly different from HD-R when combined with BEAM in B-cell aggressive R/R-NHL in terms of DFS and OS. Pre-transplant disease status (CR *vs* no CR) and numbers of treatments prior to transplant (≤2 *vs* >2) were the only significant predictors for prognosis. Patients with CR and those with ≤2 prior lines at time of SCT have the best outcomes.

P343

INTERFERON GAMMA (IFN-GAMMA) NEUTRALIZATION AS A VALUABLE THERAPEUTIC TARGET IN PRIMARY HLH
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Background: Primary HLH (pHLH) is a rare immune regulatory disorder which is lethal if untreated. High production of IFNγ has been shown to play a critical role in the pathophysiology of the disease. Immune-chemotherapy, primarily etoposide-based regimens, is currently used to control HLH and bring patients to allogeneic hematopoietic stem cell transplant (HSCT). In spite of recent

attempts to further intensify treatment regimens, mortality and morbidity remain high, also due to drug-related toxicities. NI-0501 is a fully human, high affinity, anti-IFNγ monoclonal antibody that binds to and neutralizes human IFNγ, offering a novel and targeted therapeutic approach for HLH.
Aims: To evaluate the safety and efficacy of NI-0501 in children with pHLH.
Methods: We have evaluated patients enrolled in the Phase 2 international study or who, although fulfilling the study criteria, were treated in Compassionate Use since not transferrable to study sites. NI-0501 was administered at an initial dose of 1 mg/kg every 3 days, possibly adjusted during treatment, along with dexamethasone dosed between 5-10 mg/m^2/day that could be tapered depending on clinical evolution. HLH response to treatment, NI-0501 safety profile and survival were assessed.
Results: A total of 20 patients (18 in the study, 2 in CU) have been evaluated so far: 10F/10M, median age 1.0 yr (range 2.5 mo-13 yr), mostly at the severe end of the HLH spectrum, and carrying significant toxicities from previous HLH treatments. Eighteen patients received NI-0501 as second line treatment and 2 as first line. In 18 patients a known HLH genetic defect was documented (6 FHL2, 4 FHL3, 1 FHL4, 1 FHL5, 3 GS-2, 1 XLP1, 2 XLP2). Eight patients started NI-0501 treatment in the presence of an active infection (mostly EBV and CMV), likely to be the disease trigger. Five patients had CNS signs and symptoms prior to NI-0501 administration requiring intrathecal therapy. To date, 11 patients received HSCT (2 also with additional HLH therapies), 4 achieved HLH control and are awaiting for HSCT, and 3 are at week 2 of treatment. Two patients died of HLH/MOF prior to HSCT. HLH parameters were positively impacted by NI-0501 treatment, in particular platelet and neutrophil counts, abnormal at baseline in 13 and 11 patients, normalized in 8 and 9, respectively, at end of treatment (EoT, Fig.1). CNS signs and symptoms resolved in the 4 evaluable patients. Dexamethasone dose could be at least halved in 60% of patients during the first 4 weeks of treatment. After transplantation, 9/11 patients (82%) are alive with a median follow-up of 4 mo (1 wk-2.5 yrs) Two patients died from HSCT complications (GvHD and graft rejection). IFNγ neutralization was demonstrated by a sharp decrease in CXCL9, an IFNγ-induced chemokine. NI-0501 was well tolerated and no safety concerns were identified. All infections present at start of treatment resolved upon appropriate treatment. Infections occurred during NI-0501 treatment only in patients who previously received chemotherapy. None of the infections were caused by pathogens known to be favored by IFNγ neutralization.

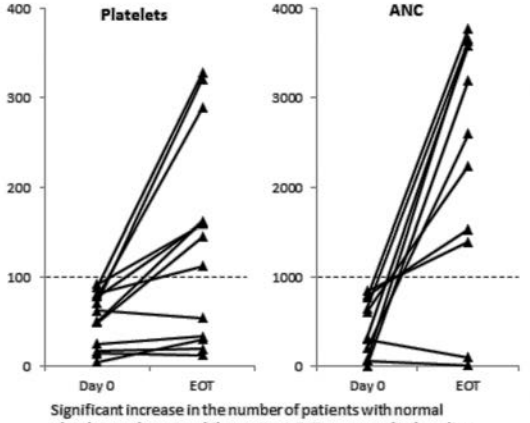

Figure 1. Response to NI-0501 treatment.

Summary/Conclusions: NI-0501 has shown to be safe and effective in patients with pHLH both in second and in first line. Efficacy of NI-0501 seems to be independent of the presence and type of causative mutations, as well as the presence and type of an infectious trigger. None of the typical etoposide-based regimen toxicities were observed, while a significant positive impact on cytopenia was noted. Based on the data available to date, IFNγ neutralization is proposed as a valuable pHLH therapy, and may be considered as first-line approach for HLH.

P344

BIDIRECTIONAL RELATIONSHIP BETWEEN CMV-R AND AGVHD: CMV-R HAS A BETTER OVERALL SURVIVAL AND T CELL RECONSTITUTION

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Background: Cytomegalovirus reactivation (CMV-R) and acute graft *versus*-host disease (aGvHD) are early complications after hematopoietic stem cell transplantation (HSCT). Multiple (m)CMV-Rs lead to an increased risk for development of aGvHD and vice-versa. Nevertheless, the interaction between aGvHD, CMV-R and their respective outcomes post HSCT is not well understood.
Aims: In a bidirectional approach we investigated the interaction of CMV-R and aGvHD on overall survival and T cell reconstituion
Methods: CMV-seropositive (R+) patients transplanted with hematopoietic stem cells derived from CMV-seropositive donors (D+) between 2006 and 2013 at Medical School Hannover were included in this analysis (n=145). All patients were monitored regularly for the development of T cells (CD8+, CD4+ T cells and CMV-CTLs) from day +25 to 1 year post-HSCT. In this study, T cell analysis was performed on day +50 and overall survival (OS) was evaluated at 2 years after HSCT.
Results: Based on the sequence of single or (m)CMV-R and aGvHD we analysed the influence of both CMV-R and aGvHD on OS. We observed that patients with aGvHD grade II-IV prior to CMV-R had a ten-fold increased risk to die (RR: 10.21, p=0.007), which was about the same as patients with aGvHD grade II-IV only (RR: 9.30, p=0.026) when compared to patients without either of these complications or to patients with CMV-R alone. Interestingly, patients with CMV-R prior to aGvHD grade II-IV (RR: 4.18, p=0.04) had only a four-fold risk to die, when compared to the patients without these complications. Patients with CMV-R alone had no increased mortality when compared to those without complications. We observed a rapid engraftment of CD8+ T cells and increased inflammatory cytokine secretion (*e.g.* IL1Ra, IL18, Hu TRAIL, MIP-1b and TNF-a) in the plasma of patient's post-CMV-R which led to the progression of aGvHD.
Summary/Conclusions: Our data suggest that the rapid reconstitution of CD8+ T cells in concert with the secretion of inflammatory cytokines post-CMV-R leads to the progression/development of aGvHD grade II or more. We could also show that CMV-R itself is not a risk factor worse OS in the R+/D+ patient group. However, as expected, aGvHD grade II-IV and CMV-R post-aGvHD grade II-IV are associated with a higher risk to die after HSCT.

P345

HIGH DOSE CHEMOTHERAPY WITH AUTOGRAFT MAY IMPROVE THE POOR PROGNOSIS OF NAIVE DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS WITH MYC/BCL2 CO-EXPRESSION

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Background: The concurrent immmunohistochemical (IHC) expression of MYC and BCL2 has recently emerged as one of the strongest and most unfavorable prognostic factor among naïve DLBCL patients after R-CHOP therapy, with a 3-year event free survival (EFS) and overall survival (OS) of 27-39% and 30-43% respectively. Nevertheless, Horn et al (Leukemia 2015) have recently suggested that the MYC/BCL2 co-expression is not strongly associated with inferior survival among young DLBCL patients treated with more intense chemotherapy regimens. This discordant result may be explained either by the lower prognostic relevance of MYC/BCL2 among young DLBCL patients, but also by the ability of more intense chemotherapy regimens to overcome the negative prognostic features of MYC/BCL2 co-expression.
Aims: We investigated the clinical impact of MYC/BCL2 co-expression among naive DLBCL patients treated with high dose chemotherapy followed by autologous stem cell transplantation in first line.
Methods: We analyzed a consecutive series of 71 naïve DLBCL patients treated with high-dose sequential chemotherapy integrated with monoclonal antibody Rituximab (R-HDS) between 2000 and 2015 at the Istituto Nazionale dei Tumori Milano. R-HDS schedule is a typical ASCT-based program, including the delivery of high-dose drugs from the start of treatment, the collection of PBSC and a final autograft. Tumor samples from all enrolled patients were analyzed for MYC and BCL2 expression by ICH with a 40% and 70% threshold, respectively.
Results: All patients had an IPI score ≥1 and 65/71 (91%) had an Ann Arbor stage ≥3. Among patients younger than 60 years, 30/47 (63%) were characterized by an age adjusted IPI≥2. No differences for IPI, ECOG score ≥2, Ann Arbor stage >2, age, extranodal sites ≥2 and LDH levels were observed between double expressor and non double expressor patients. The treatment schedule was interrupted, and the transplant was not performed, in 16 (22.5%) patients due to refractory and/or progressive disease (n=12) and adverse events (n=4), including 2 patients who died of infection during treatment. After a median follow up of 92 months (range 4-200), the overall 5-year EFS and OS were 65.2% (95% CI, 59.4-71%) and 83.5% (95% CI, 78.7-88.3%) respectively. MYC and BCL2 expression on IHC was observed in 31 (44%) and 37 (52%) patients respectively. Among them, 20 (28%) were classified as MYC/BCL2 double expressors. The Ki-67 expression, MYC or BCL2 expression alone were not associated with inferior outcome. Conversely the co-expression of

MYC/BCL2 was associated with a trend of worse outcome: 5-year EFS 49.4% (95% CI, 37.7-61.5%) *vs* 71.5% (95% CI, 65-78%); p=0.06 (Figure1a). However, this higher relapse risk did not affect final 5-year OS [76.7 (95% CI, 66.4-87) *vs* 83.4 (78-88.8)] (Figure 1b). When only the 55 transplanted patients where considered, MYC/BCL2 co-expression was not associated with any significant difference in term of EFS and OS (Figure 1c-d). By multivariate analysis (including age, bulky disease, ECOG≥2, Extra nodal sites≥2, IPI score≥2) MYC/BCL2 co-expression retained its independent prognostic value for EFS without affecting the final OS.

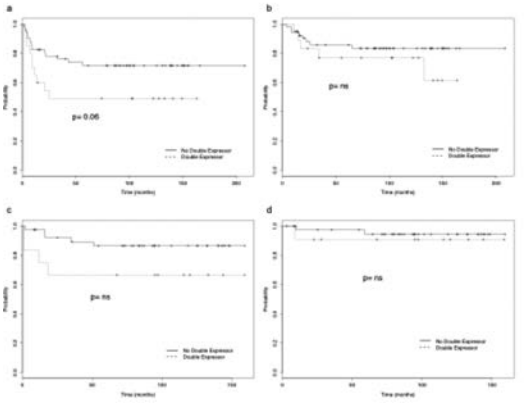

Figure 1.

Summary/Conclusions: In our R-HDS cohort the 5-year EFS and OS of double expressor patients compared better with previous reports of different DLBCL series treated with R-CHOP. This improvement was especially evident in transplanted patients, where R-HDS seems to be able to overcome the poor clinical outcome associated with MYC/BCL2 co-expression. Finally, the double expressor higher relapse risk did not affect the final whole series survival, suggesting that a significant fraction of relapsed/refractory young patients may be rescued with other intensive salvage therapies.

P346

BIOMARKERS PREDICTING ACUTE GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS: A PROSPECTIVE STUDY IN 120 PATIENTS

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Background: Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is the only curative treatment for many haematological diseases. Despite advances in supportive therapies, acute Graft-*versus*-Host Disease (aGvHD) remains the leading cause of morbidity and mortality. There are shortcomings in the prediction of aGVHD, indicating the urgent need for non-invasive and reliable laboratory tests to allow a precision-medicine tailored prophylactic approach.
Aims: We conducted a prospective observational study to ascertain the potential usefulness of the levels of ten biomarkers, measured pre-transplant and at +7 days post-transplant, in predicting the development and severity of aGvHD in allo-HSCT patients. These time-points were chosen as early risk stratification may provide a window for additional prophylactic measures.
Methods: We collected data from 120 consecutive patients (41 female and 79 male; median age 52, range 19-77 years) who underwent allo-HSCT at our institute between 2014 and 2015. Most patients were affected by acute myeloid leukaemia (n=62; 52%). Disease status at HSCT was "active disease" in 58% of patients (n=70). Stem cell donors were unrelated (n=39, HLA matching 9/10 in 14 and 10/10 in 25), family haploidentical (n=56), HLA-identical sibling (n=22), or cord blood (n=3). Post-transplant GvHD prophylaxis was with PT-Cy in 65 patients, ATG in 28 patients, both agents in 18 cases whilst 9 patients received neither. Additionally, sirolimus-based GvHD prophylaxis was used in 95 patients, while a CSA-based prophylaxis in 25 cases according to institutional guidelines. The following biomarkers were measured 7 days pre- and post-transplant: interleukin-6 (IL6), Ceruloplasmin (CER), Cholinesterase (CHE), Albumin, Immunoglobulin A, Gammaglutyl-transferase (GGT), White Blood Cells, Neutrophils, Haemoglobin and Platelets.
Results: In this cohort of 120 patients, 77 developed GvHD, of which 69 were aGvHD. Of the aGvHD cases, 49 had skin, 12 had liver and 19 had gut involvement. 39 of 120 patients died: 3/39 deaths were attributable to aGvHD. We looked for associations between in-range and out-of-range levels of our ten biomarkers with presence of aGvHD. At baseline, lower CHE concentrations were significantly related to the risk of developing aGvHD and to some of its main characteristics, such as severity (p=0.009), grade II-IV liver involvement (p=0.016), and gut involvement (p=0.036). At 7 days after HSCT, concentrations of IL6 were increased in patients who later developed grade II-IV gut GvHD (p=0.03). We found that baseline albumin concentrations were reduced in patients with subsequent liver aGvHD (p=0.027). Similarly, raised baseline GGT values were correlated with liver involvement (p=0.05). Moreover, we assessed the correlation of laboratory tests with non-relapse mortality (NRM: death from any non-relapse complication) disease relapse and overall survival (OS) at 100 days. Baseline albumin and CHE concentrations, together with post-transplant IL6 levels, were correlated to OS and TRM. Disease relapse was associated with baseline levels of albumin and IL6.
Summary/Conclusions: Pre-transplant CHE and GGT levels are predictors of aGvHD, especially with regards to grade III-IV aGvHD and liver involvement. Lower albumin levels were associated with liver aGvHD development, while raised IL6 concentration 7 days after HSCT represented a risk factor for gut aGvHD. These biomarkers will be incorporated in a treatment algorithm to increase primary GvHD prophylaxis in patients at risk of severe aGVHD after allo-HSCT.

P347

BONE MARROW WT1 LEVELS IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT (HCT) FOR ACUTE MYELOID LEUKEMIA AND MYELODYSPLASIA STUDY TIME POINTS AND THRESHOLDS WITH CLINICAL RELEVANCE

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Background: WT1 mRNA is overexpressed in most myeloid malignancies. Standardized methods of quantitation have been developed and succesfully used in minimal residual disease (MRD) assessment. The uncertaintity about the levels with prognostic and/or therapeutic impact has precluded a more widespread use of WT1 monitoring in HCT patients.
Aims: To investigate the WT1 levels and time points with clinical relevance in patiens with myeloid malignancies who received a HCT.
Methods: Prospective collection of samples from consecutive patients with acute myeloid leukemia (AML) and myelodysplasia (MDS) recipients of an allogeneic HCT in a University hospital. Normalized WT1 levels were obtained using the ELN protocol and the Ipsogen© plasmid. Statistical correlations with survival, relapse and other clinical variables were established.
Results: One hundred and ninety three consecutive allogeneic HCT recipients with myeloid malignancies (148 AML, 45 MDS) treated from 2003 to 2014 were included in the study. The analysis of pretransplant samples (n=177) showed that with WT1 levels below 100 copies had better post-transplant outcome in terms of overall survival at 5 years (OS:40±1 *vs* 29±6, p:0.004), disease-free survival (DFS:35±9 *vs* 26±6, p:0.002) and cumulative incidence of relapse (CIR:29±7 *vs* 37±6 p:0.051) than those patients with WT1>100 copies. When we analyzed the marrow samples obtained just after the neutrophil recovery (n=182), and using again the 100 copies threshold we also identified differences in OS (40±7 *vs* 31±9 p:0.025), DFS (36±7 *vs* 30±8, p:0.004) and CIR (29±6 *vs* 54±9, p<0.001). In 104 cases four consecutive follow-up samples were available. Those patients with sustained WT1 levels below 100 copies had a significantly better outcome than those with one or more determination higher than 100 copies (OS: 68±11 *vs* 26±7, p<0.001; DFS: 63±11 *vs* 20±8, p<0.001; CIR:20±8 *vs* 71±8, p<0.001).
Summary/Conclusions: Normalized bone marrow mRNA levels of WT1 provided useful information in patients with AML and MDS treated with HCT. In patients with more than 100 copies pre and/or postransplant further therapeutic interventions after the procedure are needed to avoid disease recurrence.

P348

TRANSPLANTATION FROM MATCHED UNRELATED AND HAPLOIDENTICAL DONOR IN PEDIATRIC SEVERE APLASTIC ANEMIA: EXPERIENCE WITH TCR ALPHA/BETA AND CD19 DEPLETION AS GRAFT PROCESSING METHOD

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Background: Hematopoietic stem cell transplantation from non-sibling donors

remains the only curative option for severe aplastic anemia patients refractory to ATG/CsA immunosuppression.
Aims: Although the results of MUD and haploidentical transplantation in SAA have improved significantly, graft-*versus* host disease (GVHD) remains a serious problem, associated with significant morbidity and mortality. We investigated the role of new method of graft processing - TCR alpha/beta depletion as a way to improve the results of MUD and haploidentical transplants in SAA.
Methods: Thirty seven patients with SAA were treated since November 2012 till November 2015. Median age at HSCT was 11(3-22) years, 22 male/15 female. All pts were refractory/relapsed after at least two courses of ATG/CsA, 2 pts had concurrent severe hemolytic PNH. Time from diagnosis to transplant was 4,3(1-10) years. Donors were unrelated volunteers in 29 cases, haploidentical parent in 8 cases. Preparative regimen included cyclophosphamide 100-150 mg/kg, fludarabine 150mg/kg, ATG and 2-6Gy thoraco-abdominal irradiation, in haplo transplants patients additionaly recieved thiophosphamide at 10mg/kg. Two patients recieved alemtuzumab instead of ATG because of anaphylaxis. Post-transplant GVHD prophylaxis included Tacro and Mtx on days +1,+3,+6. PBSC grafts were depleted of TCRalpha/beta cells and CD19 cells with CliniMACS device as recommended by the manufacturer. Patients recieved a median of 8,6(6,8-13,5)x10^6 CD34 per kg, 3(1-30) x10^4 TCRalpha/beta per kg.
Results: All patients engrafted with a median of 14 days for WBC and 13 days for platelets. In 4 patients after MUD transplantation secondary graft failure (rejection) developed, two of them were successfully retransplanted. Cumulative incidence of aGVHD 2-4 was 3% (95% CI: 1-17%) and 50%(95% CI: 28-89%) in MUD and haplo respectively. Cumulative incidence of aGVHD 3 was 0% and 12%(95% CI: 3-58%) in MUD and haplo respectively. Cumulative incidence of cGVHD was 3%(95% CI: 0-25%) and 37%(95% CI: 15-92%) in MUD and haplo respectively. A median follow up was 18 months. Six patients died. The reasons of death were: viral infection (3 pts), viral infection plus GVHD (one pt), and 2 pts died after second transplantation (1 – toxoplasmosis, 1 – viral infection plus GVHD). Overall survival was 81%(95% CI: 65-96%) and 87%(95% CI: 65-100%) respectively. Prolonged mixed chimerism in T-cells was detected in recipients of MUD grafts in contrast to haploidentical grafts.
Summary/Conclusions: TCR alpha/beta depletion is a robust platform for allogeneic transplantation from MUD and haploidentical donors in severe aplastic anemia. Results can be further improved by additional measures to control viral infections and prevent rejection in MUD transplants.

Stem cells and the microenvironment 1

P349

THE PRC2 COMPONENT JARID2 IS DISPENSABLE FOR HEMATOPOIETIC STEM CELLS BUT CRITICAL FOR THE MAINTENANCE OF LEUKEMIC STEM CELLS

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Background: Hematopoietic stem cells (HSCs) are under tight transcriptional regulation and erroneous regulation can confer unwanted properties to the cells and ultimately lead to malignant transformation. Several studies have implicated the epigenetic modulator Polycomb Repressive complex 2 (PRC2), which is responsible for the deposition of the H3K27me3 mark associated with transcriptional repression, in preserving correct HSC maintenance.
Aberrant function of PRC2 is shown to influence malignant transformation and propagation of disease. A number of PRC2 auxiliary factors has been identified, one being JARID2, which is involved in modulating the activity of the complex by regulating binding to target genes.The role of JARID2 in the hematopoietic system is essentially unknown.
Aims: To characterize the functional consequences of *Jarid2* deletion in both normal and malignant hematopoiesis.
Methods: Using a conditional knock out (KO) mouse model we have investigated the loss of *Jarid2* in the normal hematopoietic system. We also assayed leukemic development and maintenance in our KO model using a Notch1 induced T cell acute lymphoblastic leukemia (T-ALL) model. Both settings were studied by immunophenotypic analyses by FACS, RNAseq and micro array based gene expression profiling, and ChIPseq analyses of histone marks. Multi-lineage reconstitution and self renewal capacity of the hematopoietic stem cells were assayed by competitive and serial transplantations. Leukemic development, maintenance, and frequency of leukemic stem cells (LSC) were assessed by serial transplantation and limiting dilution transplants. Serially transplanted T-ALL was further analysed by exome sequencing to identify mutations altering LSC frequency.
Results: We found that JARID2 is dispensable for the numbers and function of adult hematopoietic stem cells and observed only mild changes for the down stream progenitor cells. These findings were further supported by a lack of changes in gene expression and H3K27me3 levels in these cells. Through transplantation assays, we found that the self-renewal capacity of normal HSCs also remained intact after loss of JARID2. These findings are in stark contrast to the requirement for JARID2 in the leukemic setting. In a model of T-ALL we observed an initial acceleration of leukemic onset, possibly explained by improved homing abilities of the *Jarid2* KO cells. However, KO T- ALL showed a marked decrease in the ability to propagate leukemia in secondary recipients, and the increased latency was reflected in a dramatic reduction in leukemic stem cells (LSC) frequency. Furthermore, gene expression profiles for these cells were inversely correlated with relapse signatures for childhood T-ALL. Surprisingly, the latencies were reverted upon re-transplantation into tertiary recipients, and this corresponded to a rise in LSC numbers. To study this further we performed exome sequencing of serially transplanted T-ALL and identified mutations in *Pten* and *Ikzf1*. These are likely candidates responsible for rescuing the LSC deficiency observed for JARID2 depleted T-ALL.
Summary/Conclusions: JARID2 is, contrary to other PRC2 components, dispensable for normal HSC function, but plays a key role in LSC maintenance in NOTCH1 induced T-ALL. Loss of *Jarid2* leads to a marked loss of LSCs but their numbers can recover as a consequence of secondary mutations.
Thus, this study provides new insights into the function of PRC2 in normal and malignant hematopoiesis as well as into mechanisms governing the behavior of LSCs in T-ALL.

P350

HHEX PROMOTES HSC SELF-RENEWAL AND STRESS HEMATOPOIESIS VIA REPRESSION OF CDKN2A

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Background: The Hematopoietically-expressed homeobox transcription factor (Hhex) is important for the maturation of definitive hematopoietic progenitors and B-cells during development. We have recently shown that in adult hematopoiesis Hhex is dispensable for maintenance of hematopoietic stem cells (HSCs) and myeloid lineages but essential commitment of common lym-

phoid progenitors to diverse lymphoid lineages. However, whether Hhex plays a role in HSC self-renewal and myeloid expansion during hematopoietic stress is unknown.
Aims: The aim of this is to determine the roles of Hhex in HSC self-renewal and emergency hematopoiesis.
Methods: We employed inducible knock out mice for Hhex to assess the requirement of this transcription factor in adult haematopoiesis. Serial bone marrow transplantation was used to test the effect of Hhex deletion on HSC self-renewal. In addition, the bone marrow response to myeloablation was tested using 5- fluorouracil (5-FU) treatment, and the proliferation of immature blast colony-forming units was tested *in vitro* using soft agar cultures.
Results: Here we show that Hhex-deleted HSCs are progressively lost during serial bone marrow transplantation, revealing an intrinsic defect in HSC self-renewal. Moreover, Hhex-deleted mice show markedly impaired hematopoietic recovery after myeloablation using 5-FU. *In vitro* cultures demonstrated that although Hhex-null donor bone marrow contained elevated numbers of hematopoietic progenitors, immature blast colonies were specifically incapable of replating. Thus, Hhex is essential for HSC self-renewal and immature myeloid progenitor expansion *in vitro* and *in vivo*. RNAseq analysis of Hhex-null LSK cells showed Hhex deletion led to the dysregulation of cell cycle regulators, including an upregulation of Cdkn2a-encoded $p16^{Ink4a}$ and $p19^{Arf}$. Consequently we crossed Hhex inducible knock-out mice with those lacking Cdkn2a. Loss of Cdkn2a completely restored the expansion of Hhex-null progenitors and blast colony replating *in vitro*, as well as hematopoietic reconstitution following myeloablation *in vivo*.
Summary/Conclusions: These finding show that Hhex is a key transcriptional mediator of HSC self-renewal and stress hematopoiesis, mediating these effects by regulation of cell cycling via repression of Cdkn2a.

P351

WNT5A FROM THE NICHE REGULATES THE ACTIN REGULATORY PATHWAY IN HEMATOPOIETIC STEM CELLS
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Background: The niche regulates the quality of hematopoietic stem cells (HSCs) that are regenerated under conditions of stress, such as transplantation. We found earlier, that WNT5A is overexpressed in stromal cells capable of maintaining HSC activity in an *in vitro* model of culture stress.
Aims: We here wished to delineate the role of stromal WNT5A in hematopoietic processes *in vivo*.
Methods: For this purpose, we performed transplantation experiments in which wild-type (WT) HSCs were transplanted into a WNT5A haploinsufficient environment. After trasnplantation the regenerated HSCs were subjected to rigorous molecular and cellular asessment, as well as transplanted into secondary WT recipients.
Results: We find that although HSCs efficiently find and lodge in the Wnt5a-deficient niche, the reduced level of WNT5A in the niche regenerates HSCs, which do not successfully engraft secondary recipients. In particular, RNA sequencing shows a dysregulated Zeb1- and Nfatc-associated gene expression of multiple genes involved in the small GTPase- dependent actin polymerization pathway. Misexpression of these genes results in reduced ability to direct polarized F-actin localization, leading to defects in adhesion, migratory behavior and homing to the bone marrow of secondary recipients. Our study also shows that the Wnt5a-deficient environment similarly affects p185-BCR-ABL cells, which, in 42% of the studied recipients, fail to generate leukemia and, in the remaining cases, fail to transfer leukemia to secondary hosts.
Summary/Conclusions: Thus, we show that Wnt5a in the niche is required to regenerate HSCs and leukemic cells with functional ability to rearrange the actin cytoskeleton which is required for successful engraftment.

P352

NPM1 IS REQUIRED FOR NORMAL HEMATOPOIESIS
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Background: The NPM1 gene encodes a multifunctional protein that shuttles between nucleus and cytoplasm and is frequently mutated in hematological malignancies. Recurrent mutations of NPM1 occur in 30% of acute myeloid leukemia (AML) patients, causing a frame-shift in exon 12 that results in aberrant localization of the mutated protein, designated NPMc+, to the cytoplasm. Although NPM1 is the most frequently mutated gene in AML, its role in leukemogenesis is poorly understood, given its multiple cellular functions and attribution as a proto-oncogene and a tumour suppressor. Previous studies showed that constitutive homozygous deletion of NPM1 in mice is embryonic lethal due to aberrant organogenesis and defects in primitive hematopoiesis, whilst heterozygous loss of a single NPM1 allele causes a hematological condition resembling human myelodysplastic syndrome (MDS), a preleukemic condition often progressing towards AML.
Aims: We aim to study the role of NPM1 in adult hematopoiesis.
Methods: We describe a conditional mouse model using Tamoxifen-inducible Cre recombinase to delete *NPM1* in adult hematopoiesis.
Results: We found that deletion of the *NPM1* gene in adult mice resulted in multiple hematopoietic abnormalities. Loss of *NPM1* compromised hematopoietic stem and progenitor cell (HSPCs) function, causing depletion of progenitors and mature lineages, particularly the erythroid lineage. *NPM1*-null HSPCs exhibited aberrant cell cycle status with increased number of cells in the synthesis phase. Competitive transplantation and *in vitro* differentiation assays revealed that *NPM1*-null HSPCs had reduced capacity to engraft and produce mature hematopoietic lineages, including B-cells, T-cells, and myeloid cells compared to wild-type controls, indicating impaired self-renewal and differentiation of the stem cells. Phenotypically, *NPM1*-null mice were anemic and leukopenic with splenomegaly, symptoms that resemble human MDS.
Summary/Conclusions: In summary, this mouse model highlights the important role of *NPM1* in adult hematopoiesis. Knowledge of the role of *NPM1* in normal hematopoiesis is likely to improve our understanding of AML pathogenesis associated with mutations in *NPM1*.

P353

PLZF MUTATION ALTERS MOUSE HEMATOPOIETIC STEM CELL FUNCTION AND CELL CYCLE PROGRESSION
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Background: Hematopoietic stem cells (HSCs) give rise to all blood populations due to their long-term self-renewal and multipotent differentiation capacities. Because they have to persist throughout an organism's life span, HSCs tightly regulate the balance between proliferation and quiescence. As with most tissues that have a high cellular turnover, hematopoietic tissue is sensitive to aging, which results in multiple functional defects that accumulate in HSCs from older individuals. Aging HSCs have a reduction in their regenerative capacity and support decreased lymphoid lineage output together with increased myeloid output. Surprisingly, the number of phenotypically-defined HSCs increases with age; while it has long been known that aging has a major impact on the frequency and cell-cycle kinetics of hematopoietic cell compartments, mechanisms underlying this phenomenon remain poorly understood.
Aims: Here we investigated the role of the transcription factor, plzf in HSC fate using the Zbtb16lu/lu mouse model, harboring a natural spontaneous mutation that inactivates plzf.
Methods: We used the Zbtb16lu/lu mouse model in order to study the role of plzf in HSCs. We performed serial and competitive transplantation assays to evaluate Zbtb16lu/lu HSC function. Effect of Zbtb16lu/lu mutation at the genomic level was addressed by transcriptomic analyses and plzf role on cell cycle regulation was studied through a combination of flow cytometry and BrdU injection.
Results: Regenerative stress revealed that Zbtb16lu/lu HSCs had a lineage skewing potential from lymphopoiesis towards myelopoiesis, an increase in the long-term-HSC pool and a decreased repopulation potential. Comparison of young Zbtb16lu/lu HSCs with one-year-old HSCs for their competitive repopulation ability showed that the Zbtb16lu/lu HSCs have a myeloid competitive advantage and contribute more to the LT-HSC compartment relatively to ST-HSC and MPP1 fractions. Furthermore, old plzf-mutant HSCs present an amplified aging phenotype. Comparative microarray-based gene expression data from Zbtb16lu/lu and control HSCs revealed that Zbtb16lu/lu HSCs harbor a transcriptional signature associated with a loss of stemness and cell-cycle deregulation suggesting changes in HSC cell cycles induced by plzf mutation. Indeed, cell-cycle analyses revealed an important role for plzf in the regulation of the G1-S transition of HSCs. We also demonstrated a significant increase in apoptosis in Zbtb16lu/lu HSC in comparison to WT cells that could account for the limited accumulation of Zbtb16lu/lu HSCs in young PLZF-mutant. Interestingly, apoptosis was not as increased in transplanted Zbtb16lu/lu HSCs.
Summary/Conclusions: These results suggest that Zbtb16lu/lu promotes premature-aging-like syndrome in mice after regenerative stress and imply a role for plzf in the control of age-related mechanisms in HSC. Our study reveals a new role for plzf in regulating HSC function that is linked to cell-cycle regulation and positions plzf as a key player in controlling HSC homeostasis.

P354

IL-1 INDUCED ACTIVATION OF QUIESCENT HEMATOPOIETIC STEM CELLS *IN VIVO*

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Background: The pro-inflammatory cytokines IL-1α and IL-1β play important roles in the acute inflammatory response to infection and injury. Intracellular IL-1α protein is highly expressed in endothelial cells and platelets, suggesting that its release may function as a danger signal. In contrast, IL-1β is highly secreted by monocytes and macrophages upon infection to initiate a systemic pro-inflammatory response. In addition, IL-1 is involved in a variety of autoinflammatory diseases, which are often associated with hematopoietic dysfunctions. However, thus far the effect of IL-1α and IL-1β on hematopoietic stem cells (HSCs) remains unclear.
Aims: To investigate the effect of the pro-inflammatory cytokines IL-1α and IL-1β on quiescent HSCs *in vivo*.
Methods: To analyze the effect of IL-1α or IL-1β on quiescent HSCs, wild-type (wt) or IL-1R$^{-/-}$ (IL-1 receptor knock-out) mice were injected with IL-1α or IL-1β. HSC number, proliferation and cell cycle status were analyzed by FACS 18 hours following treatment. HSCs from treated mice were also isolated and transplanted into lethally irradiated recipients to analyze the function of these HSCs.
Results: Here, we could show that IL-1α and IL-1β efficiently induced the activation of quiescent HSCs *in vivo*. Within 18 hours after treatment of mice, HSCs exited G_0 phase and entered an active cell cycle. This effect was abrogated in mice lacking the IL-1 receptor (IL-1R$^{-/-}$), demonstrating the requirement of the IL-1 receptor. Competitive bone marrow (BM) transplantations and serial transplantation assays showed no functional impairment or reduced self-renewal potential of HSCs upon short-term IL-1 treatment. Moreover, forward and reverse BM chimeras showed that IL-1 signaling via the IL-1R in cells of the BM niche is dispensable for the IL-1 effect on HSCs. Interestingly, mixed BM chimeras with wt and IL-1R$^{-/-}$ HSCs indicated a direct effect of IL-1α on HSCs, whereas the effect of IL-1β on HSCs was both direct and indirect via other cells of the hematopoietic system. Furthermore, we could link the effect of IL-1 on HSCs *in vivo* to NFkB signaling.
Summary/Conclusions: Our data show a strong effect of IL-1α and IL-1β on quiescent HSCs *in vivo.* In addition to previous studies on other pro-inflammatory cytokines, our data indicate that *in vivo* activation of HSCs by pro-inflammatory cytokines is a more general phenomenon, strongly linking the host response to systemic inflammation to activation of quiescent HSCs.

P355

SFRP2 FROM THE NICHE IS REQUIRED TO MAINTAIN THE REGENERATION OF THE HEMATOPOIETIC STEM CELL POOL

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Background: We previously found that *Sfrp2* was overexpressed in stromal cells, which maintain hematopoietic stem cells (HSCs) in *in vitro* culture.
Aims: Here, we determined the relevance of Sfrp2 expression by niche cells for the maintenance of hematopoiesis under stress conditions *in vivo*.
Methods: To determine the role of Sfrp2 in HSC responses under stress conditions, we studied *in vitro* cultures, 5-FU treatment *in vivo*, and HSC engraftment in transplantation experiments.
Results: In in-vitro co-cultures with sh*Sfrp2* stromal cells, the number lineage-negative Kit+ Sca-1+ (LSK) and progenitors increased. The LSK cells showed higher levels of Ki-67 expression, BrdU incorporation, and catenin-dependent Wnt signaling. Yet, total repopulating activity of these cultures was diminished, suggesting exhaustion of HSCs. These *in vitro* results were mirrored in *in vivo* models of stress, such as aging, 5-FU treatment and hematopoietic regeneration in *Sfrp2*-/- recipients. In all three *in vivo* situations of stress, we noted an increase of LSK cells, which in accompanied by increased levels of ®- catenin and cyclin D1. In the transplantation experiments, the increase in LSK cells was accociated with a progressive loss of HSCs in serial transplantation recipients. Similarly, genotoxic stress in 5-FU-treated *Sfrp2*-/- mice showed LSK cells with a higher cycling activity than wild-type (WT) littermates, as shown by higher levels BrdU incorporation, and a higher expression of Ki-67 and canonical Wnt signaling mediators. Importantly, increased cycling of LSKs was accompanied with stress-induced senescence-associated DNA damage response as indicated by ©H2A.X staining and depolarized localization of acetylated H4K16.
Summary/Conclusions: Our experiments are consistent with the view that *Sfrp2* expression in the niche is required to limit stress-induced DNA damage and canonical Wnt-mediated HSC activation, thus preventing HSC exhaustion.

P356

CLEC-2 EXPRESSION IS A NEW MARKER FOR A SUBSET OF HEMATOPOIETIC STEM/PROGENITOR CELLS THAT CONTRIBUTES TO INFLAMMATION-INDUCED EMERGENT MEGAKARYOPOIESIS

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Background: Recent studies have suggested that megakaryocytes can be generated from multiple pathways, through some processes bypassing multipotent common myeloid progenitor (CMP) and/or bipotent megakaryocyte/erythrocyte progenitor (MEP) stages. Furthermore, a recent research demonstrated the presence of a subset of megakaryocyte-committed cells within hematopoietic stem cell (HSC) compartment that are activated by inflammatory stress. However, the immunophenotype of these megakaryocyte-biased HSCs remains poorly understood. Here, we show that the authentically identified HSC population includes a functionally distinct subset expressing the C-type lectin-like receptor 2, CLEC-2, which contributes to inflammation-induced emergent megakaryopoiesis.
Aims: The present experiments were designed to characterize biological significance of CLEC-2$^+$ cells within the immunophenotypically defined HSC compartment.
Methods: Bone marrow (BM) cells were obtained from femurs and tibias of 6-8 weeks old C57BL/6J mice. To induce inflammation, we injected a single dose of 5 mg/kg pI:C into mice intraperitoneally. HSCs and stem/progenitor cells were isolated by staining unfractionated BM cells. In this experiment, we defined Lin$^-$Sca1$^+$c-Kit^{high} (LSK) CD150$^+$CD34$^-$ cells as HSCs, Lin$^-$Sca1$^-$c-Kit$^+$IL-7Rα$^-$FcRII/III^{low}CD34$^+$ cells as CMP, and Lin$^-$Sca1$^-$c-Kit$^+$IL-7Rα$^-$FcRII/III$^-$CD34$^-$ cells as MEP, respectively.
Results: We first evaluated the expression of CLEC-2 on unfractionated BM cells. CLEC-2 expression was significantly high on CD41(Itga2b)$^+$ megakaryocytes and endothelial cells, but very low on multi- and bipotent committed precursors, such as CMPs and MEPs, that were sequentially placed downstream of HSCs. On the other hand, we found that LSK CD150$^+$CD34$^-$ HSCs fraction contained a substantial number of CLEC-2$^+$ cells. Interestingly, the treatment with pI:C significantly augmented CLEC-2$^+$ fraction from 0.6% to 4.9% in the HSCs compartment, while it reduced CLEC-2$^-$ population. Cell cycle analysis revealed that most CLEC-2$^+$ HSCs are in G0/G1 and only a small population are in S/G2/M phase of the cell cycle before stimulation with pI:C, indicating that this population is also in a quiescent state as well as CLEC-2$^-$ HSCs without stimulation. Although CLEC-2$^+$ HSCs didn't express CD41 protein, an early marker of megakaryocytic maturation, before stimulation with pI:C, its expression was induced by pI:C treatment drastically on CLEC-2$^+$ HSCs, suggesting that inflammatory signals efficiently activated quiescent CLEC-2$^+$ HSCs and imposed commitment to the megakaryocyte lineage on these cells. To assess whether CLEC-2 distinguished functionally different subpopulations of HSCs, we evaluated colony types yielded from CLEC-2$^+$ and CLEC-2$^-$ HSCs after 14-day *in vitro* semiliquid cultures. While CLEC-2$^-$ HSCs produced numerous CFU-Mix, CFU-GM/G/M, BFU-E colonies, CLEC-2$^+$ HSCs were less potent to produce these colonies. On the other hand, CLEC-2$^+$ HSCs had great potential to produce CFU-Meg. Furthermore, qRT-PCR analysis revealed that CLEC-2$^+$ HSCs exhibited a differential lineage profile from CLEC-2$^-$ HSCs, with increased expression of megakaryocyte/platelet genes such as *CD41*, *pf4*, and *vwf*, as well as transcription factors *gata1, Fog-1*, and *klf1*.
Summary/Conclusions: We identified viable megakaryocytes-biased HSCs in the most primitive hematopoietic cell fraction of adult BM with a new marker CLEC-2, which contributes to emergent megakaryopoiesis upon inflammatory stimulation. Our findings provide a new insight into the hematopoietic hierarchy.

P357

IN VITRO AND *IN VIVO* STEM CELL PROPERTIES OF HIGHLY ENRICHED HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS

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Background: Human bone marrow contains a rare population of non-hematopoietic stem/progenitor cells, the so-called mesenchymal stromal cells (BM-MSCs). BM-MSCs are essential constituents of the hematopoietic niche and give rise to skeletal tissues and the hematopoietic stroma. Although BM-MSCs have been suggested to be stem cells, it has thus far not been thoroughly investigated, whether or not BM-MSCs fulfill stringent cell properties, *i.e.* self-renewal and differentiation *in vitro* and, importantly, *in vivo*.
Aims: Based on our recent finding that BM-MSCs were highly enriched in lin$^-$/CD45$^-$/CD271+/CD140a$^-$ BM cells (Li et al, Stem Cell Reports 2014), the current study aimed to study the stem cell properties of this putative stem cell population.
Methods: BM aspirates were obtained from healthy donors. Following lineage depletion, mononuclear cells were antibody-labeled, FACS sorted (single-cell and bulk), analyzed for single cell gene expression (Fluidigm), differentiation potential, and propagated as either CFU-F in standard adherent MSC cultures

or as non-adherent mesenspheres, the latter of which has been reported to better conserve immature MSC features. Adherently-cultured and sphere-derived cells were then compared side-by-side for their self-renewal and differentiation potential *in vitro* and *in vivo* by serial xenotransplantation into NSG mice.
Results: Single cell Fluidigm analysis of lin$^-$/CD45$^-$/CD271+/CD140a$^-$ BM-MSCs showed homogeneous mRNA expression levels of MSC associated genes, such as VCAM1, BMP5, CXCL12, LEPR, and others. Furthermore, similar progenitor cell frequencies and *in vitro* self-renewal capacities were observed under CFU-F and mesensphere assays conditions. CFU-Fs and mesenspheres displayed similar surface marker profiles and comparable *in vitro* tri-lineage differentiation potential towards osteogenesis, adipogenesis and chondrogenesis. *In vivo* self-renewal and differentiation potential of primary BM-MSC were assessed by s.c. transplantation of lin$^-$/CD45$^-$/CD271+/CD140a$^-$-derived CFU-Fs and spheres into NSG mice. Whereas CFU-F numbers decreased after transplantation, mesensphere *in vivo* self-renewal was documented by increasing numbers of spheres recovered after primary and secondary transplantation. Additionally, lin$^-$/CD45$^-$/CD271+/CD140a$^-$ -derived spheres displayed full *in vivo* differentiation capacity in primary and secondary transplantations.
Summary/Conclusions: Our data provide definite evidence that primary human lin$^-$/CD45$^-$/CD271+/CD140a$^-$ cells fulfill stringent stem cell criteria, *i.e. in vitro* and *in vivo* self-renewal and differentiation. However, BM-MSCs stem cell properties were preserved only when cells were propagated as non-adherent cells, but not under standard adherent culture conditions.

P358

GENOME WIDE DYSREGULATION OF GENE EXPRESSION BY TRISOMY 21 IN FETAL LIVER HAEMATOPOIETIC STEM AND PROGENITOR CELLS
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Background: Trisomy 21 (T21) causes multilineage perturbation of fetal and neonatal haematopoiesis. We have previously demonstrated that 2nd trimester Down Syndrome (DS) fetal liver (FL) samples have increased numbers of immunophenotypic haematopoietic stem cells (HSC) and megakaryocyte erythroid progenitors (MEP) together with a severe reduction in committed B progenitors (CBP). Functional studies and Fluidigm gene expression assays support lineage bias favouring megakaryocyte-erythroid over B lymphoid differentiation and proliferation but did not identify causative genes on chromosome 21 (Hsa21) reflecting the limited number of Hsa21 genes which can be included on the panel[1]. Determining the mechanisms underlying these defects is likely to be important for understanding why young children with DS are more prone to myeloid and lymphoid leukaemias.
Aims: To investigate the impact of the additional copy of chromosome 21 (Hsa21) on the transcriptome of primary FL HSC and progenitor cells (HSPC). Specifically, to determine (a) whether there is a T21 'gene expression signature' and whether this is consistent across all HSPC types and samples and (b) whether T21 causes consistent patterns of genome-wide dysregulation of gene expression in HSPC.
Methods: Genome-wide transcriptome profiling was performed by RNA sequencing of 8 flow-sorted FL HSPC populations (immunophenotypic HSC, MPP, LMPP, CMP, MEP, GMP, ELP and CBP as previously described[1]) from 7 gestation-matched 2nd trimester FL samples: DS (n=4) and normal (n=3). Indexed cDNA libraries were multiplexed and sequenced using Illumina HiSeq2500. Raw reads generated were subjected to an in house RNASeq analysis pipeline including adaptor trimming, QA, filtering and alignment of genome and transcriptome using TopHat2. Read-count based expression analysis was performed using DESeq2, and HTSeq-count; and differential expression compared in each HSPC population between the DS and normal FL samples. LOESS (Local polynomial regression fitting) was used for up/down regulated chromosomal domains. Data analysis and visualisation was performed with R.
Results: Using ANOVA, ~2700 genes were differentially expressed (DE) between HSPC populations from all 7 samples (FDR<0.001). Principal Component Analysis (PCA) showed good spatial segregation of HSPC into distinct lineage-specific populations in both normal and DS FL. The best spatial segregation was found using the top 300 DE genes which also showed that while PC1 separated normal and T21 HSPC equally, PC2 accounted for a greater segregation of T21 than normal FL HSPC. Although lineage-specific gene expression patterns were largely maintained in DS HSPC, there were differences in levels of gene expression between DS and normal HSPC with the number of significantly (FDR<0.05) DE genes ranging from 10 in CMP to 1225 in CBP. Perturbation of lineage-associated gene expression was particularly prominent in CBP, HSC, MPP and MEP. More than half of all protein coding Hsa21 genes (154/243) were expressed in both DS and normal FL HSPC. Within each HSPC population, a consistent Hsa21 gene expression profile was seen in all 7 samples and most of the differences in Hsa21 gene expression between DS and normal HSPC were an exaggeration of the 'normal' HSPC profile rather than an aberrant expression of a different set of Hsa21 genes or a single Hsa21 gene. Using Loess smoothing, Hsa21 gene expression was consistently higher in DS HSPC compared to normal HSPC along the entire length of Hsa21 rather than in clearly defined domains. Finally, the majority of DE genes in all HSPC populations were located on chromosomes other than Hsa21.
Summary/Conclusions: These data show that global perturbation of fetal haematopoiesis by T21 is matched by genome-wide dysregulation of gene expression affecting most chromosomes in all HSPC, particularly lymphoid progenitors. Although DS FL HSPC populations all showed a 'T21 gene expression signature', this reflected more pronounced expression of the same Hsa21 genes as in normal HSPC rather than being driven by aberrant expression of a small subset, or single, Hsa21 gene(s).

Reference
1. Roy A *et al.*, PNAS 2012 Oct 23; 109(43):17579-84.

P359

GPR56: A NOVEL MOLECULE INVOLVED IN HEMATOPOIETIC STEM CELL GENERATION
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Background: Knowledge of the key factors that drive hematopoietic stem cell (HSC) generation is of particular importance for current hematopoietic regenerative approaches and reprogramming strategies. At mouse embryonic day (E)10.5, the permanent adult hematopoietic system initiates with the formation of HSCs in clusters lining the major embryonic vasculature (aorta, vitelline and umbilical arteries) in a process called endothelial-to-hematopoietic transition (EHT). From all hemogenic endothelial cells (HECs) only few generate an HSC.
Aims: We aime to find novel regulators of EHT and possibly a new and more faithful HSC marker.
Methods: By CRISPR Cas9 genome editing we have generated a Gpr56 knockout zebrafish model to study its function during embryonic development
Results: A comparative RNA sequencing analysis of HEC *versus* HSCs from mouse tissue by our lab has resulted in the identification of several candidate regulatory genes of EHT. The top candidate, *gpr56,* showed a 4.88 log fold (30x) increase during endothelial to HSC transition and was recently implicated in HSC function in the adult mouse. Upon *gpr56* knockdown, zebrafish embryos showed severe reduction of *cmyb* expressing cells (HSC marker, a reduction in CD41 expressing cells (expressed in HSCs and progenitors) and a reduction in EHT events, showing a requirement for Gpr56 in HSC generation. Rescue with both *gpr56* zebrafish and mouse RNA resulted not only in the restoration of *cmyb* expressing cells, but in an expansion and ectopic expression of *cmyb* expressing cells to the axial vein. This shows that Gpr56 is sufficient for HSC generation and suggests that the function of Gpr56 is conserved between mouse and zebrafish.
Summary/Conclusions: Since Gpr56 is required for HSC generation and functions at the membrane, it is a perfect target for manipulation by bioactive compounds. Therefore, we want to identify the direct binding partners of Gpr56 (both its ligand and downstream signaling partners) and identify bioactive compounds which regulate Gpr56 function and thus manipulate HSC generation for possible therapeutic regenerative purposes.

Gene therapy, cellular immunotherapy and vaccination

P360

DENDRITIC CELL THERAPY FOR MALIGNANT PLEURAL MESOTHELIOMA (MPM): FINAL RESULTS OF A PHASE I/II CLINICAL TRIAL

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Background: Cellular therapies, including the use of dendritic cells, are gaining momentum as a therapeutic option in cancer patients, especially in light of recent data showing that such therapies have the potential to improve survival often without causing significant toxicity. Except for a small group of long-term survivors (*i.e.* survival beyond 36m), patients with Malignant Pleural Mesothelioma (MPM) face little prospect of survival by any of the mainstream treatments.
Aims: The aim of this study was to establish the potential utility of dendritic cell therapy for immune modulation in MPM patients.
Methods: Ten MPM patients with non-resectable disease (median age 62 years, stage 1-2, PS 0-1) were included in this open-label phase I/II clinical trial (NCT01291420) after completion of frontline platinum/pemetrexed-based chemotherapy. Autologous DCs were propagated *in vitro* from fresh peripheral blood monocytes obtained by leukapheresis, according to our previously published protocol (Van Tendeloo *et al*. PNAS 2010). Antigen loading of the DCs was performed by electroporation of mRNA encoding the mesothelioma-associated tumor antigen Wilms' tumor-1 (WT1). DCs ($10x10^6$ cells per injection) were administered 4 times on a biweekly basis, after which response evaluation was performed by various immunological assays and imaging studies.
Results: All patients completed the intended treatment schedule of 4 biweekly DC vaccinations; most patients continued to receive DC vaccinations with a mean number 18 vaccines administered per patient (range 5-44). The vaccinations were well tolerated; none of the patients showed overt signs of systemic toxicity. All patients except one demonstrated a significant delayed-type hypersensitivity skin reaction, providing evidence of vaccine-elicited immunity *in vivo*. Progression-free survival, determined by repeated imaging studies, was 5 months. Median overall survival calculated from start of chemotherapy was 36 months. After exclusion of 4 long-term survivors, median overall survival was corrected to 32 months.
Summary/Conclusions: DC therapy in chemotherapy-treated MPM patients is feasible, safe and immunogenic. Overall survival data of this study compare favorably to the 22-month overall survival reported for a similar cohort of patients (Hillerdal *et al.* J Thorac Oncol 2008). This is in line with other recent studies showing that DC therapy can confer overall survival benefit to cancer patients.

P361

EXPLORING CHIMERIC NATURAL KILLER RECEPTOR NKP30 EXPRESSING HUMAN T LYMPHOCYTES FOR ADOPTIVE IMMUNOTHERAPY TO ACUTE LEUKEMIA

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Background: Adoptive cellular therapy (ACT) of chimeric antigen receptor (CAR)-reprogrammed T cells has advanced as a valuable personalized and effective immunotherapy for leukemia and solid tumors. In particular, clinical trials on $CD19^+$ lymphomas using CD19 CAR expressing T lymphocytes have revealed highly promising results. However, suitable antigens for an effective and specific CAR-mediated therapy to acute myeloid leukemia (AML) are still warranted as *e.g.* CD33 and CD123 CAR expressing T cells induce potent immune responses not only to AML blasts but also recognize normal hematopoietic stem cells (HSC). In contrast, B7H6, a member of the B7 family, is frequently expressed on various tumor cells including AML blasts but not constitutively expressed on normal tissues and recognized by the natural killer (NK) cell activating receptor NKp30. Moreover, NKp30 recognizes human leukocyte antigens (HLA)-B-associated transcript 3, a nuclear factor that is secreted and translocated to the cell surface in stressed and transformed cells.
Aims: In the current study, we therefore explored human T cells redirected by retroviral transduction of a NKp30-based CAR for effective antileukemic immunity *in vitro* and adoptive immunotherapy to AML *in vivo* using humanized mice.
Methods: Peripheral blood mononuclear cells (PBMC) or immunomagnetically purified human T cells were polyclonally stimulated and reprogrammed with a CAR based on the external region of the NKp30 receptor fused to the CD3ζ chain signaling domain (kindly provided by Dr. S. Klobuch, Dept. of Internal Medicine 3, Medical University Regensburg, Germany) by retroviral gene transfer. Transduced cells were further selectively expanded utilizing puromycin resistance. NKp30 expression was determined by flow cytometry. IFN-γ ELISPOT analyses and cytotoxicity assays were performed to assess antileukemic responses to acute leukemia lines and primary AML blasts *in vitro* and *in vivo* by adoptive transfer of redirected T cells into leukemia engrafted NSG mice. Expression of B7H6 in target cells was examined by RNA-based RT PCR.
Results: Following transduction and puromycin selection ≥90% of $CD3^+$ T cells expressed the NKp30 CAR. Upon coculture with B7H6 expressing targets such as K562 and HL-60 (myelogenous leukemia cell lines), NALM 16 (pre-B-ALL) and AML MZ987 NKp30-redirected T cells elicited potent IFN –γ release and exhibited cytolytic activity to both leukemia lines and primary AML blasts. These responses were specific as no reactivity to the B7H6 negative myeloma line U266 was observed. Furthermore, Mock transduced T cells used as controls did not reveal significant alloreactive responses. To test for antitumor immunity *in vivo*, we first examined reactivity of NKp30-redirected T cells to K562 cells engrafted into NSG mice and observed clear reduction of tumor burden. Moreover, first studies revealed, that adoptive transfer of 1 - $5x10^6$ HLA-matched, NKp30-redirected $CD3^+$ T cells into NSG mice engrafted with patient derived AML blasts for up to 5% thus resembling minimal residual disease at time of transfer resulted in significant leukemia regression. Further experiments to elaborate the therapeutic efficacy and what extent $CD4^+$ and CD8 T cells contribute to this antileukemic immunity are in progress.
Summary/Conclusions: These studies demonstrate that human T lymphocytes can be successfully redirected to acute leukemia by NK cell activating receptor based CARs such as the NKp30-CAR. As its ligand B7H6 has not been reported to be expressed on $CD34^+$ HSC, this antigen might be an interesting target for adoptive immunotherapy to AML.

P362

TARGETING CD123+ ACUTE MYELOID LEUKEMIA CELLS WITH REDIRECTED NATURAL KILLER CELLS

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Background: The utility of immune cell modification via chimeric antigen receptors (CARs) has been demonstrated clinically for T cells (*e.g. versus* $CD19^+$ lymphatic leukemia) and is also currently investigated for natural killer (NK) cells. The observation that CD123 is highly expressed on the surface of many primary human acute myeloid leukemia (AML) cells makes CD123 a clinically interesting target.
Aims: The aim of this work was to engineer NK92 cells to express a CAR that detects and eradicates $CD123^+$ AML cells.
Methods: NK92 cells, an NK cell line, were transduced with state-of-the-art alpharetroviral self-inactivating (SIN) vectors encoding EGFP alone as control or a third generation CAR engineered with an anti- CD123 single chain variable fragment (scFv) (with or without an HA-tag) and containing the CD28 transmembrane domain, the 4-1BB costimulatory domain, the CD3ζ signaling domain and an internal ribosomal entry site (IRES) element for EGFP expression. EGFP, CD123-CAR and HA-CD123-CAR protein expression were quantified by flow cytometry and verified by immunoblotting after separation by SDS-PAGE. The kinetics of cytotoxicity elicited by modified NK92 cells was monitored over 48 hours at effector:target ratios of 5:1 and 10:1 and quantified by flow cytometry.
Results: Transduction efficiencies were 76% for the EGFP control vector at multiplicity of infection (MOI) of 0.3, 25% for the CD123-CAR vector at MOI of 0.3 and 30% for the HA-CD123-CAR vector at MOI of 1. Transduced NK92 cells were enriched to >95% purity by flow cytometric sorting based on EGFP expression and sorted NK92 cell populations were used for the following experiments. Using an anti-HA antibody, the HA-CD123-CAR was detected in >85% of sorted NK92 cells modified with the HA-CD123-CAR but not in the EGFP controls or in cells modified with the CD123-CAR lacking the HA-tag. The CD123-CAR was detected with a goat-anti-human IgG antibody (approximately 60% of sorted cells), but the HA-CD123-CAR was less efficiently recognized with this antibody (approximately 30% of sorted cells). Untransduced NK92 and EGFP-transduced NK92 cells served as negative controls for antibody detection. Flow sight analysis provided evidence that the HA-CD123-CAR was expressed on the surface of NK92 cells. SDS-PAGE and immunoblotting experiments further demonstrated HA-CD123-CAR and EGFP transgene expression in the respective samples. Transduced populations were stably cultivated and expanded for several months. Compared with the EGFP-modified NK92 cells used as a control, CD123-CAR- and HA-CD123-CAR-modified NK92 cells exhibited greatly enhanced cytotoxicity against the $CD123^+$ AML target cell line KG1a upon coincubation at effector:target ratios of 5:1 and 10:1. The AML-specific cytotoxicity of NK92-CD123-CAR cells is currently being confirmed with primary AML cells.
Summary/Conclusions: We demonstrate efficient modification of NK92 cells with a clinically relevant CAR designed to target CD123-expressing cells. Importantly, CD123-CAR modified NK92 cells exhibited greatly improved ability to eliminate $CD123^+$ AML cells. Thus, our results support the continued investigation of NK cell redirection with molecules designed to target AML-specific antigens.

P363

PRECLINICAL EVALUATION OF CHIMERIC ANTIGEN RECEPTOR LYMPHOCYTES MODIFIED BY SLEEPING BEAUTY TRANSPOSON FOR THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Chimeric antigen receptor (CAR)-modified T-cell adoptive immunotherapy is an emerging therapeutic option proven effective in the treatment of hematological malignancies. However, the success of CAR-engineered T cells strictly depends on the optimization of several critical parameters related to cell manufacturing and gene therapy.
Aims: We sought to develop a novel gene-modification protocol to engineer cytokine-induced killer cells (CIKs) with CD19 CAR using the *Sleeping Beauty* (SB) transposon system.
Methods: With an improved SB transposon platform, we genetically modified CIK cells to express the CAR specific for acute lymphoblastic leukemia (ALL) CD19+ blasts and evaluated their preclinical efficacy and safety.
Results: The non-viral transduction protocol combined to the large scale production process minimally affected the phenotype of the CD19 CAR CIK-cell final product. Stable expression of CD19 CAR (average 60%) was achieved together with an efficient T cell expansion suitable for clinical application. Furthermore, modified cells displayed persistence of cell subsets with memory phenotype, specific and effective anti-tumor activity. Upon comparison with conventional T-SB platforms, our method achieved superior results in terms of expansion, CAR expression and functionality. Adoptive transfer of CD19.CAR lymphocytes led to a significant antitumor response *in vivo*. CD19.CAR CIK cells also controlled leukemia in xenograft models of human ALL, bearing the high-risk features of MLL–ENL and Ph-like (PAX5/AUTS2) gene rearrangements. Frozen/thawed CD19.CAR CIK cells remained active *in vitro* and *in vivo* with efficacy comparable with that of fresh CIK cells. Furthermore, NOD-SCID-γchain-/- (NSG) mice were treated with CD19.CAR CIK cells to evaluate general toxicity, tissue damage, and biodistribution. Notably, we found no evidence of integration enrichment near cancer-related genes and transposase expression in the final cell product.
Summary/Conclusions: Taken all together, our findings describe a novel donor-derived non-viral CAR approach characterized by efficient cell transfection and expansion that may widen the range of applications of T cell-based immunotherapy. We are currently designing a phase I/II study for relapsing and remitting ALL after Hematopoietic Stem Cell Transplantation (HSCT).

P364

CORD BLOOD DERIVED NK CELLS TRANSFER HISTONE H2AZ TO MULTIPLE MYELOMA CELLS CAUSING DIRECT AND INDIRECT CYTOTOXICITY

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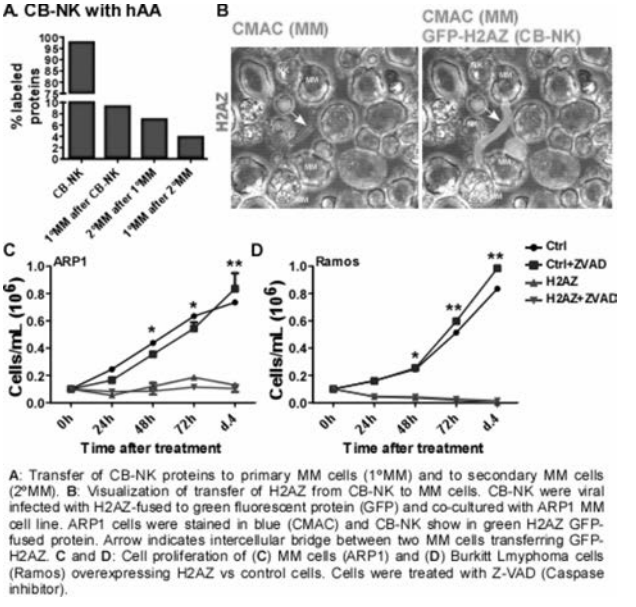
Background: Cord blood derived natural killer cells (CB-NK) are a feasible source of NK cells to use in immune cell therapy for hematological malignancies. We previously showed that CB-NK exert a transmissible cytotoxicity towards multiple myeloma (MM) cells, which involves lipid-protein vesicle transfer between initial MM cells exposed to CB-NK (primary MM cells) and the recipient MM cells exposed to primary MM cells (secondary MM cells).
Aims: We aimed to determine which proteins with cytotoxic activity were transferred from CB-NK to primary MM cells, and from primary to secondary MM cells.
Methods: CB-NK and MM cells (ARP1) were labeled with heavy aminoacids (hAA) in independent experiments, then they were co-cultured and FACS sorted before we performed TRANS-SILAC proteomics to identify proteins transferred from CB-NK to MM cells and from MM to CB-NK. Transfer of these proteins was validated by confocal fluorescence microscopy. Functional assays by lentiviral overexpression of these proteins were performed to evaluate cytotoxic effects in CB-NK and MM cells.
Results: We demonstrated CB-NK protein transfer between primary and secondary MM cells, which represents a dilution of the cytotoxic CB-NK material from primary to secondary MM cells (Figure 1A). Interestingly, under the presence of CB-NK, primary MM cells transfer a high amount of their own proteome to CB-NK and to secondary MM cells. A high number of Histone proteins were on the list of transferred proteins. We focused on the functional role of Histone *H2AZ* variant 1 which was transferred from CB-NK to primary and then to secondary MM cells. Time lapse *in vivo* confocal microscopy showed the direct and secondary transfer of H2AZ between MM cells associated to cell death. This transmission occurred either through vesicles or large intercellular structures (Figure 1B). H2AZ overexpression in CB-NK increased CB-NK cytotoxicity against MM cells. Consistently, H2AZ induced overexpression was lethal not only in MM cells but also in Burkitt Lymphoma (Ramos) and acute promyelocytic leukemia (HL60) cell lines. This cell death was caspase independent (Figure 1C-D). H2AZ-mediated cytotoxicity was also transmissible from H2AZ-overexpressing MM cells to neighboring MM cells causing cell death in this secondary MM population.



A: Transfer of CB-NK proteins to primary MM cells (1°MM) and to secondary MM cells (2°MM). **B**: Visualization of transfer of H2AZ from CB-NK to MM cells. CB-NK were viral infected with H2AZ-fused to green fluorescent protein (GFP) and co-cultured with ARP1 MM cell line. ARP1 cells were stained in blue (CMAC) and CB-NK show in green H2AZ GFP-fused protein. Arrow indicates intercellular bridge between two MM cells transferring GFP-H2AZ. **C** and **D**: Cell proliferation of (**C**) MM cells (ARP1) and (**D**) Burkitt Lmyphoma cells (Ramos) overexpressing H2AZ vs control cells. Cells were treated with Z-VAD (Caspase inhibitor).

Figure 1.

Summary/Conclusions: Our findings reveal a novel mechanism of malignant cell cytotoxicity performed by NK cells which is caspase independent and mediated by histones. We demonstrate the relevance of cell-cell communication mediated by intercellular structures as a mechanism leading to MM cell death. Molecules transferred from CB-NK to MM cells can lead to the discovery of novel therapeutic targets such as H2AZ in hematological malignancies.

P365

ALLOGENEIC TCRA/CD38 DOUBLE KNOCKOUT T-CELLS BEARING AN ANTI-CD38 CHIMERIC ANTIGEN RECEPTOR: AN IMPROVED IMMUNOTHERAPY FOR THE TREATMENT OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND MULTIPLE MYELOMA

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Background: Adoptive immunotherapy with autologous T-cells expressing chimeric antigen receptors (CARs) targeting CD19 has achieved long-term remissions in patients with B-cell leukemia, pointing out that CAR technology may become a game changer in cancer treatment.
Aims: In the present study we have assessed the feasibility of CAR-mediated targeting of the CD38 antigen, which is highly expressed on tumors cells from most patients with acute lymphoblastic leukemia (T-ALL) and multiple myeloma (MM). However, expression of CD38 on normal activated T-cells is a significant hurdle for the development of CAR T-cells against this protein, since antigen-expressing T-cells will be targeted, potentially preventing the efficient production of anti-CD38 CAR T-cells.
Methods: To circumvent this issue we have used Transcription Activator-Like Effector Nuclease (TALEN®) gene editing technology to inactivate the CD38 gene (CD38 KO) in T-cells, prior to transduction with a lentiviral vector encoding an anti-CD38 CAR. To validate this approach, we have examined the capacity of CD38 KO cells expressing an anti-CD19 CAR to eliminate CD19+ cells in order to determine if the absence of CD38 has an impact on T-cell activity. Experiments in an orthotopic Burkitt's lymphoma mouse model showed that CD38 disrupted T-cells expressing anti-CD19 CAR were able to mediate an *in vivo* anti-tumor activity similar to unmodified T-cells expressing an anti-CD19 CAR. These results demonstrate that T-cells lacking CD38 are capable of mediating efficient *in vivo* anti-tumor activity.
Results: Gene editing technology can also be used to manufacture T-cells from healthy donors to generate allogeneic "off-the-shelf" engineered CAR+ T-cell-based frozen products. We have previously demonstrated that TALEN® mediated inactivation of the TCRa constant (TRAC) gene can be achieved at high frequencies and eliminates the potential for edited T-cells to mediate Graft

versus Host Disease (GvHD). Furthermore, multiplex genome editing can lead to the production of double KO (TRAC and CD38) T-cells, allowing large scale manufacturing non allo-reactive CD38 specific T-cells. We will present data demonstrating that such gene-edited anti-CD38 CAR T-cells can be efficiently produced and display high cytotoxic activity.
Summary/Conclusions: Thus, large-scale manufacturing of allogeneic, non-alloreactive CD38 specific T-cells from a single healthy donor could offer the possibility of an off-the-shelf treatment that would be immediately available for administration to a large number of T-ALL and MM patients.

P366

OPTIMIZED AAV-MEDIATED HUMAN FACTOR VIII GENE THERAPY IN HEMOPHILIA A MICE AND CYNOMOLGUS MACAQUES

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Background: Hemophilia A is an X-linked bleeding disorder driven by a deficiency in human coagulation factor VIII (hFVIII), which occurs at a frequency of 1 in 5,000 live male births. The bleeding manifestations of hemophilia, which directly correlate with circulating FVIII activity, can result in significant disability or even death. Protein replacement therapy is effective in maintaining a mild or moderate phenotype. However, infusion frequency remains burdensome to patients. AAV-based gene therapy has the potential to provide long-term improvement of the disease phenotype following a single administration of vector.
Aims: In an effort to optimize expression of hFVIII for the treatment of hemophilia A, an extensive study was performed combining liver-specific promoter and enhancer elements with a codon-optimized human B-domain-deleted hFVIII transgene.
Methods: Due to the large size of the FVIII coding sequence, it is critical for gene expression control elements to be as short as possible while retaining hepatocyte-restricted transcription. Therefore, several strong liver-specific promoters were shortened and combined, with combinations consisting of up to three liver-specific enhancer sequences, to generate 42 enhancer/promoter combinations. These 42 liver regulatory gene cassettes were then packaged into the AAVrh10 capsid and tested in FVIII KO mice. Following intravenous (IV) administration of 10^{10} genome copies (GC), mice were bled every 2 weeks to follow hFVIII activity and antibody generation to the transgene.
Results: At week 2 post-injection, mice showed a range in hFVIII activity from 0.12-2.12 IU/ml. FVIII KO mice developed antibodies to hFVIII at week 4, and by week 8, mice in most of the 42 vector groups had detectable anti-hFVIII IgG levels. Based on the FVIII KO mouse studies and a small pilot rhesus macaque study, 2 of the original 42 enhancer/promoter combinations were selected for further evaluation in cynomolgus macaques, using 2 different Clade E capsids for expression. Each of the 4 vector combinations were administered IV at a dose of $1.2x10^{13}$ GC/kg into 5 macaques per group. With one capsid plus enhancer/promoter combination, peak expression of 37% of normal FVIII levels was seen at week 2 post-vector administration, which then plateaued at 20% of normal. While antibodies to the hFVIII were detected in the majority of macaques by week 8, antibodies remained undetectable in 2 animals at week 30 post-vector administration.
Summary/Conclusions: The use of an AAVrh10 vector to deliver a codon-optimized, B-domain-deleted hFVIII transgene results in a substantial increase in FVIII expression in both FVIII KO mice and cynomolgus macaques. The majority of animals generated an anti-FVIII antibody response by week 8 post vector delivery. However, 2 of the macaques dosed were free of a detectable antibody response through week 30. Overall, our study supports the continued development of AAV-based gene therapeutics for hemophilia A.

P367

A DOSE-ESCALATING PRECLINICAL STUDY TO DETERMINE THE SAFETY, EFFICACY, AND MINIMUM EFFECTIVE DOSE OF A CLINICAL CANDIDATE VECTOR IN A MOUSE MODEL OF HEMOPHILIA B

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Background: While safe and effective recombinant FIX therapeutics are currently available, patients with Hemophilia B may gain additional clinical benefit from longer-acting therapeutic options. The use of rAAV-based gene therapy to obtain long-term expression of a human FIX (hFIX) transgene may be ideal in this setting. In recent years, academic institutions have begun to generate promising clinical data in this area. However, there is an ongoing need to replicate and extend these results in a commercial setting in order to allow such new therapies to become broadly available to patients.
Aims: Dose-dependent inflammatory responses that impact transgene expression have been reported in clinical trials with rAAV FIX vectors. This highlights the need for clinical rAAV vectors to have the highest possible potency, allowing the overall vector dose to be reduced. The goal of this study is to investigate the potential of a clinical candidate AAV vector to achieve potent, safe, and effective levels of hFIX expression in a mouse model of Hemophilia B, while also mapping the vector's minimum effective dose (MED).
Methods: We have developed a potent AAV serotype rh10 vector containing codon-optimized human FIX (hFIXco) cDNA under the control of a highly active liver-specific promoter with the goal of achieving approximately 10% of normal FIX levels in patients. In preparation for a clinical trial, we tested this AAVrh10.hFIXco vector for aspects of efficacy and safety in the factor IX knockout (FIX-KO) mouse model of Hemophilia B.
Results: A dose-dependent increase in FIX protein expression and activity was observed following intravenous administration at vector doses between $1.6x10^{10}$ and $5.0x10^{13}$ GC/kg. FIX-KO mice that received AAVrh10.hFIXco at $1.6x10^{10}$ GC/kg achieved between 5% and 8% of normal hFIX expression by 2 weeks after dosing and maintained this level for the 90-day study period. In comparison, with a dose of $5.0x10^{10}$ GC/kg, FIX-KO mice achieved between 30% and 42% of normal hFIX expression by day 14, which was also maintained for the 90-day study period. From these results, we can estimate that the vector dose required to achieve the stated goal of 10% of normal FIX expression levels would be between $1.6x10^{10}$ GC/kg and $5.0x10^{10}$ GC/kg. Levels of hFIX antigen expression correlated well with measured hFIX activity levels, where doses between $1.6x10^{10}$ GC/kg and $5.0x10^{10}$ GC/kg corresponded with 8% to 35% of normal hFIX activity. FIX-KO mice also provide an excellent model to examine the effects of AAVrh10.hFIXco on hemophilia B-related complications, where mice are prone to spontaneous bleeds and often suffer from fatal hemorrhages after handling or trauma. In this study, 4 out of 7 animals in the Day 90 control group succumbed to hemophilia-related complications. In contrast, FIX-KO mice receiving a single dose intravenous administration of AAVrh10.hFIXco at doses of $1.6x10^{10}$, $5.0x10^{10}$, $1.6x10^{11}$, $5.0x10^{11}$, $5.0x10^{12}$, and $5.0x10^{13}$ GC/kg showed no test article-related mortality, clinical pathology, gross pathology, or histopathologic findings.
Summary/Conclusions: Overall, our study demonstrates the safety, efficacy, and MED for a clinical candidate AAVrh10.hFIXco vector in FIX-KO mice. These findings support the progress of a gene therapy based treatment for Hemophilia B in its transition to clinical use.

P368

THE LCR-FREE GAMMA-GLOBIN LENTIVIRAL VECTOR COMBINING TWO HPFH ACTIVATING ELEMENTS CORRECTS MURINE THALASSEMIC PHENOTYPE *IN VIVO*

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Background: The β-thalassemias result from reduced or absent expression of the β-chain of adult hemoglobin (HbA;$\alpha_2\beta_2$) causing precipitation of excess α-chains and eventually apoptosis. Thus, factors that reduce the degree of chain imbalance such as an innate ability to increase fetal hemoglobin (HbF;$\alpha_2\gamma_2$), as in the HPFH phenotype, have an ameliorating effect on the disease. Hence, gene therapy of β-thalassemia based on γ-globin addition via viral vectors, displays a considerable advantage.
Aims: In this study, we assessed the efficiency of our previously generated LCR-free γ-globin self-inactivating vector GGHI (Papanikolaou *et al.* Hum Gene Ther 23:15-31, 2012) to correct the thalassemic phenotype *in vivo* in the $Hb^{th3/+}$ C57BL/6J mouse model (thal3 model).
Methods: Recipient mice aged about 12 weeks, were treated with the myelosuppresant factor busulfan, while donor mice, of the same age and of the opposite gender, were treated with 5-fluorouracil, prior to transplantation. Total bone marrow was isolated from 5-fluorouracil–treated donors and was transduced with GGHI in X-VIVO™ medium containing cytokines (mIL-1a, mIL-3, mIL-6 and mSCF) at an MOI=30, employing the spinoculation method. The transduced cells were then transplanted via tail vein intravenous injection to the recipients. To evaluate the therapeutic effect of GGHI, blood was collected from recipient mice prior and post transplantation for 4 months, hemoglobin levels (g/dl), hematocrit and total red blood cell count were assessed by a hematological analyzer. The expression of human γ-globin in peripheral blood was assessed by flow cytometry using an anti-HbF monoclonal antibody.
Results: Our results documented that transplanted thalassemic mice (n=4) with GGHI-corrected hemopoietic stem cells, exhibited an increase in hematocrit values by 22.3% (ranging from 24.5% to 35.7%, p=0.02) with a concomitant increase in hemoglobin levels, reaching an average of 11.1 g/dl in transplanted mice *vs* 8.8 g/dl to those prior to transplantation, which corresponds to a 25.5% increase (p=0.008). Human γ-globin was detected in the peripheral blood of all transplanted animals by flow cytometry and ranged from 20 to 45%. Transduction efficiency in these experiments was estimated to be 35-50% as assessed *in vitro* in CFUs by PCR for vector-specific sequences.
Summary/Conclusions: In summary, these results demonstrate for the first time that viral-mediated globin gene transfer via an LCR-free γ-globin lentiviral vector in hemopoietic stem cells effectively corrects a severe hemoglobin disorder.

P369

BALANCE OF ANTI-CD123 CHIMERIC ANTIGEN RECEPTOR (CAR) BINDING AFFINITY AND DENSITY IN AN *IN VITRO* MODEL OF ACUTE MYELOID LEUKEMIA

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Background: Chimeric Antigen Receptors (CARs)-redirected T lymphocytes are a promising novel immunotherapeutic approach, nowadays object of accurate preclinical evaluation also for the targeting of Acute Myeloid Leukemia (AML). In this context, we recently developed a CAR against the CD123, overexpressed on AML blasts and leukemic stem cells (LSCs). However, the potential recognition of low CD123-positive healthy tissues, through the so called "on-target-off-organ" effect, limits the safe clinical employment of CAR-redirected T cells. In order to address this issue, among the CAR design variables under investigation, it has been found that CAR-T cell functional profiles can be modulated by tuning the binding affinity to the target antigen, in an extremely context-dependent manner.
Aims: We aimed therefore to unravel how the interplay existing between CAR affinity, antigen density and CAR expression could impact on anti-CD123 CAR-redirected effector cells efficacy against leukemic cells and safety towards healthy cells.
Methods: We developed a novel integrated model for the functional screening of *in silico*-selected CAR mutants, starting from predicted antibody binding properties, that are then *in vitro* validated based on CAR-redirected T cell related biological requirements. We therefore generated and tested anti-CD123 lower CAR affinity mutants by single residues substitution on the wt CAR. In this way, the affinity mutants produced should provide the same binding site as the original molecule, representing the binding properties variation the only variable in the system.
Results: In particular, exploiting a panel of anti-CD123 CAR affinity mutants, we defined both "lytic" and "activation" antigen thresholds showing that, while the early cytotoxic activity is not affected neither by CAR expression nor by CAR affinity tuning, the CAR expression represents the main variable impairing later effector functions. In view of a future clinical translation of this approach, the lowest affinity mutant suggested a potential threshold of affinity below which, even if the safety profile is preserved, the antileukemic efficacy could be impaired.
Summary/Conclusions: Overall, the full dissection of all these variables offers additional knowledge for the proper design of suitable anti-CD123 CAR-based approaches for the treatment of AML.

Red blood cells and iron - Clinical 1

P370

PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) OF GBT440, A NOVEL HEMOGLOBIN S (HBS) POLYMERIZATION INHIBITOR FOR THE TREATMENT OF SICKLE CELL DISEASE (SCD), IN HEALTHY VOLUNTEERS AND SCD PATIENTS

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Background: SCD is caused by a point mutation in the β-globin gene producing hemoglobin S (HbS) that polymerizes upon deoxygenation with subsequent formation of sickled red blood cells (RBCs). GBT440 is a novel, orally bioavailable small molecule that inhibits HbS polymerization by increasing the affinity of O_2 to hemoglobin (Hb). Based on this mechanism, a left-shift of the oxygen equilibrium curve (OEC) at p20 or p50 (partial pressure of oxygen that results in 20% or 50% saturation of Hb with oxygen, respectively) can potentially be used as a PD marker of GBT440.
Aims: To determine PK of GBT440 in healthy volunteers (HV) and SCD patients and correlate the GBT440 concentrations in RBCs to p20 and p50 values in these two populations.
Methods: A Phase 1/2, randomized, placebo-controlled, double-blind, single and multiple ascending dose study of the tolerability and PK of GBT440 was conducted in HV and SCD patients (6 treated and 2 placebo subjects in each cohort). Part A of the study consisted of a single ascending dose evaluation in healthy subjects (100 to 2800 mg) and SCD patients (1000 mg). Part B consisted of a multiple ascending dose evaluation for up to 15 days in HV (300 to 900 mg QD) and up to 28 days in SCD patients (700 mg QD). Whole blood and plasma concentrations of GBT440 were analyzed by LCMS. Samples were obtained pre-dose and at various time points post-dose. OECs were measured by a TCS Hemox analyzer and p20 and p50 values were determined.
Results: Both the maximum concentration (C_{max}) and the area under the curve (AUC) increased dose proportionally following single and multiple doses. When a single dose of 1000 mg GBT440 was administered to HV and SCD patients, GBT440 exposure was lower and the half-life ($T_{1/2}$) was significantly shorter in SCD patients relative to HV (Table 1). RBC/plasma ratio were 95:1 and 75:1 for HV and SCD subjects, respectively. The difference in PK is likely due to a lower total Hb in SCD patients to which GBT440 binds, and possibly the more rapid turnover of RBCs. Inter-subject variability at steady state was low in the intended dose range (%CV <15%). A linear relationship between concentrations of GBT440 in RBC ($[GBT440]_{RBC}$) and p20 and p50 for both HV and SCD patients was observed. Due to the biphasic nature of GBT440's OECs, p20 appears to be a more sensitive parameter and has better correlation than p50 within the expected target therapeutic range (<40% Hb modification). p20 correlated well with $[GBT440]_{RBC}$ at ≥0.25 mM (~5% Hb modification; r^2=0.83 and 0.55 for HV and SCD, respectively) and p50 at ≥0.5 mM (~10% Hb modification; r^2=0.48 and 0.40 for HV and SCD, respectively) (Image/Pictures).

Table 1

PK Parameters	HV	SCD
Cmax (µg/mL)	58.2	36.0
AUC0-∞ (µg hr/mL)	6730	2480
T1/2 (hr)	69.1	38.8

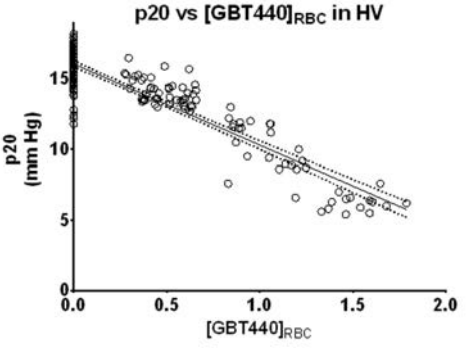

Figure 1.

Summary/Conclusions: GBT440 is an orally bioavailable compound that has potential to be used to treat SCD by once daily administration. The exposure of GBT440 increased proportionally with dose. There was a linear relationship between $[GBT440]_{RBC}$ and the degree of left-shifting of the OEC curve which

demonstrates that GBT440 enhances O_2 affinity of Hb. The high correlation between $[GBT440]_{RBC}$ and the p20 indicates that the change in Hb-O_2 affinity can be used as a PD marker for GBT440.

P371

GBT440, A NOVEL HBS POLYMERIZATION INHIBITOR, INCREASES HB OXYGEN AFFINITY AND RESULTS IN A RAPID IMPROVEMENT IN HEMOLYSIS AND ANEMIA

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Background: Sickle cell disease (SCD) is caused by polymerization of Hemoglobin S (HbS), resulting in hemolysis and vaso-occlusion. No therapy achieving pancellular, direct inhibition of HbS polymerization is available. GBT440 is a novel small molecule which increases hemoglobin oxygen affinity and inhibits HbS polymerization and prevents sickling *in vitro*.
Aims: This study explored safety, pharmacokinetics (PK) and pharmacodynamics (PD) of GBT440 in healthy volunteers (HV) and subjects with SCD and anti-hemolytic and anti-sickling effects in SCD subjects.
Methods: This randomized, placebo-controlled, double-blind, phase I/II study enrolled HV and HbSS and HbSB^0 SCD subjects. GBT440 was dose orally. The study was conducted in three parts: Part A, single ascending doses, Part B multiple ascending doses for 28 days and Part C 90-day dosing (ongoing). The primary endpoint was safety. Secondary endpoints included PK, PD and hematologic response.

Table 1. Biomarker Results

Day 28, or change from baseline	GBT440 500 mg (n=10)	GBT440 700 mg(n=12)	Placebo (n=8)
Hb Modification, d28 (%, mean)	~13	~17	NA
p50 d28 (mmHg, mean)	30	29	34
Hb (g/dL)	0.5	0.7	-0.1
Unconjugated Bilirubin (%)	-31	-43	2
LDH (%)	-20	-12	-7
Reticulocytes(%)	-31	-37	7
P-Selectin(%)	ND	-19	20
Erythropoietin (mU/mL)	-9	-18	28
Sickle Cells Counts (%)	-56	-46	14

ND=Not yet done, data still to be analyzed, NA=not applicable, LDH=lactate dehydrogenase

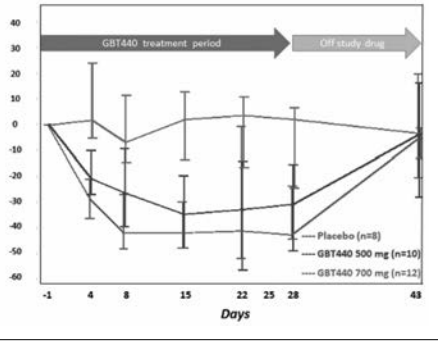

Figure 1. Relative chenge in unconjugated bilirubin from baseline (Median and 25th and 75th percentile).

Results: As of 22 February 2016, 30 SCD subjects had completed Part B (10 at 500 mg; 12 at 700 mg; 8 received placebo [pbo]). The 1000 mg (28 days) and 700 mg (90 days) cohorts in Parts B and C, respectively, were still under evaluation; 60% were male, 40% were female; 20% were on hydroxyurea (HU); 80% had 0-1 painful crisis in the prior year (range 0-7); median age was 32 yrs. GBT440 was well tolerated; there were no drug-related severe or serious adverse events and the most common AEs were headache and pain. PK exposures increased dose-proportionally; mean GBT440 blood half-life after a single dose was 1.6 days in SCD subjects and RBC:Plasma ratio was 75:1. GBT440-treated subjects demonstrated increased hemoglobin oxygen affinity (p50 moved towards the normal range) and a sustained decrease in unconjugated bilirubin, a marker of hemolysis, during the treatment period, with return to baseline by day 43 (Table 1, Figure 1). Other markers of hemolysis, hemoglobin level, sickle cell counts, and erythropoietin levels improved concordantly, as did the inflammatory marker P-Selectin (Table 1, change to day 28). At the EHA conference, additional results, including hemolysis markers and exploratory markers of RBC function, will be presented from Part B 1000 mg and Part C 700 mg cohorts.

Summary/Conclusions: GBT440 was well tolerated with dose-proportional PK and increases in hemoglobin oxygen affinity. GBT440 resulted in a marked and sustained reduction in clinical markers of hemolysis, an increase in hemoglobin, and decreases in erythropoietin, circulating sickle cells, and the inflammatory marker P-Selectin. These results are consistent with inhibition of HbS polymerization leading to decreased RBC damage, improved RBC lifespan, improvement in inflammation and tissue oxygen delivery, and support further investigation of GBT440 as a potential disease-modifying therapy for SCD.

P372

CEREBROVASCULAR RESERVE IN SICKLE CELL DISEASE ASSESSED WITH PERFUSION MAGNETIC RESONANCE IMAGING

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Background: Arterial Spin Labelling (ASL) MRI is a non-invasive perfusion MRI technique that circumvents the need for PET-or-SPECT evaluations of cerebral tissue viability, and allows us to assess haemodynamics in relation to stroke risk in Sickle Cell Disease (SCD). SCD is a hereditary form of anaemia leading to red blood cell damage, hampered oxygen transport and neurovascular tissue damage. Local deficits in perfusion lead to silent ischaemic infarcts as a result of impaired cerebrovascular reserve (CVR).
Aims: Our aim was to gain understanding of cerebral haemodynamics in SCD by utilising ASL MRI. We investigated the acute cerebral blood flow (CBF) response to an administration of acetazolamide (ACZ)-a potent vasodilator-in order to probe CVR in patients with SCD who were in a steady-disease state. We hypothesised that patients would have impaired CVR due to pre-existing increased vasodilation and elevated resting CBF[1].
Methods: 23 SCD patients (aged 33±13y) with HbSS/HbSß^0-thalassaemia, and 6 age- and ethnicity-matched healthy controls (aged 30±14y; Wilcoxon rank sum, p=0.38) were recruited from an outpatient clinic and invited to undergo MRI. Blood samples were drawn from an antecubital vein prior to MRI. ASL-MRI was performed prior to and 10 minutes after ACZ on a 3.0Tesla Philips Ingenia system (Philips Healthcare, Best, the Netherlands). To avoid overestimation of CBF, T1 of blood – a parameter used in the quantification of CBF from ASL, and which is influenced by blood haematocrit(Hct) - was measured in the sagittal sinus with a separate MRI sequence. ASL data were processed with in-house developed software and CBF was quantified using a single-compartment flow model[2]. Wilcoxon rank-sum tests were performed to compare groups on CBF at baseline, and CVR. CVR was calculated as $(CBF_{ACZ}–CBF_B)/CBF_B*100\%$, where CBF_B is baseline and CBF_{ACZ} is post-ACZ CBF. Pearson's correlation was performed between baseline CBF, and Hct and CVR. P<0.05 was considered statistically significant.
Results: Whole brain CBF was significantly higher in patients (mean 55±12 mL/100g/min) compared to controls (mean 31±3 mL/100g/min) at baseline (p<0.001) and was significantly correlated with Hct in all subjects (R=-0.83, p<0.001) and patients alone (r=-0.63, p=0.002). Mean CVR in patients (24±13%) was significantly lower than in controls (69±11%; p<0.001). Figure 1 shows the inverse relationship between CBF and CVR.

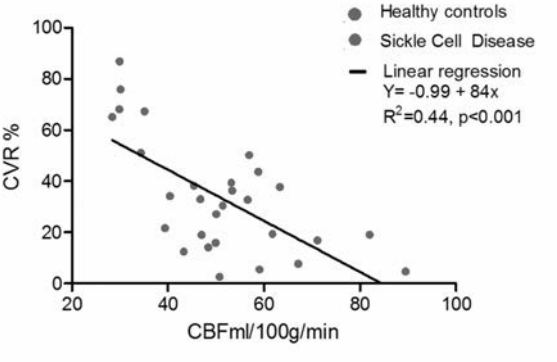

Figure 1.

Summary/Conclusions: The finding that patients had higher CBF at baseline compared to controls complements previous studies in which CBF increases are associated with anaemia in SCD[3]. The finding that Hct was inversely correlated with CBF confirms previous findings in which perfusion increases compensate for anaemia to cater for adequate oxygen delivery. The relatively low CVR in SCD patients is in line with literature suggesting that maximum vasodilation has been reached in SCD. Since this was observed in SCD patients in a steady-state disease course, it is very imaginable that the CVR will become exhausted during times of increased metabolic demand. The results show that CVR could potentially be a cerebrovascular biomarker in SCD for treatment

stratification in the future. In conclusion, ASL-based CBF measurements with ACZ show robust CVR results and indicate that cerebral haemodynamics may be impaired in SCD.

References
1. Nur *Blood* 2009.
2. Alsop *MRM* 2015.
3. Gevers *JMRI* 2012.

P373

RESTING STATE FUNCTIONAL MRI SHOWS HOW COGNITIVE PERFORMANCES COULD BE PRESERVED IN CHILDREN WITH SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) is the most common genetic disorder worldwide. Cerebrovascular complications are frequent events in children with SCD. Overt ischemic stroke occurs in 11% of untreated children and cerebral silent infarcts (CSI) affect 40% of children by the age of 14. In the past years improvements have been made in the management of stroke and CSI and algorithms for screening, prevention and management based on Transcranial Doppler (TCD) and Magnetic Resonance Imaging/Angiography (MRI/MRA) are routinely used. Less progress has been made in the management of cognitive dysfunction, a major morbidity among children with SCD. Impairment of cognitive function is reasonable in children who experienced an overt stroke or present CSIs, due to the anatomical damage of the brain. But the pathophysiology of cognitive impairment in children with normal neuroimaging is less clear. Functional MRI allows the evaluation of resting state brain networks. The Default Mode Network (DMN) is a brain network involving the precuneus and posterior cingulate, the bilateral inferior–lateral–parietal and ventromedial frontal cortex and is invoolved in the cognitive impairment of various diseases
Aims: We aimed at evaluating DMN connectivity by means of resting state functional MRI hypothesizing that neurocognitive scores and parameters linked to cognition in SCD, such as mean steady state hemoglobin and Oxygen saturation (SatO2), could be related to brain connectivity in the DMN.

Table 1

	N° of Patients
Phenotype	
SS	39
Sβ°	1
Gender	
M	21
F	19
Age (years)	8,08 (4,6-15,0)
Transcranial Doppler	
Normal	40
Conditional/Abnormal	0
History of Condition/Abnormal	7
MRI	
Normal	20
Silent Infarcts	20
MRA	
Normal	12
Stenosis	28
Neurocognitive evaluation	
Full scale IQ	
Normal	33
Low	7
Verbal IQ	
Normal	29
Low	11
Performance IQ	
Normal	35
Low	5
Hemoglobin (g/dL)	
≥8	30
<8	10
02 pulse oxymetry	
≥97%	31
<97%	9

Methods: In this cross-sectional study 40 children with SS-Sβ° (mean age 8 years) underwent neurocognitive evaluation and comprehensive brain imaging assessment with TCD, MRI, MRA, Resting State (RS) Functional MRI with evaluation of the DMN. Sixteen healthy age-matched controls underwent MRI, MRA and RS functional MRI. Brain connectivity was compared between patients and controls and analyzed in patients according to TCD, MRI/MRA results, haemoglobin and SatO2 values
Results: Patients characteristics are displayed in Table 1. Children with SCD displayed increased brain connectivity in the DMN compared to controls, even in the absence of alterations in standard imaging techniques. Patients with low neurocognitive scores presented higher brain connectivity compared to children without cognitive impairment or controls, suggesting an initial compensatory mechanism to maintain performances. In our cohort steady state haemoglobin level was not related to increased brain connectivity, but SatO2<97% was (Fig. 1 Functional MRI group analysis. (A) Increased connectivity in the DMN, in the posterior precuneus, in patients *versus* controls; (B) Increased connectivity in the DMN, in the medial prefrontal regions, in patients with normal Verbal IQ compared to controls; (C) Increased connectivity in the DMN, in the right prefrontal region, in patients with normal MRI compared to controls; (D) Increased connectivity in the posterior calcarine region in patients with SatO2≤97% compared to patients with higher SatO2).

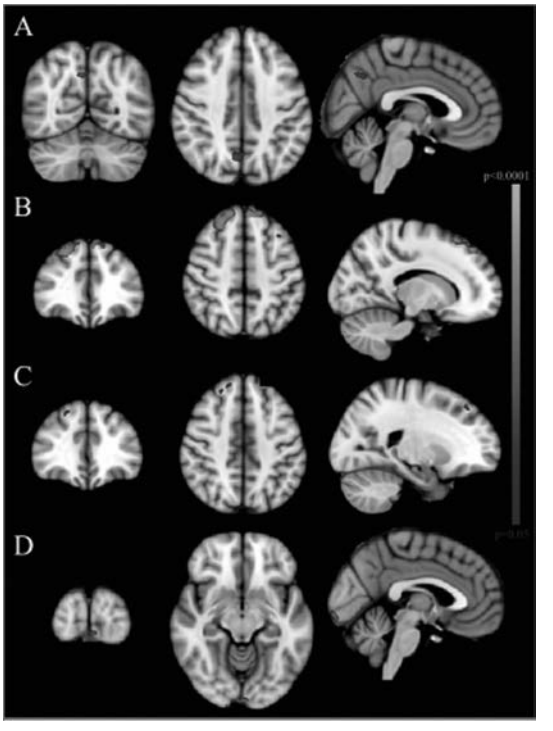

Figure 1.

Summary/Conclusions: Our findings provide novel evidence that SCD is characterized by functional abnormalities in brain connectivity that are independent from the known mechanisms of cerebral vasculopathy and are thus not revealed by routinely used imaging techniques. fMRI might represent a useful complimentary analysis for investigating the disease natural course in studies linking physiopathology, anatomical and functional changes.

P374

INFLUENCE OF UNDERWEIGHT STATUS ON THE CLINICAL PHENOTYPE OF AN INTERNATIONAL COHORT OF SICKLE CELL DISEASE PATIENTS: THE CASIRE GROUP ANALYSIS

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Background: The Consortium for the Advancement of Sickle Cell Research is an international, multi-institutional group evaluating clinical severity of adults and children with SCD through a validated questionnaire and medical chart review. Sites include academic centers in Italy, U.S., and Ghana. Chawla *et al* published on the clinical phenotype of underweight pediatric SCD patients in a U.S. cohort and revealed male gender, older age, lower hemoglobin and SS phenotype were statistically associated with being underweight. Although malnutrition and SCD are both relatively common in parts of Ghana, the impact of comorbid malnutrition on the SCD phenotype has not been published in an international cohort.
Aims: The aim of this study was to determine the relationship between underweight status (<5th percentile body mass index (BMI)) and clinical markers of SCD severity in the CASIRE cohort.
Methods: Subjects were enrolled following informed consent/assent. Categories for underweight, normal, overweight and obese were calculated using the formula: weight (kg)/[height (m)]^2. BMI alone was used to classify adults into weight categories. For children, weight categories were determined using gender- and age-associated BMI percentile charts. Demographic data included age, gender, and country of residence. Clinical data included room air oxygen saturation, hemoglobin phenotype (SS, Sβ0, Sβ+, SC), hemoglobin level, leukocyte count, urine microalbumin, pain crisis frequency, emergency department (ED) utilization frequency, as well as history of leg ulcers, acute chest syndrome (ACS), priapism, stroke, and avascular necrosis (AVN). The relationship between BMI and both demographic and clinical data was analyzed using SPSS statistical software.
Results: There were 676 patients in the study and 56% were under age 18. Patients were equally distributed between developed and developing countries (48% U.S./Italy *vs* 52% Ghana). 16% of the patients were underweight (n=109). Compared to normal, overweight and obese patients, underweight SCD patients were more likely to be male (79% *vs* 52%, *p<0.001*), Ghanaian (83% *vs* 47%, *p<0.001*), report less frequent pain crises/ year (<3/year 82.2% *vs* 64.6%, *p<0.001*), have higher ED utilization for pain (>3/year 34.8% *vs* 21.6%, *p=0.01*), have no prior history of acute chest syndrome (35.5% *vs* 18.7%, *p=0.001*), have a history of leg ulcers (11% *vs* 4.4%, *p=0.005*), higher leukocyte count (12.2 *vs* 10.5 x1000/ul, *p=0.007*), and lower hemoglobin (8.1 *vs* 9.2 g/dL, *p<0.001*). In the U.S./Italy cohort only, underweight BMI was associated with greater microalbuminuria (60.8 *vs* 25.2 mg/gm, *p=0.04*). In the SS and Sβ0 thalassemia group only, underweight patients were more likely to have AVN of hips (25% *vs* 13%, *p=0.02*) or humeral head (13.8% *vs* 4.3% *p=0.03*) compared to non-underweight patients. There was no significant relationship between underweight BMI and age, hemoglobin phenotype, stroke, priapism, or room air oxygen saturation.
Summary/Conclusions: We are the first to describe the clinical severity markers of an underweight SCD patient cohort that includes adults as well as individuals from both developed and developing nations. We have identified that underweight SCD patients demonstrate a distinct clinical phenotype. Although less likely to experience pain crises and develop ACS, they are more likely to have far less clinically apparent complications such as AVN and early signs of kidney damage. The strikingly high prevalence of underweight BMI in the Ghanaian cohort is particularly noteworthy and also warrants further investigation.

P375

IMPROVEMENT IN CARE QUALITY FOLLOWING DEVELOPMENT OF A NEW ADULT SICKLE CELL DISEASE CENTER WITHIN AN ACADEMIC CANCER CENTER

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Background: In the United States, a national shortage of specialized centers with expertise in the management of adults with sickle cell disease (SCD) remains a concerning public health disparity. As a result, poor quality of care is delivered to this vulnerable population as the emergency department emerges as the primary mode of care delivery with little emphasis on chronic disease management. Yet, across the country there is an abundance of cancer centers whose operational infrastructure is not only well-suited to the treatment of the cancer patient, but also can provide medical and procedural care essential to the management of SCD. In light of this, a new adult SCD center was formally established within our academic hospital-based cancer center in 2009.
Aims: To describe the development of the new center and measure its impact on patient volume, acute care utilization patterns, and quality of care delivered.
Methods: A single-institution retrospective chart review was conducted of all SCD patient encounters occurring five years pre- and six years post-SCD center establishment (2004-2015). Programmatic, demographic, clinical as well as hospital utilization and care quality data were compared.
Results: Following establishment of the SCD center, the adult SCD population grew from 22 to 193 patients over six years. Total visits increased from 153 to >2500 per annum. The center was staffed by one primary academic hematologist, shared cancer center nursing staff, and a full-time dedicated social worker. Patients received both preventative visits for chronic disease management including hydroxyurea, scheduled blood transfusions where appropriate, as well as treatment for acute painful episodes with intravenous opioids. Additionally, four programs were developed to enhance care: a formal transition program with the affiliated children's hospital, a specialized red blood cell apheresis program, a fast-track emergency department pain management protocol, and a day hospital for individualized management of acute painful episodes. Post-SCD center establishment, patients experienced increased average annual outpatient preventative visits for chronic disease management (1 *vs* 5) and fewer average hospitalizations yearly (2.4 *vs* 0.8). There was a decrease in hospitalization rates for management of acute pain (50% *vs* 15%), average hospitalization length of stay (12 *vs* 4.95 days), and the proportion of hospital discharges resulting in readmission within thirty days (60% *vs* 26.5%). Ninety percent of children were successfully transitioned to adult care. Hydroxyurea use among eligible patients increased from 30% to 90%.
Summary/Conclusions: We conclude that embedding adult SCD centers within existing cancer center infrastructures can improve patterns of health care utilization and positively impact the delivery of quality care.

P376

KLOTHO GENE POLYMORPHISMS AND THEIR ASSOCIATION WITH CLINICAL MANIFESTATIONS IN PATIENTS WITH SICKLE CELL ANEMIA

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Background: Sickle cell anemia (SCA) is the most common genetic disease worldwide. Although patients with SCA present the same genotype, the clinical course may significantly vary among patients. Several evidence suggest that such heterogeneity occurs due to environmental, social, and genetic factors. Therefore, several studies aim to identify molecular markers that might explain the different clinical courses of patients with SCA. An important gene to be evaluated in these patients is *KLOTHO*. This gene encodes for a transmembrane and secreted proteins involved in many important processes in vascular homeostasis, such as suppression of expression of adhesion molecules, nitric oxide production and suppression of oxidative stress. These points are important in the pathophysiology of SCA, justifying the importance of studying *KLOTHO* on these patients.
Aims: The aim of this study was to investigate the clinical impact of two tag single nucleotide polymorphisms (rs211239, G>A and rs685417, G>A) in *KLOTHO* gene of the 703 patients with SCA followed in a single reference center in northeast Brazil.
Methods: Patients with SCA were divided into two groups: cases (incidence of osteonecrosis, priapism, leg ulcers, stroke, heart disease and/or acute chest syndrome) and controls (patients without occurrence of such events). The entire cohort were genotyped by PCR-RFLP.
Results: The recessive genotype (AA) of polymorphism rs211239 was associated with the number of clinical complications of patients: only 2% of patients with wild type genotype (GG) or heterozygous (GA) had three or more complications and 5% with genotype AA showed this phenotype (P=0.036). For the other variables, no association was found. In contrast, for the polymorphism rs685417, the wild genotype (GG) was significantly associated with higher frequency of vaso-oclusion crises (P=0.009) and heart disease (P=0.033). In addition, heterozygous genotype (GA) was significantly associated with the occurrence of leg ulcers (P=0.006), osteonecrosis (P=0.038) and priapism (P=0.02).
Summary/Conclusions: Our results showed that the over dominant model (GA genotype) of rs685417 is associated with priapism, leg ulcers and osteonecrosis, while the dominant model (GG genotype) is associated with heart disease and higher frequency of vaso-oclusion. Futhermore the recessive model (AA genotype) of rs211239 is associated with the number of complications in SCA patients.

P377

ASSOCIATION BETWEEN ENVIRONMENTAL FACTORS AND HOSPITAL ADMISSIONS FOR SICKLE CELL DISEASE: A RETROSPECTIVE TIME SERIES ANALYSIS

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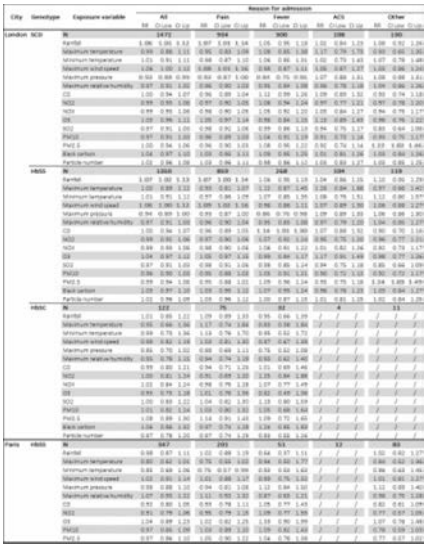
Background: The clinical severity of sickle-cell disease (SCD) is extremely variable. Genetic and genome wide association studies does not entirely account for phenotypic variability. Investigations of the impact of environmental factors, including climate and air quality, on the severity of the disease conducted across a range of countries have provided inconsistent results. Accurate identification of environmental factors triggering clinical complications in urban settings could lead to better prevention measures, which could result in improved quality of life for patients with SCD and their relatives as well as in reductions in hospital admissions and health care costs.
Aims: To investigate the impact of climate change and air quality on the rate of hospital admissions in urban European cities.
Methods: We extracted anonymised daily hospital admission records over 5 years (2008-2012) for patients with (SCD) less than 18 years living within a radius of ten kilometres from each of the following hospitals: Kings College Hospital (Camberwell), Evelina London Children's Hospital (Lambeth) and Royal London Hospital (Whitechapel) in London, and the Necker Hospital for Sick Children in Paris. Recorded reasons for hospital admissions were pain, fever, acute chest syndrome and other. Information on the genotype of patients, either HbSS or HbSC, was available for the three London hospitals, but not in Paris. Official meteorological data of daily rainfall (mm); air temperature (°C), relative humidity (%), wind speed (knots) and pressure (hPa) were extracted from the British Atmospheric Data Centre for several monitoring stations, including Heathrow– the reference station in London - and St James Park– the nearest station to the three hospitals. Data were purchased from Météo France only for one meteorological station, Paris Montsouris (48°49'18"N, 2°20'12"E).
Official daily air quality data on carbon monoxide (CO, mg/m^3), nitrogen dioxide (NO2, µg/m^3), sulphur dioxide (SO2, µg/m^3), ozone (O3, µg/m^3), particulate matter ≤10 µm in diameter (PM_{10}, µg/m^3) and particulate matter ≤2.5 µm in diameter ($PM_{2.5}$, µg/m^3), black carbon (µg/m^3) and particle number (µg/m^3) were extracted from the London Air Data and AirParif websites. Missing data were an issue: we kept only data from the most complete monitoring stations (records available for ≥80% of days) and filled the gaps using an expectation–maximization imputation algorithm for multivariate normal time series implemented in the *mnimput* function of the *mtsdi* R package. Pollutant's measurement data were normalised using a log transformation. Cross-validation based on a left-out sample of 100 daily records were conducted and the root mean squared error (RMSE) and normalised root mean square error (NRMSE) were calculated. All meteorological and air quality data were normalised for the analyses. Statistical differences between admission rates per year, season, month and day of the week were identified by ANOVAs with Tukey's significant difference (HSD) test.
Results: Over the five-year study period, 1,887 and 346 hospital admissions for SCD were recorded in London and Paris, respectively. In London, increased admissions for acute pain in children with SCA was associated with increased rainfall and wind speed, although no such pattern was seen in Paris, where temperature had a significant effect. No air quality effects were seen in either city, and there were no clear environmental effects on admissions for ACS or in children with HbSC disease.

Table 1.



Summary/Conclusions: Using high-quality data from London and Paris, combined with rigorous time-series analysis methods, our results do not support associations between hospital admissions for SCD and temperature. Environmental factors, which consistently appeared significant throughout our analyses, were rainfall, wind speed and pressure. A better understanding of the environmental factors triggering clinical complications in patients with SCD could provide insight into prevention of acute events in SCD.

Infectious diseases, supportive care

P378

MULTICENTER, PROSPECTIVE, RANDOMIZED TRIAL OF INTRAVENOUS ITRACONAZOLE *VS* LIPOSOMAL AMPHOTERICIN B AS EMPIRICAL ANTIFUNGAL THERAPY FOR HEMATOLOGICAL MALIGNANCY WITH PERSISTENT FEVER AND NEUTROPENIA

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Background: Febrile neutropenia, one of the most serious complications that can occur during the treatment of hematological malignancies, is sometimes caused by fungal pathogens. Prospective, randomized studies indicated the utility of liposomal amphotericin B or caspofungin as empirical antifungal therapy. Itraconazole is a broad spectrum triazole antifungal agent, effective against *Candida*, *Aspergillus*, *Trichosporon* and *Mucorales*; however, its oral capsules are poorly absorbed in the intestines, which makes it difficult to achieve a stable serum drug concentration. An intravenous formulation of itraconazole has been developed to allow a sufficient serum concentration to be achieved, which may, therefore, offer a potentially safer and effective alternative.
Aims: The aim of this multicenter, open-label, randomized, non-inferiority trial was to confirm the safety and efficacy of intravenous itraconazole *versus* liposomal amphotericin B as empirical antifungal therapy in patients with hematological malignancies aged 20-79 years, who developed fever refractory to broad-spectrum antimicrobial agents during neutropenia.
Methods: Patients were recruited from 19 institutes in Japan and randomly assigned to either liposomal amphotericin B 3.0 mg/kg once daily group or intravenous itraconazole 200 mg per dose group with five stratification factors (risk, previous antifungal prophylaxis, age, sex and institute). The primary endpoint was defined as success of all components of a five-part composite secondary endpoint (overall favorable response), consisting of successful treatment of baseline infection by the end of treatment, absence of breakthrough infection, no discontinuation of the antifungal treatment due to drug-related toxicity, resolution of fever during neutropenia (axillary temperature <37.5 °C for at least 48 hours), and survival 7 days after termination of the antifungal treatment. The non-inferiority margin for the difference between the experimental and control arms for an overall favorable response was -10%, implying that intravenous itraconazole is non-inferior if the lower limit of the 90% confidence interval of the difference is larger than -10%.
Results: Between April 2011 and February 2015, 103 patients were registered. The study was terminated early due to low patient recruitment. There were no baseline fungal infections in both groups. The mean duration of antifungal therapy was 14.4 days for liposomal amphotericin B (52 patients) and 14.0 days for intravenous itraconazole (50 patients). Overall favorable response rates were 32.7% for liposomal amphotericin B and 36.0% for intravenous itraconazole, with a difference of 4 percent (90% confidence interval, -12% to 20%), which doesn't fulfill the statistical criterion for non-inferiority of intravenous itraconazole. These rates were independent of the antifungal prophylaxis or risk. All patients survived until 7 days after termination of the antifungal treatment. The outcomes were similar between liposomal amphotericin B and intravenous itraconazole with respect to documented breakthrough fungal infections (3.8% *vs* 0.0%, P=0.50), discontinuation of treatment due to drug-related toxicity (19.2% *vs* 10.0%, P=0.26), and resolution of fever during neutropenia (38.5% *vs* 42.0%, P=0.84). Five patients who received liposomal amphotericin B were later diagnosed with probable invasive fungal disease, while none of the patients who received intravenous itraconazole developed fungal infections (P=0.06). Significantly fewer patients in the intravenous itraconazole (14

patients) than the liposomal amphotericin B (29 patients) group had events associated with grade 3-4 hypokalemia (P<0.01). The incidence rates of other adverse events were similar.
Summary/Conclusions: Intravenous itraconazole showed similar efficacy and better safety outcomes compared to liposomal amphotericin B as empirical antifungal therapy in hematological malignancy patients with persistent fever and neutropenia, although its non-inferiority could not be demonstrated due to the small sample size.

P379

PREVALENCE, STRUCTURE, AND PUTATIVE MECHANISM FOR TEMPLATED INSERTIONS IN VDJ RECOMBINATION

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Background: Functional immunoglobulin heavy chain genes are assembled in pre-B cells by genetic VDJ recombination. Random N nucleotides are inserted at the junctions of the participating gene segments by terminal desoxynucleotidyl transferase. Together, combinatorial and junctional diversity create an extremely diverse B-cell repertoire. Recently, insertion of long sequences of a LAIR1 exon template at the V-D junction was described as a novel mechanism to generate broadly neutralizing antibodies to parasital RIFIN antigens on *P. falciparum*-infected erythrocytes (Tan *et al.*, Nature 2016). In these antibodies, the binding specificity of the LAIR1 element was shifted from its natural ligand collagen to RIFINs by extensive somatic hypermutation, indicating passage through a germinal center reaction and antigenic selection.
Aims: To investigate whether templated insertions (TI) occur in healthy donor B-cell repertoires and to elucidate the mechanism behind these insertions.
Methods: We searched for TI in BCR repertoires from 6 healthy donors comprising >32,000 unique VDJ sequences. These repertoires were generated by ARTISAN PCR, an anchored RT-PCR primed on constant immunoglobulin regions and employing a template-switching reverse transcriptase. ARTISAN amplicons were barcoded and analysed as full-length single molecules by PacBio massive parallel sequencing. With its unbiased amplification and an error rate of $0.126x10^{-3}$, ARTISAN PCR in combination with PacBio sequencing accurately displays the entire repertoire of VDJ sequences in complex biological samples (Koning *et al.*, EHA 2015). VDJ sequences (n=719) belonging to the highest percentile with respect to total length, length of the CDR3 region, or combined length of N1 and N2 regions as identified by IMGT V-QUEST were selected. Predicted N regions were submitted to BLAST search with an E cut-off value of 0.00139 to correct for multiple testing.
Results: We searched for TI in BCR repertoires from 6 healthy donors comprising >32,000 unique VDJ sequences. These repertoires were generated by ARTISAN PCR, an anchored RT-PCR primed on constant immunoglobulin regions and employing a template-switching reverse transcriptase. ARTISAN amplicons were barcoded and analysed as full-length single molecules by PacBio massive parallel sequencing. With its unbiased amplification and an error rate of $0.126x10^{-3}$, ARTISAN PCR in combination with PacBio sequencing accurately displays the entire repertoire of VDJ sequences in complex biological samples (Koning *et al.*, EHA 2015). VDJ sequences (n=719) belonging to the highest percentile with respect to total length, length of the CDR3 region, or combined length of N1 and N2 regions as identified by IMGT V-QUEST were selected. Predicted N regions were submitted to BLAST search with an E cut-off value of 0.00139 to correct for multiple testing.
Summary/Conclusions: TI is a novel mechanism of antibody diversification for which we propose the term "T region". Unlike suggested by Tan *et al.*, T regions appear not to originate through repair of double-stranded DNA breaks introduced by activation-induced deaminase in germinal center reactions. Their presence in unmutated VDJ sequences that are presumably derived from naïve B-cells, their exclusive positioning in V-D, D-J, or V-J junctions, and the universal presence of cryptic RSS sites rather point to primary VDJ recombination as the generating event. The combined evidence also suggests that certain loci (*e.g.* the LAIR1 exon) and certain individuals are more prone to generate antibody T regions. We speculate that juxtaposition of IGHV and T region templates is mediated by RAG1/RAG2 via RSS binding during VDJ recombination, followed by introduction of a double-strand break on the IGHV allele only and aberrant repair by using the template in *trans*. As shown by Tan *et al.*, VTDJ sequences may be selected by antigenic stimulation if undergoing somatic hypermutation, and may participate in protective immune responses.

P380

FLUOROQUINOLONE PROPHYLAXIS SIGNIFICANTLY AFFECTS BACTERIAL EPIDEMIOLOLOGY AND ANTIBIOTIC RESISTANCE BUT DOES NOT IMPACT ON INCIDENCE AND MORTALITY OF BLOODSTREAM INFECTIONS IN ACUTE LEUKAEMIA

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Background: The efficacy of fluoroquinolones (FQ) prophylaxis in neutropenic patients, including those with acute leukaemia (AL), has been demonstrated more than ten years ago (Bucaneve 2005). With the recent emergence of multiresistant Gram-negative (G-neg) bacteria its usefulness has been challenged. Indeed, FQ have been associated to an increased incidence of Extended Spectrum Beta-lactamases producing (ESBL+) enterobacteria and carbapenem-resistant (CR) G-neg.
Aims: In order to better understand the impact of FQ prophylaxis on recent bacterial epidemiology, we analyzed all BSI occurring during chemotherapy-induced neutropenia in AL patients treated in 7 centres participating to the regional network Rete Ematologica Lombarda (REL).
Methods: From Dec-12 to Dec-14, all febrile/infectious episodes were prospectively recorded. Data concerning incidence, bacterial epidemiology and resistance, and outcome of BSI occurring during neutropenic phases of chemotherapy cycles were analysed according to the administration or not of FQ prophylaxis (FQ cycles and nFQ cycles, respectively). The number of treatment cycles delivered to AL patients during the period of observation was also requested to each participating centre.
Results: During the period of observation, 472 AL (376 myeloid and 96 lymphoblastic) patients underwent 1356 chemotherapy cycles. FQ prophylaxis was administered in 946 (69.8%) of all cycles. Overall incidence of BSI did not differ with or without FQ prophylaxis, as BSI occurred in 258/946 (27.3%) and in 121/410 (29.5%), respectively (p=0.43). Gram-positive (G-pos) BSI were significantly more frequent with prophylaxis (FQ: 53.1% *vs* nFQ: 38.3%, p=0.0075), whereas G-neg BSI were less frequent, although not significantly (FQ: 37.2% *vs* nFQ: 44.6%, p=0.169), as well as polymicrobic (PMB) BSI (FQ: 9.3% *vs* nFQ: 15%, p=0.1). Fungaemia was rare in both groups (FQ: 1/258, 0.4%; nFQ: 3/121, 2.5%, p=0.06). Among G-pos strains, coagulase-negative staphylococci (CoNS) showed a trend to a higher frequency with FQ (FQ:33.7% *vs* nFQ:24.8%, p=0.07). Considering G-neg, FQ significantly reduced the frequency of Enterobacteria other than *E. coli* (Enterob n/coli) BSI (FQ:5% *vs* nFQ:16.5%; p=0.0002), as well as of *P. aeruginosa* (FQ: 5% *vs* nFQ: 15.7%; p=0.0005). Considering resistant strains among G-neg, FQ significantly increased the proportion of ESBL+ Enterobacteria (FQ: 29.2% *vs* nFQ: 14,8%; p=0.048) whereas the frequency of CR G-neg BSI remained similar in the two groups (FQ:8.3% *vs* nFQ:11.1%; p=0.57). Overall 30-day mortality was 23/379 (5.8%); it was 13/258 (5%) in FQ BSI and 10/121 (8.3%) in nFQ (p=0.22). However, Enterob n/coli and/or *P. aeruginosa* BSI were fatal in 5/10 cases in nFQ group (50%) (15,4%) as compared to 2/13 in the FQ (p=0.07). The 30-day fatality rate of ESBL+/CR G-neg BSI were similar in the two groups (FQ 5/13, 38.5% *vs* nFQ 4/10, 40%).
Summary/Conclusions: Our study shows that FQ prophylaxis did not influence the overall incidence of BSI but it markedly affected bacterial epidemiology. G-pos BSI, particularly CoNS, were significantly increased whereas G-neg BSI by Enterob n/coli and *P. aeruginosa* were significantly decreased, although ESBL+ strains became relatively more common. Overall 30-day mortality was similar in both groups. However, a worrisome 50% mortality due to Enterob n/coli and/or *P. aeruginosa* BSI among nFQ patients suggests extreme caution and the need for close epidemiologic surveillance and controlled studies when considering FQ prophylaxis discontinuation.

P381

VIRAL ENCEPHALITIS AFTER HAPLO-IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION: CAUSATIVE VIRAL SPECTRUM, CHARACTERISTICS AND RISK FACTORS

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Background: Hematopoietic stem cell transplantation (HSCT) is increasingly used to treat hematologic, oncologic, autoimmune, immunodeficiency, and genetic diseases. Up to 70% of patients develop neurological complications after an allogeneic HSCT, of which approximately 25% of patients suffer from more severe neurological complications involving the central nervous system (CNS) and frequent infections, which are associated with high morbidity and mortality. With an allo-HSCT, a recipient's immunocompromised state and the potential for donor-transmitted infections can result in a unique epidemiology of encephalitis, including infection by certain uncommon causative viruses, whichfeatures an increased risk of high morbidity and mortality due to encephalitis regardless of the specific cause compared with immunocompetent individuals. Currently, most reports focus on HHV-6 and limited data are available on the characteristics and independent risk factorsfor viral encephalitis (VE) in patients after a haplo-identical HSCT.
Aims: To retrospectively identify characteristics and risk factors of VE in patients who underwent a haplo-identical HSCT.

Methods: A nested case-control study was designed. Cases with VE and controls matched for years of HSCT and length of follow-up were identified from a cohort composed of 1274 patients who underwent a haplo-identical HSCT from 2012 to 2015.
Results: VE was identified in 30 patients (2.4%, 95% confidence interval 1.6%>3.2%).The median time from HSCT to diagnosis was 144.5 days (range 24–1077 days), but the onset times were lowest in patients with human herpesvirus-6 encephalitis and greatest in patients in which more than one virus was detected.The viruses detected included RSV-B (43.3%), BKV (23.3%), more than one virus (10%), CMV (6.7%), RSV-A (6.7%), HSV (3.3%), HHV-6 (3.3%) and CVB3 (3.3%). Seizures and alterations of consciousness were the most frequently presented symptoms, followed by symptoms, including fever (13 patients, 43.3%), tremor (8 patients, 26.7%), delirium (5 patients, 16.7%), dysesthesia (4 patients, 13.3%), headache (4 patients, 13.3%), paralysis (4 patients, 13.3%), vomiting (2 patients, 6.7%), apathy (1 patient, 3.3%), and irritability (1 patient, 3.3%).Neuroimaging detected abnormalities in 19 (76%) patients,9 of whom showed focal white matter hyperintensity in T2-weighted images and FLAIR, which is consistent with CNS demyelinating diseases. These demyelination abnormalities were detected in encephalitis caused by RSV-B or BKV, with especially high incidence in RSV-B encephalitis (63.6%). The CSF cell count, protein and glucose concentration were elevated in 8 (26.7%), 18(60%), and 9 (30%) patients, respectively. Multivariate analyses revealed that acute GVHD (grad III-IV), CMV viremia and engraftment syndrome were significantly and independently associated with VE. The probability of a sustained response to treatment was 76.7%, but the treatment efficacy and prognoses varied considerably based on the different causative viruses. The cumulative mortality for patients suffering with viral encephalitis was significantly higher than in the controls (p=0.003). Of the 22 patients who survived, 3 (14.3%) continued to exhibit prosopoplegia, spastic paralysis and anterograde amnesia at the last follow-up.
Summary/Conclusions: Acute GVHD (grad III-IV), CMV viremia and engraftment syndrome were associated with an increased risk of viral encephalitis after a haplo-identical HSCT. Characteristics, such as onset time, response to treatment and outcome, varied based onthe different causative viruses.

P382

COMPREHENSIVE MONITORING OF GUT MICROBIOTA DURING THERAPY FOR ACUTE LEUKEMIA

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Background: Colonization and infection by multi-resistant bacteria increasingly challenges the treatment of critical ill patients as well as the infrastructure of care giving facilities. Especially patients with leukemia, where intensive antibiotic therapy is necessary to manage infectious complications during disease and treatment associated cytopenias, are at high risk for acquiring resistant pathogens.
Aims: In this study we longitudinal analyzed stool microbiota of patients with acute leukemia beginning from admission to hospital to obtain insights in the development of dysbiosis and correlated findings with clinical parameters.
Methods: We used a comprehensive strategy including 16s sequencing, quantitative (q) PCR, and cultivation techniques. A total number of 108 stool samples were collected from 3 cohorts: (1) patients undergoing treatment for acute leukemia (n=56), (2) outclinic patients with known multi-resistant bacteria colonization in anal swab (n=26) and (3) healthy controls (HC) (n=26).
Results: All leukemia patients experienced infectious complications requiring antibiotic treatment with at least three different antibiotic regimens. Four out of 14 patients established multiresistent gram-negative bacteria (MRGN) and/or vancomycin-resistent enterococcacae (VRE) in cultures from anal swab during their course of disease. Considering quantitative assessment leukemia patients displayed a significant decreased bacterial load when compared to HC or outclinic patients (5 *vs* 75 *vs* 43ng bacterial DNA /mg stool; p<.001) indicating a decreased colonization resistance as risk factor of acquiring multi-resistant bacteria. Dysbiosis in acute leukemia was characterized by a shift towards enterococcacae including cases with >90% colonization. In contrast outclinic patients and HC showed no relevant enteroccocacae fraction. Furthermore, gram-negative bacteria increased during treatment of leukemia as it was also observed in outclinic patients. Both, enterococcacae and gram-negative pathogens were responsible for infectious complications with proof of the respective bacteria in primary sterile liquids such as blood cultures. In 6/10 positive blood cultures the causative organism was found in gut microbiota before. Interestingly, morbidity of enteroccocacae was only seen in patients with >50% intestinal colonization whereas gram-negative infections also occurred in patients with significant reduced proportion thereof. In some samples collected late during leukemia treatment staphylococcus aureus as well as other staphylococcacae were found. On the other hand protective bacteria such as blautia or prevotella disappeared. In general, a dramatic loss of diversity could be observed. Outclinic patients with known multi-resistant bacteria particularly showed a gram-negative fraction of intestinal bacteria as expression of a sustained dysbiosis.
Summary/Conclusions: During treatment of leukemia a clinically relevant dysbiosis was observed responsible for infectious complications.

P383

INVASIVE FUNGAL INFECTIONS IN LYMPHOPROLIFERATIVE DISORDERS: REVISION OF INCIDENCE IN THE "MODERN TREATMENT" ERA

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Background: Invasive fungal infections (IFIs) are serious, life-threatening complications of hematological malignancies. While epidemiological data on patients (pts) with acute myeloid leukemia have been updated during the past few years, little information is available on IFIs in lymphoproliferative malignancies.
Aims: Aim of the study was to identify the incidence and variety of IFIs, risk factors, prognosis and outcome of IFIs in lymphoproliferative disorders.
Methods: We performed a monocentric retrospective analysis on 1191 adult pts affected by lymphoproliferative disorders [Chronic lymphocytic leukemia (CLL), n=305; Hodgkin lymphoma (HL), n=188; diffuse large B cell lymphoma (DLBCL), n=350; follicular non Hodgkin lymphoma (FL), n=100; and multiple myeloma (MM), n=248] diagnosed and treated in our hospital from 2006 to 2014. We included only probable or proven IFI (*De Pauw, CID 2008*). Data were collected from routine clinical practice in order to provide information regarding fungal infections and in particular epidemiology, diagnosis, treatment and outcome. Clinical data were matched with microbiological (fungal culture or serology) and radiological data.
Results: Thirty-eight patients (3.2%) developed a proven/probable IFI. Invasive Aspergillosis (IA) was the most frequent fungal infection (31 cases, 2.6%), while the other 7 infections were caused by yeasts (0.6%) (Table 1). Fever was present in 84% of patients with IFI and upper respiratory tract symptoms in 47% of patients. Lung was the most frequent organ involved (76%). We recorded five proven infections affecting the gastrointestinal system, blood and urinary tract. IFI was the cause of death in only one patient (2.6%), while 7 patients died from the hematological disease with a concomitant IFI. The prevalence of IFI was highest in pts with MM (5.6%), followed by HL (3.7%) and DLBCL (3.1%). Patients with FL and CLL patients were at lower risk for IFI (2% and 1.3%, respectively) (Table 1) The majority of IFI (25/38, 66%) occurred during first-line treatment, while 13 (34%) IFI occurred in patients treated with salvage regimens for progressive or relapsed disease. Treatment-related factors that were frequently present in patients with IFI were: HSCT within 100 days (47% of pts), corticosteroid treatment (50% of pts) and neutropenia (47% of pts). Restricting the analysis to patients with DLBCL, we observed that the majority of patients (7/11) who developed IFI had a high-risk international prognostic index (IPI) and developed IFI during consolidation phase including HSCT that was our standard approach in these years for patients with young patients with high-risk DLBCL. In a logistic regression analysis, this group of patients had a 6.1-fold risk (OR; 95% CI 1.2-30, *p=0.02*) for IFI with respect to young standard risk DLBCL (<65 years, treated with R-CHOP). In the same line, the group of younger MM pts (<65 years) undergoing HSCT as part of their first-line treatment were at higher risk for IFI than older patients (>65 years) treated without HSCT (OR 15.4; 95% CI 1.9-127.4, *p<0.01*). In contrast, IFI were rare during first-line treatment in patients with FL and CLL who developed IFI more often during salvage treatment for relapsed/progressive disease.

Table 1. Incidence of fungal infections in lymphoproliferative disorders.

UNDERLYING DISEASE	Patients (n)	Incidence IFI (n)	Molds (all IA) (n) Probable/Proven	Yeasts (n) Probable/Proven
CLL	305	4 (1.3%)	1(0.3%) 1/0	3 (1%) 1/2*
HL	188	7 (3.7%)	6 (3.2%) 6/0	1 (0.5%) 0/1*
NHL aggressive	350	11 (3.1%)	8 (2.3%) 8/0	3 (0.8%) 1/2*
NHL Indolent	100	2 (2%)	2 (2%) 2/0	0 (0%) 0/0
MM	248	14 (5.6%)	14 (5.6%) 14/0	0 (0%) 0/0
Overall incidence	1191	38 (3.2%)	31 (2.6%)	7 (0.6%)

* Proven IFI:
Rhodotorula mucilaginosa sepsis (CLL-relapse)
Candida Tropicalis cystitis (CLL-onset)
Candida Norvegensis peritonitis (HL-onset)
Candida Parapsilosis sepsis (NHL-ASCT)
Candida Albicans colitis (NHL-ASCT)

Summary/Conclusions: Treatment strategies in lymphoproliferative disorders that include HSCT in addition to combined treatments of cytotoxic and immunomodulating and immunosuppressive agents can increase the risk of mycoses and require continuous monitoring and revision of the specific risk factors for fungal infections.

P384

INFECTIOUS AND THROMBOTIC COMPLICATIONS IN PERIPHERALLY INSERTED (PICCS) COMPARED TO SHORT TERM CENTRAL VENOUS CATHETERS IN ONCOHEMATOLOGIC PATIENTS: A REL GROUP (RETE EMATOLOGICA LOMBARDA) STUDY

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Background: PICCs are vascular devices inserted in a peripheral vein providing a central venous access with an intermediate duration between jugular or subclavian short term central venous catheters (STCVCs), and long term ones (port-a-caths). Few clinical data from large oncohematologic patients (pts) series are available on infectious and thrombotic complications occurring in PICCs *versus* STCVCs.
Aims: To analyze the results of a large multicenter, retrospective study of the REL group (Rete Ematologica Lombarda-Lombardy Hematologic Network, Italy) aimed at comparing infectious and thrombotic complications in PICCs *vs* STCVCs in oncohematologic pts management.
Methods: Four REL Hematology Centres participated to the study. The clinical data of implanted PICCs and STCVCs from January 2010 to June 2015, were retrospectively collected.
Results: 464 PICCs and 441 STCVCs were compared. Total *in situ* days were 48293 for PICCs and 9471 for STCVCs. Median age PICC *vs* STCVCs: 61 (range 10-88) *vs* 53 years (range 18-99). Underlying diseases in PICCs *vs* CVCs: 199/133 non-Hodgkin's lymphoma, 10 chronic lymphocytic leukemia (PICCs), 110/203 acute myeloid leukemia, 46/54 acute lymphoblastic leukemia, 36/5 Hodgkin's lymphoma, 40/22 multiple myeloma, 11/10 myelodysplastic/ myeloproliferative syndrome, 10 miscellaneous/14 severe aplastic anemias. The median PICC *vs* STCVC life-span was 76/19 days (range 1-800; 1-78; p<.0001). Complications (PICC *vs* STCVC) occurred in 185 *vs* 316 cases (40%, 3.8/1000 cath days *vs* 71.6%, 33.3/1000 cath days); in detail: 97 *vs* 284 infectious (21%, 2/1000 cath days; 64.4%, 30/1000 cath days), 38 *vs* 6 thrombotic (8%, 0.8/1000 cath days; 1.36%, 0.63/1000 cath days), 44 *vs* 24 mechanical (9.5%, 1/1000 cath days; 5.4%, 2.5/1000 cath days) and 6 *vs* 2 miscellaneous (1.2%, 0.1/1000 cath days; 0.5%, 0.2/1000 cath days). Among infectious complications in PICC *vs* STCVC, we reported 18 *vs* 122 cases (3.8%, 0.37/1000 cath days *vs* 27.6%, 13/1000 cath days) of fever of unknown origin (FUO) and 79 *vs* 125 cases (17%, 1.6/1000 cath days *vs* 28%, 13.2/1000 cath days) of BSI. Globally 400/463 (86%) PICCs were removed. Catheter related complications was the cause of PICC removal in 132/464 (28.4%) *vs* 154/441 (35%) STCVC cases. In 55/97 (56.7%) PICCs and in 124/284 (50%) STCVCs (86 infectious and 38 local inflammation), the infectious episode led to catheter removal. Thromboses caused removal in 28/38 (73%) PICCs and 6/6 (100%) STCVCs. Comparing PICCs and STCVCs outcomes, PICCs presented a longer duration (p<.0001), lower total complications rate (p<.0001), lower removal rate for complication (p=.0363), fewer infectious complications (p<.0001), more thromboses (p<.0001).
Summary/Conclusions: Our data suggest that PICCs, as an alternative to STCVCs, are safe and effective in patients at high risk of hemorrhagic and infective complications. In particular PICCs, compared to STCVCs, presented a lower rate of infections (21%, 2/1000 cath days *vs* 64.4%, 30/1000 cath days), and a higher rate of thrombotic complications (8%, 0.8/1000 catheter days *vs* 1.36%, 0.63/1000 catheter days). In particular, in terms of cath days, STCVCs increase in infection complications largely overcome PICCs increase in thrombosis (30 *vs* 2/1000 cath days for infections compared to 0.63 *vs* 0.8/1000 cath days for thrombosis). Therefore we conclude that PICCs compare favorably with STCVCs in terms of complications (in particular infections), and facilitate the proper management of complex and prolonged therapeutic programs in oncohematologic patients.

P385

USEFULNESS OF SERUM 1,3-B-D-GLUCAN FOR DIAGNOSIS OF INVASIVE FUNGAL DISEASE IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RETROSPECTIVE ANALYSIS

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Background: Serologic detection of circulating 1,3-b-D-glucan (BG) fungal biomarker shows promise for improving the diagnosis of invasive fungal diseases (IFDs). Numerous studies have evaluated the diagnostic performance of BG and proved its suboptimal sensitivity when used alone for early diagnosis of IFDs. Moreover, the major limitation of this test mainly in hematological patients (pts.) is the high frequency of false positive results.
Aims: We have evaluated the usefulness of the serum BG assay in real practice for the diagnosis of IFDs in pts. with hematological malignancies.
Methods: We retrospectively analyzed serum BG samples collected from patients with hematological malignancies in high risk of IFDs treated at our department between 2010 and 2014. The Fungitell assay was used for detection of BG. We performed retrospective cross check of probable and proven IFDs recorded in the Fungal INfection Database - FIND. BG results were not used for the diagnosis of IFDs.
Results: Fungitell assay was performed on 1912 serum samples from 630 patients with hematological malignancy (3.0 samples/pt.). Depending on the cut-off used (≥60; ≥80; ≥100; ≥150pg/ml) there were 231 positive BG samples in 78 pts.; 185 samples in 64 pts.; 155 samples in 52 pts. and 108 samples in 33 pts, respectively. Proven and probable cases of IFD occurred only in 49 (21.2%) out of 231 positive samples (in 19 out of 78 patients) with BG ≥60 pg/ml - 11 probable invasive aspergillosis (IA), 4 proven IA, 3 invasive candidiasis and 1 invasive fusariosis. Eight pts. (1.5%) from the group with negative BG samples (<60pg/ml) developed any IFD (6 probable IA, 1 proven IA and 1 invasive trichosporonosis). Depending on the criterion of positivity of BG used (≥60/ ≥80/ ≥100/ ≥150pg/ml) sensitivity was 67.1/63.6/63.1/53.9%, specificity was 90.1/92.9/93.6/95.4%, negative predictive value (NPV) was 98.6/98.6/ 98.6/98.6% and positive predictive value (PPV) was 21.2/21.3/26.5/25.9%, respectively.
Summary/Conclusions: Our study proved high specificity and NPV of serum BG allowing to exclude patients with IFD. On the other hand we confirmed limited sensitivity and mainly very low PPV of the test as its major limitations. We didn't observe any significant differences in sensitivity and PPV between groups with different cut-offs. Thus positive BG assay results in high risk hematological patients should be carefully evaluated together with other clinical and microbiological findings. This work was supported by grant MUNI/A/1028/2015.

P386

DIFFERENT ISOFORM LOCALIZATION GOVERNS SIGNALING QUALITY OF DECTIN-1 IN THE REGULATION OF ANTI-FUNGAL IMMUNITY

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Background: Dectin-1, a C-type-lectin-like receptor, is recognized as a major receptor for fungal ß-glucans and contributes to anti-fungal immunity. Two major human isoforms (isoform A and B) are expressed in human monocyte populations by alternative splicing. They differ by the presence of a stalk region and its N-linked glycosylation site.
Aims: The incidence of invasive fungal infections (IFI) in hematological patients has substantially increased during the last decades. Thus, there is a need for a better understanding of antifungal immunity. Here, we focus on dissecting the specific characteristics of Dectin-1 isoform A and B concerning subcellular localization, ligand binding capacity and protein signaling.
Methods: Expression of Dectin-1 isoform A and B was analyzed in monocyte-derived cells. Cell lines stably expressing Dectin-1 isoform A or B were created and characterized concering localization using flow cytometry and confocal laser scanning microscopy and signaling quality using immune blotting.
Results: While glycosylated isoform A of Dectin-1, was found to be predominantly localized at the cell surface, Dectin-1 isoform B missing a N-glycosylation site was retained intracellularly and remained unglycosylated. Inhibition of using Tunicamycin resulted in efficient abrogation of surface expression of Dectin-1 isoform A. Binding of Zymosan and Syk- and p38- dependent signaling quality of Zymosan stimulated cells expressing Dectin-1 isoform B was drastically reduced compared to cells expressing Dectin-1 isoform A.
Summary/Conclusions: Glycosylation of Dectin-1 plays an important role for surface expression and consequently Zymosan mediated signal transduction. Different expression profiles of human Dectin-1 isoforms on monocyte-derived cells may indicate distinct isoform usage as a cell-type specific mechanism of regulating anti-fungal immunity.

P387

PRELIMINARY REPORT OF FUNGEMIA IN HEMATOLOGICAL MALIGNANCIES FROM SEIFEM-2015 SURVEY

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Background: In the era of mold active azoles prophylaxis, the incidence of invasive fungal infections has varied.
Aims: We evaluated characteristics and outcome of patients (pts) with fungal bloodstream infections (FBSI) during treatment of hematological malignancies (HMs) on a retrospective/prospective study.
Methods: We gathered all consecutive documented FBSI diagnosed between January 2011 and December 2015 in 29 Italian Hematology Departments (both adults and pediatrics), referring to SEIFEM (Sorveglianza Epidemiologica Infezioni Fungine Emopatie Maligne).
Results: We collected 198 cases: M/F 116/82, median age 55 y.o. (range 3-88). FBSI were detected in 82 AML pts (41.4%), 53 (26.8%) NHL, 24 (12.1%) ALL, 14 (7.1%) MM, 13 (6.5%) MDS, 4 (2.1%) MPN, 5 (2.5%) CLL and 3 (1.5%) HL. Regarding the underlying malignancies' treatment at FBSI onset, 64 pts (35.3%) experienced the first cycle of chemotherapy, 55 pts (43.9%) salvage treatment for refractory/relapsed HMs, 21 pts (20.7%) subsequent treatment after complete or partial remission; 58 pts (29.3%) underwent HSCT (16 autologous and 42 allogeneic). Central venous catheter had been placed in 182 pts (92%), urinary catheter in 75 pts (37.8%) and nasogastric tube in 8 pts (4%); 76 pts (38.3%) were supported with parenteral nutrition. Among 114 (57.6%) neutropenic pts at FBSI diagnosis (neutrophils count <500/mmc), 45 (39.5%) recovered at the end of observation period, (median duration of neutropenia 13 days, range 1-100). Use of steroids was reported in 105 pts (53%), with a median duration of 12 days (range 3-106). In 43 pts (21.7%) a skin, oral and/or rectal fungal colonization was detected. FBSI was most frequently caused by yeasts: 158 (79.8%) *Candida* spp. (49 *albicans* and 109 *non albicans*), 10 (5.1%) *Geotrichum*, 5 (2.5%) *Trichosporon*, 6 (3%) *Rhodotorula*, 4 (2%) *Saprochaeta* and 1 (0.5%) *Blastoschizomyces*. Molds have been detected rarely: 12 (6%) *Fusarium*, 1 each (0.5%) *Scedosporium* and *Mucor*. Antifungal prophylaxis was administered in 121 pts: 11 received only topic antifungals. In 101 pts systemic antifungals were administered as follow: 38 (37.6%) posaconazole, 38 (37.6%) fluconazole, 8 (7.9%) itraconazole, 6 (5.9%) liposomal amphotericin B (L-AmB), 8 (7.9%) echinocandins (6 caspofungin and 2 micafungin), 3 (3%) voriconazole. The median duration of prophylaxis was 15 days (range 4-120). The residual 86 pts (43.5%) did not received any antifungals. In 20 pts (10%) no systemic antifungal therapy was administered: 14 of them experienced early mortality. The residual 178 pts were treated as follow: 89 (44.9%) echinocandins (77 caspofungin, 6 anidulafungin, 6 micafungin), 50 (25.2%) L-AmB, 31 (15.5%) azoles (20 fluconazole, 1 posaconazole, 7 voriconazole, 3 itraconazole) and 9 (4.4%) combo therapy (4 posa+L-AmB, 4 caspo+L-AmB, 1 vori+caspo). The median duration on treatment was 12 days (range 2-180). At 30 days from FBSI diagnosis 81 pts (40.9%) were dead: 37 (18.6%) from fungal infection, 19 (9.6%) from HMs, 11 (5.5%) from other causes; in 14 pts (7.1%) we cannot discriminate the cause of death (FBSI *vs* HMs). Of note, 33 dead pts (16.6%) received no antifungal prophylaxis and 6 (3%) received neither prophylaxis or therapy.
Summary/Conclusions: Our study confirms the rarity of FBSI in hematological pts. The most frequently identified strains are *Candida* spp, especially *non albicans*, yet molds are identified not so rarely, *i.e. Fusarium* spp. Although the wider use of broad spectrum azoles prophylaxis and the early institution of antifungal therapy, overall mortality in this setting is still high.

P388

PROGNOSTIC FACTORS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES ADMITTED TO THE INTENSIVE CARE UNIT: EXPERIENCE WITH 335 PATIENTS FROM A SINGLE CENTER

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Background: In the last years, the prognosis of patients with hematological malignancies has improved substantially due to the availability of more effective treatments and better management of complications associated with the disease and treatments, including stem cell transplantation (SCT). Despite this progress, patients with hematologic malignancies still have serious complications that requires admission to the intensive care unit (ICU).
Aims: The aim of this study is to analyze the survival outcomes and identify the prognostic value of different variables of patients with haematological malignancies requiring admission to the ICU at our institution during the past fourteen years.
Methods: Three hundred thirty-five patients (140 F/195 M) with a diagnosis of hematologic malignancy that required admission to the ICU from January 2000 to September 2013 were included. Thirthy-nine percent of patients had undergone SCT, mostly allogenic (73%), and approximately one half of the patients were receiving front-line treatment. The main causes of admission were septic shock (45.7%) and acute respiratory failure (37.6%). The most common hematologic malignancy was acute myeloid leukemia (29.3%).
Results: Overall ICU and hospital mortality were 37.6% and 54.6%, respectively, and a decrease in ICU mortality beyond 2005 (52% 2000-2004, 31% 2005-2008 and 32% 2009-2013, p <0.001) was observed (Figure 1). The variables associated with a worse overall survival in the ICU were being admitted to ICU from the hematological unit, relapsing disease, severe thrombocytopenia, allogeneic SCT, high APACHE II (>19 points) and SOFA (>8 points) score, previous use of antibiotics, use of vasopressors, non-invasive and invasive mechanical ventilation (IMV) and renal replacement therapy. By contrast, the potential myelotoxicity of previous chemotherapy as well as neutropenia had no significant impact. In the multivariate analysis the need of IMV (HR 16.3; p <0.001), renal replacement therapy (HR 3.28; p=0.003), previous use of antibiotics (HR 2.08; p=0.022) and APACHE II score greater than 19 points (HR 1.99, p=0.024) retained their independent prognostic value. Allogeneic SCT was also associated with a shorter overall survival compared to other therapeutic modalities (p<0.001); however the characteristics associated with the transplant (donor type, source of stem cells, conditioning regimen intensity or the development of graft-*versus* host disease) had no effect on survival. The prognosis of patients undergoing acute leukemia induction was similar to those treated with autologous SCT.

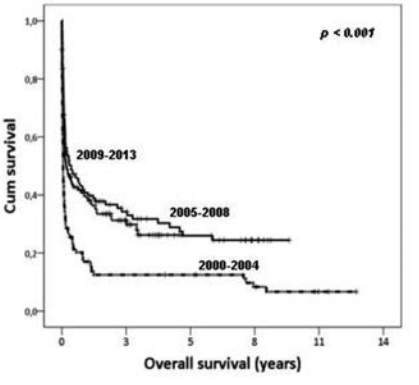

Figure 1. Overall survival of patients admitted to ICU according to the period.

Summary/Conclusions: The survival of patients with hematological malignancies admitted to the ICU has improved in the last years. A previous allogeneic SCT transplantation, the need for mechanical ventilation and renal replacement therapy, prior use of antibiotics and high APACHE II score are factors associated with a worse prognosis in this group of patients.

Transfusion Medicine

P389

SAFETY AND EFFICACY OF HUMAN T-LYMPHOTROPIC VIRUS 1 (HTLV-1) HYPERIMMUNE GLOBULIN AGAINST HTLV-1 INFECTION IN A HUMANIZED MOUSE MODEL

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Background: Adult T-cell leukemia (ATL) is a malignant disease caused by infection with human T-lymphotropic virus 1 (HTLV-1). Prevention of HTLV-1 infection is the most effective strategy for eradicating ATL. However, there is currently no effective vaccine or anti-viral agent for HTLV-1.
Aims: In this study, we aimed to develop a safe and effective HTLV-1 hyperimmune globulin (HIG) by using donations from HTLV-1-positive carriers that were screened at the Japanese Red Cross. Additionally, we assessed the viral safety of HIG during the manufacturing process by spiking the samples with HTLV-1.
Methods: First, we developed two *in vitro* screening methods to evaluate and characterize the anti-viral effect of HTLV-1-positive plasma. We modeled HTLV-1 infection by using an HTLV-1-infected cell line, MT-2, along with Jurkat or SLB-1 cells. We then purified HIG from 30 typical HTLV-1-positive serum samples from the excluded blood donations to the Japan Red Cross, and we evaluated its effect in a humanized mouse (Hu-PBL NOG) model to assess its ability to prevent HTLV-1 infection *in vitro*. Hu-PBL NOG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic) mice were treated with HIG for 5 days before HTLV-1 infection by injection with mytomicine C-treated MT-2 cells ($1x10^5$) 3 days after the mice received human peripheral blood mononuclear cells ($1x10^7$). To observe the viral clearance ability throughout the HIG manufacturing process, we performed a viral clearance study in which we purified HIG from HTLV-1-negative or -positive plasma that had been spiked with $1x10^5$ MT-2 cells.
Results: We found that HTLV-1-positive plasma, defined as plasma isolated from an HTLV-1 carrier with a proviral load above four, can effectively block both HTLV-1 infection and syncytia formation *in vitro*. In *in vivo* experiments, HTLV-1 was detected in the untreated HTLV-1-infected mice, but not in the HIG-treated HTLV-1-infected mice on days 11 and 38 post-infection. HTLV-1-infected cells ($CD3^+/CD4^+/CCR4^+/CD25^+/CD30^+$) could be seen in the spleen, liver, and lungs of the untreated HTLV-1-1infected mice, but no HTLV-1-infected cells were observed in these tissues in the HIG-treated mice, even though their normal T cells ($CD3^+/CD4^+$ and $CD8^+$) were observed. We were still able to detect the inhibitory effect of HIG treatment on HTLV-1 infection when we treated mice with HIG 20 days after the HTLV-1 infection. The results of a viral clearance study show that high log reduction values were obtained during the HIG manufacturing process. We were unable to detect any HTLV-1 genomes in the purified HIG, and there was no HTLV-1 infectivity in the HIG purified from source plasma spiked with $1x10^5$ MT-2 cells.
Summary/Conclusions: We found that HTLV-1 HIG may be an effective and safe therapy for the prevention of HTLV-1 infection.

P390

STABLE AND FULL PRODUCTION OF FETAL HAEMOGLOBIN AFTER BONE MARROW TRANSPLANT FAILURE IN PATIENTS WITH THALASSAEMIA MAJOR AND CLINICAL REMISSION: THE GENETIC BACKGROUND

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Background: High fetal haemoglobin (HbF) levels ameliorate morbidity and mortality in Sickle Cell Anaemia (SCA) and β-thalassaemia. The variability of HbF levels is genetically controlled by multiple genes and recent studies provide new insight into the molecular mechanisms in order to induce the HbF production in adult haemopoietic cells as a promising therapeutic approach to ameliorate the severity high levels of HbF ameliorate the severity of the β -disorders. A strong support to such novel approaches comes from recent clinical observations carried out by our group
Aims: To study the fetal haemoglobin levels and genetic background of three thalassemic patients who underwent bone marrow transplant (BMT) and exhibited high HbF level after BMT failure and autologous reconstitution.
Methods: Out of 292 consecutive patients with β-haemoglobin disorders undergoing allogeneic Bone Marrow Transplant at our institution, we observed 3 BMT recipients who developed the reactivation of HbF synthesis after BMT failure. We analyzed the blood samples of three patients for the genetic polymorphisms associated to increased level of HbF, genomic DNA sequencing, western blotting, electrophoretic mobility shift assays, surface plasmon resonance (SPR) biospecific interaction analysis, bioinformatics analyses based on docking experiment.
Results: Three patients with β°-thalassaemia major underwent BMT and rejected at +40, +90 and +18 days after transplant respectively. The autologous recovery was documented (0% residual donor cells) in all cases. Transfusion therapy was required to support anaemia until +118, +162 and +178 days after transplant respectively. Afterwards the Hb levels were steadily over 10.2 g/dl (range 10.2-11.8 gr/dL) without the use of transfusion support and the Hb electrophoresis revealed HbF 99.8% in all 3 cases. At +93, +82 and +17 months respectively of ongoing follow-up after graft failure, all 3 patients maintain the sustained and full (99.8%) production of HbF and are transfusion-free. (See the table).The genetic analysis documented that all patients were carrier of the non-deletion form of hereditary persistence of HbF. The 3 thalassaemic patients exhibit the homozygosity for the –158 (C->T) point mutation in the G γ promoter sequence and we found in all three a novel polymorphism of the Aγ-globin gene at position +25(G→A). This region belongs to a sequence recognized by DNA-binding protein complexes, including LYAR (Ly-1 antibody reactive clone), a zinc-finger transcription factor previously proposed to be involved in down-regulation of the expression of γ-globin genes in erythroid cells decreasing the efficiency of the interaction between this sequence and specific DNA binding protein complexes.
Summary/Conclusions: Our study showed that the reactivation of HbF synthesis can occur in the adult age and the high levels of HbF provide a therapeutic benefit to the β-disorders. We found a novel polymorphism of the Aγ-globin gene in all three thalassemic patients who failed BMT and developed high levels of HbF. It is likely that the favorable genetic background in these patients concurred the full HbF production.

P391

AUDIT OF TRANSFUSION PRACTICES IN PATIENTS RECEIVING AUTOLOGOUS STEM CELL TRANSPLANTATION AT UNIVERSITY HOSPITALS BIRMINGHAM

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Background: Autologous stem cell transplantation (autoSCT) is an effective consolidation therapy, improving outcomes in a number of haematological conditions. Blood product support is often required to treat anaemia and reduce risk of haemorrhage. However many patients undergoing autoSCT have previously been treated with chemotherapy regimens that do not routinely require support with blood products.
Aims: We examined transfusion practice at our transplant centre to investigate whether patients undergoing autoSCT were receiving potentially avoidable transfusions.
Methods: 121 consecutive patients who underwent autoSCT at University Hospitals Birmingham between 01/09/14 and 31/10/15 were identified using the Bone Marrow and Stem Cell Transplant registry.
Of the 26 patients receiving their pre transplant treatment in our centre, 16 (58%) received their first transfusion during the transplant period. Clinical and laboratory data was collected from these patients from the electronic patient record, and paper records when necessary.
Results: 69% were male, median age 60 years (range 31 to 68 years). Myeloma was the most frequent indication for transplant (81%). There were 40 platelet transfusions delivered to 16 patients, median 3 per patient (range 1-6). Of these, 29 were apheresis and 11 were pooled doses. All were single unit transfusions. There were 13 units of red cells transfused in 6 episodes to 5 patients, median 2 per patient, with one single unit transfusion. Transfusions were delivered a median of 3 days before engraftment (range day -12 to day +53), with 15%, 45% and 75% being delivered within 1, 3 and 5 days of engraftment respectively and a median of 4 days before discharge (range day -9 to day +56), with 15%, 37% and 59% occurring within 1, 3 and 5 days respectively.
23 (57.5%) doses of platelets were assessed to be clinically justified based on the available information. Of these, 11 were for platelet count less than or equal to $10x10^9$/L, 3 were for minor bleeding and the remaining 9 were for prophylaxis during a septic episode to maintain platelet count greater than $20x10^9$/L. The 16 platelet transfusions where no clinical justification was evident amounted to 28 potentially unnecessary donor exposures in 12 patients. 3 of the 6 red cell episodes were justified by haemoglobin less than 80g/L. Two episodes were not assessable due to lack of clinical noting. There were 3 patients who only had a single platelet transfusion, involving 6 donors, and there was no apparent clinical justification in any of these.
Summary/Conclusions: In conclusion we found transfusion practice during autoSCT to be liberal, resulting in high donor exposures, of which at least 39% were potentially avoidable. 12 (75%) patients (who were previously transfusion naïve) were exposed to at least one transfusion which we believe was not clinically justified. The median potentially avoidable donor exposures in these

patients was 1.5 (range 1-6). Strict adherence to recent NICE guidelines and local policy is expected to improve the donor exposure rate in autoSCT, particularly in those not previously transfused.

P392

COAGULATION PROFILE AND OUTCOME OF BLOOD COMPONENT THERAPY IN SNAKE BITE VICTIMS

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Background: Bites from poisonous snakes cause substantial human morbidity and mortality in tropical and subtropical countries. Hemotoxic envenoming is quite common in many parts of India, but only limited data exist on its accurate incidence in the country, and clinical effects. Herein we present findings from an observational study on coagulation profile and outcomes of blood component therapy in patients with hemotoxic snake bites
Aims: To assess the coagulation profile and outcome of blood component therapy in snake bite victims
Methods: We undertook an observational study between January 2013 and June 2014, on patients admitted to the medical ICU of our tertiary hospital, with a history of snake bite and satisfying the inclusion criteria. The patients underwent detailed evaluation of envenoming by clinical and laboratory investigations. The patients were screened by a standard, 20-minute Whole Blood Clotting Time (WBCT) test (repeated four times at 30 min. intervals) and prolongation of at least one test result indicated hemotoxic envenoming. Routine blood counts, Bleeding Time (BT), Prothrombin Time (PT), International Normalized Ratio (INR), activated Partial Thromboplastin Time (aPTT), fibrinogen and D-dimer levels were also monitored. Samples of plasma separated from a citrated blood sample collected at the occurrence of deranged WBCT were flash frozen and assayed for coagulation factors. Blood component therapy and its outcomes were also monitored in the patients.
Results: Among 595 patients reporting with snake bites, 445 were adults. Absence of signs of envenoming suggested non-venomous bites 282 patients. One hundred and sixty three patients manifested features of envenoming, and received Anti-Snake Venom (ASV). Species identification of the biting snake was not possible in 50% of cases. Among the rest, Russel's viper and Pit viper accounted for 66 and 9 cases, respectively. Only the cases where the biting species could be identified were studied further. Delayed treatment resulted in severe local and systemic manifestations in patients. Hematological manifestations developed following bites from Russel's viper, pit viper and unidentified species as well. Acute Renal Failure (ARF) due to Disseminated Intravascular Coagulation (DIC) and capillary leak syndrome developed only in patients with bites from Russels viper, and necessitated hemodialysis. Coagulopathies occurred in most patients with bites from Russel's viper and pit viper. Patients bitten by Russel's viper manifested severe deficiency of Factor V and Factor X, while it did not occur in pit viper bites. Qualitative testing for Factor XIII revealed its deficiency specifically in cases of Russel's viper bite. Most patients with hemotoxic envenomation received transfusion of fresh frozen plasma (FFP), 24 hours after administration of ASV. Severe thrombocytopenia necessitated the administration of platelet concentrate in seven patients. Packed Red Blood Cells (PRBC) was administered in 4 patients with anemia. Blood component therapy significantly increased hospital stay of the patients. No statistically significant complications or mortality occurred after transfusion therapy.
Summary/Conclusions: Rural areas had higher incidence of snake bites. Though BT and CT were sufficient to identify hemotoxicity, costlier and time-consuming assessment of PT, aPTT, serum fibrinogen and D-dimers had more sensitivity. Hematological manifestations occurred following bites from Russel's viper and pit viper, while the former produced major bleeding diatheses and a higher incidence of DIC and ARF. The public need to be made aware of the need for early identification and appropriate treatment of snake bites

P393

IMPACT OF PROTHROMBIN COMPLEX CONCENTRATE (PCC) USE ON TRANSFUSION REQUIREMENTS AND BLOOD LOSS IN CARDIAC SURGERY

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Background: Cardiac surgery is associated with significant coagulopathy and bleeding. This has led to off-label Prothrombin Complex Conccentrate (PCC) use, despite minimal data regarding its efficacy in this setting. There have been retrospective studies investigating use of four-factor PCCs in Europe showing variable efficacy, however the only PCC available in Australia is Prothrombinex-VF, a three-factor PCC containing factors II, IX and X.
Aims: We aimed to retrospectively review the perioperative off-label use of PCCs during cardiac surgery in our institution, to evaluate if PCC use reduced postoperative bleeding or transfusion requirements, and its effect on other clinical and laboratory parameters compared to patients who did not receive PCC.
Methods: A retrospective audit was performed, with the approval of the hospital Research Ethics Committee, of all patients undergoing cardiac surgery from 1st June 2012 to 31st December 2012. Data was collected via the hospital's cardiac surgery database and medical records.
Results: 193 patients underwent cardiac surgery during the study period. 66 (34%) received PCC at a median dose of 22 IU/kg. There was a significant reduction in the INR (medain INR reduced from 1.5 pre-PCC to 1.3 post-PCC), with the change in INR greatest for patients with the highest pre-PCC INRs), but no change to APTT following PCC use. Post-operative blood loss, as measured by the 4-hour chest drain output, was comparable to international studies and did not decrease significantly with PCC (290mL *versus* 270mL, p=0.29). The PCC group also had higher transfusion requirements, including more red blood cells (median 4 units *versus* 0 units), more platelet transfusions (median 2 bags *versus* 0), more Fresh Frozen Plasma (both receiving a median of no units however the PCC group had a greater IQR of 0-2 units), and more cryoprecipitate transfusions (median 3.5 units *versus* 0) (p <0.0001). These results were, however, potentially confounded by longer median cardiopulmonary bypass time and a greater proportion of aortic surgeries in the PCC group.
Summary/Conclusions: A substantial proportion of patients undergoing cardiac surgery are treated off-label with PCCs. This is associated with a reduction in the INR, but no significant difference in the post-operative blood loss or blood product transfusion rate.

P394

COMBINED PLASMA EXCHANGE AND ERYTHROCYTAPHERESIS FOR TREATMENT OF MULTIORGAN FAILURE DUE TO SUSPECTED FAT EMBOLISM SYNDROME IN SICKLE CELL DISEASE

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Background: Fat embolism syndrome (FES) as a consequence of bone marrow necrosis during a vaso-occlusive crisis is a devastating complication of sickle cell disease (SCD) that affects all age groups and phenotypes with a high mortality. Clinically FES is characterised by acute respiratory failure, neurological impairment, cutaneous/mucosal petechiae, acute kidney injury, and hepatic dysfunction. Additional markers that aid diagnosis include hyponatraemia, thrombocytopenia and coagulopathy. Retrospective case reviews of FES in SCD have highlighted the role of red cell exchange transfusion in improving clinical outcome though mortality remains ~30%. These have not differentiated between manual and automated red cell exchange. Previous studies have shown reduction in inflammatory mediators after manual exchange transfusion. Such an effect would not be expected from selective removal of red cells during automated erythrocytapheresis (aRCE) increasingly the modality of choice in specialist haemoglobinopathy centres. Since inflammatory mediators are likely to play a role in its pathogenesis we reasoned that combined plasma exchange (PEX) and erythrocytapheresis could improve the outcome of FES in SCD.
Aims: To report the outcome in adult SCD patients who developed multiorgan failure and met diagnostic criteria for FES treated with combined aRCE and PEX.

Table 1. Clinical details at the onset of MOFS.

	Case 1	Case 2	Case 3
Gender	F	F	F
Age	60	47	33
Genotype	HbS-beta thal	HbS-O Arab	HbSS
WC x10^6/L	2,41	7	11
Hb g/l	66	68	65
Plt x10^6/L	64	48	50
Creatinine (umol/L)	112	122	>110
ALT (iU/L)	72	113	>40
Arterial pO2 (kPa)	7.7	8.2	6.5
Glasgow Coma Score Before PEX	11	9	8
HbS%	28	23	25
Courses of PEX	3	2	1
Outcome at 2 months	Alive	Alive	Alive
Recovery (days)	9	18	16

Methods: A retrospective analysis of 3 SCD patients aged 33-67 years with suspected FES treated at Hammersmith Hospital. Clinical details of the patients are summarized in table 1. Apheresis was performed using the Spectra Optia Apheresis System according to our standard operating procedure with a target HbS of <30% and plasma volume exchanged of 3L. Consecutive aRCE and PEX were performed initially and a decision to repeat PEX subsequently based on clinical response (median number PEX 2;range 1-3). Time to recovery was defined by reversal of neurological and respiratory impairment and normalisa-

tion of platelet count, liver and renal function and coagulation parameters. Overall survival (OS) was assessed 2 months post-treatment with a median follow up of 16 months (range 2-21).
Results: All patients exhibited features of multiorgan failure compatible with the diagnosis of FES. OS was 100% at 2 months. Rapid improvement in neurological status was seen with normalisation of Glasgow Coma Scale within 24 hours of completing PEX. No long-term neurological sequelae were observed. Median time to recovery as defined above was 16 days (range 9-18).
Summary/Conclusions: Based on these favourable preliminary results in SCD patients with multiorgan failure due to suspected FES it is recommended combined aRCE/PEX is evaluated in a larger cohort of patients to determine its efficacy in treatment of this life-threatening complication.

P395

REDUCTION OF ABO GLYCOSYLTRANSFERASE CAUSED BY SYNONYMOUS VARIATION IN B309 ALLELE

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Background: Non-synonymous single-nucleotide polymorphism in the *ABO* gene can affect the enzyme characteristics and protein quantity producing many kinds of ABO blood type subgroups. B3 phenotype including 11 variant alleles reveals characteristic mixed field agglutination. Synonymous variations, formerly called 'silent' mutation, can result in aberrant protein expression and protein folding. Interestingly, *B309* variant (*255C>T* on exon 6 in the background of *B101*) was recently reported to have synonymous SNP (sSNP). We found B309 alleles recurrently in Korean B3 phenotype individuals.
Aims: B309 allele's effect on B antigenic expression was investigated in patients and confirmed by the experimental model.
Methods: Individuals with mixed field agglutination with anti-B were tested by direct sequencing. *B309* allele were isolated and confirmed by TA cloning. cDNAs from exon 5 to exon 7 were amplified and sequenced in B309 individuals to exclude out the possibility of splice variant. *B101* and *B309* coding sequence with *255C>T* were cloned, inserted to the vectors with fluorescent tags and transduced into the HeLa cells that have O/O genotype. The protein expression and antigenic changes by sSNP were evaluated with fluorescent protein expression and flowcytometric analysis.
Results: We found two unrelated Korean individuals and three family members in one pedigree who have *B309* alleles and all of them revealed B3 phenotypes. Exon 6 of ABO transferese gene was not spliced out in their cDNAs. Protein expression and antigenic expression were reduced in B309 variant (the median MFI: 36.85) compared to B101 (MFI: 50.03).

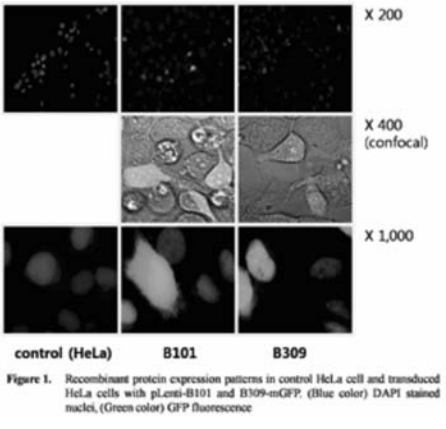

Figure 1. Recombinant protein expression patterns in control HeLa cell and transduced HeLa cells with pLenti-B101 and B309-mGFP. (Blue color) DAPI stained nuclei, (Green color) GFP fluorescence

Figure 1.

Summary/Conclusions: Individuals with *B309* allele revealed B3 phenotypes and their *255C>T* synonymous polymorphism didn't contribute to the alternative splicing. *255C>T* polymorphism was experimentally proven to cause the reduced expression of ABO transferase and the subsequent weakening of B antigens. This phenomenon implies that sSNP could also affect the expression and function of the protein, and finally alter the immunohematologic reactions.

P396

EFFECTS OF IMPLEMENTATION OF A MASSIVE TRANSFUSION PROTOCOL IN A TERTIARY REFERRAL HOSPITAL

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Background: Following the study in 2007 of Borgman et al regarding early plasma product support in trauma patients receiving massive transfusion, the implementation of massive transfusion protocols (MTPs) has become the norm.Aims of utilising a MTP include pre-emptive transfusion with plasma products platelets in an effort to be proactive and overcome the effects of transfusing mostly large amounts of packed red cells on coagulation, in patients with critical bleeding.
Aims: The effect of implementing a MTP in a tertiary referral hospital, for both trauma and non-trauma–related critical bleeding was examined. Parameters include the number of MTP 'episodes' or 'activations', the proportion of 'true' critical bleeding episodes, the ratio of blood products transfused (Red Blood Cells:Fresh Frozen Plasma:Cryoprecipitate:Platelets) and blood product wastage.The appropriateness of platelet transfusion and 28-day mortality were of interest.
Methods: Data regarding the issuing of blood products which was collected by Blood Bank staff at the time of the MTP, as well as computer records regarding laboratory parameters and blood product use was assessed retrospectively.The first dataset period, denoted Period 1(P1), was prior to the implementation of the MTP and the second period (P2) post-implementation.
Results: During P1 (16.5 months), the total number of massive transfusion 'episodes' was 53 and during P2 (7.5 months) there were 59 MTP 'activations'.During P1 the average number of Red cells (RBC), Fresh Frozen Plasma (FFP), Cryoprecipitate(Cryo) and Platelets (Plt) transfused per patient were:15.1, 13.3, 13.6 and 1.5 respectively.4 RBCs or less were transfused in only 3 episodes during P1.During P2 (7.5 months), the total number of activations included 57, and the corresponding average numbers of RBC, FFP, Cryo and Plt per patient were 7.9, 6.9, 7.4 and 0.5.4 RBCs or less were transfused in 27 episodes during P2.The proportion of deaths for the 53 patients during P1 was 28% and during P2 the proportion of deaths was 31.6%.The RBC:FFP ratio in P1 was 1.13:1 and in P2 it was 1.18:1. RBC:Cryo ratio was 1.11:1 for P1 and 1.09:1 for P2. RBC:Plt ratios for P1 and P2 were 10.06:1 and 16.2:1.
Summary/Conclusions: The 2 periods contain similar numbers of patients, however the time periods during which the 'critical bleeding' episodes were recorded differ markedly (16.5/12pre *vs* 7.5/12post).This is mainly due to a large number of MTP activations in which 4 or less RBC units were transfused in the post-implementation period.The high proportion of patients who did not have truly 'massive bleeding' but for whom the MTP was activated (47%), is similar to that previously reported.Correspondingly, the number of blood products transfused per patient was much higher in the pre-MTP protocol period. A cardinal reason for the wastage of blood products is that in patients who are severely injured, the MTP is activated after they have undergone an initial assessment and plasma products are thawed, however they succumb to their injuries before receiving these.Patient mortality did not differ in the periods before and after the MTP implementation (28.3% v 31.6%).The ratio of RBC to plasma products is similar in the pre- and post-MTP implementation periods.Fewer platelets were transfused per patient in the post-MTP period than pre-implementation (0.5 v 1.5);this is most likely because the 1st MTP 'shipment' of blood products containing 4 RBCs, 4 FFPs and 4 Cryo, would not normally include platelets.In summary, implementation of a formal MTP protocol has resulted in more 'activations' than had been the case before, an increased number of 'false' activations, increased wastage of blood product, no significant changes in mortality or ratio of plasma product to RBCs transfused.Possible ways to mitigate plasma product wastage could include alteration of MTP activation criteria, extending the lifespan of thawed products and the use of lyophilised fibrinogen.

P397

LOW PREVALENCE OF ALLOIMMUNIZATION AFTER RED BLOOD CELL TRANSFUSION IN 182 CHILDREN WITH SICKLE CELL DISEASE

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Background: A high prevalence (around 30%) of red blood cell alloimmunization has been reported in patients with sickle cell disease (SCD), but few studies have focused on children.
Aims: Our objectives were to study prevalence and risk factors of red blood cell alloimmunization in a population of children affected with SCD.
Methods: We analyzed the medical and transfusion files of 245 children with SCD, hospitalized in our center in 2014, and identified 182 patients aged 1 to 18 years who received at least one red cell concentrate in their lifetime. The main clinical and immuno-haematological characteristics of alloimmunized and non-alloimmunized patients were compared.
Results: The 182 patients received 1 to 339 red blood cell units with a median of 10 (IQR: 4-46.7) units per patient. Six patients out of the 33 in whom a red cell genotyping was performed (18.2%) had a partial antigen in the Rh blood group system. Five patients (2.7%) had a S-s- rare blood type (either $U+^{var}$ or U-). Excluding autoantibodies and the naturally-occurring antibodies identified before any transfusion, the prevalence of alloimmunization was 13.7% (95% CI [8.6-18.6]). After excluding the probable irregular natural antibodies (anti-M, anti-Le^a, anti-Le^b), the prevalence dropped to 7.4% (95% CI [3.5-11.3]) and the alloimmunization rate was 0.17/100 units received. These antibodies were

directed against antigens of Rh (D, C, Ce and C^w), Kell (Kp^a), Duffy (Fy^a, Fy3), MNS (S, U) and Colton (Co^b) blood group systems. Two anti-D alloimmunizations involving partial antigens could have been prevented by systematic *RHD* genotyping. Main risk factors for alloimmunization were a higher number of red blood cell units (median of 65 *vs* 10 units per patient; p=0.01) and the presence of one or more red cell autoantibodies (46.2 *vs* 4.7%; p<0.0001).
Summary/Conclusions: The relatively low frequency of alloimmunization in our study is probably due to the fact that it is a pediatric population exclusively transfused with blood systematically matched for C, c, E, e and K antigens, and probably also for other blood group antigens as the number of donors of African-Caribbean origin is high in the Paris region. The prevalence of red blood cell alloimmunization is relatively low among SCD patients followed in our pediatric center but it could still very likely be lowered by the implementation of a systematic genotyping for the major blood group systems. The presence of red cell autoantibodies appears to be a major risk factor for alloimmunization and could justify specific transfusion guidelines.

P398

INTRAUTERINE BLOOD TRANSFUSIONS IN FETAL ANEMIA CAUSED BY PARVOVIRUS B19 INFECTION

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Background: Parvovirus B19 has an affinity for hematopoietic system cells, including erythroid progenitor cell. The virus attacks cells of the red blood cell lines in the bone marrow, causing hemolysis and red blood cell aplasia. Fetal anemia is a serious complication in pregnancy associated with perinatal mortality and morbidity. The immaturity of the fetal immune system asociated to its inability of overproducing red blood cells leads to a physiological immunodeficiency that can be life threathening producing serial fetus conditions such as non inmune hydrops fetalis and spontaneous abortions. We present 8 cases of fetal anemia induced by Parvovirus B 19 (PV19) infection diagnosed during routine prenatal ultrasounds examination using the measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) and their subsequent treatment by intrauterine blood transfusions (IBT).
Aims: To determine the impact of intrauterine blood transfusions on the correction of fetal anemia in fetal PV19.
Methods: A retrospective descriptive study was performed of all intrauterine blood transfusions for PV19 between January 2014 and December 2014. The study was carried out in the Complejo Hospitalario Materno- infantil de Las Palmas de Gran Canaria. The patient's clinical, laboratory and transfusion data were obtained from hospital records. Incidental findings at routine second trimester ultrasounds concerning an increase of the MCA- PSV>1.5 MoM correlated with fetal anemia. MCA-PSV is a sensitive, noninvasive means to determine the degree of fetal anemia that can be used accurately to time the first and subsequent intrauterine transfusions. Pregnant women at risk were assessed for the presence of PV19 infection by determining their IgM anti Parvovirus status and PCR. The primary management tool was cordocentesis to assess fetal hemoglobin and performing IBT. Serial hematocrit determinations were taken and IBT where performed until the resolution of anemia.
Results: In 2014, an outbreak of parvovirus B19 infection occurred. Eight asymptomatic pregnant women were diagnosed. Their median gestational age at diagnosis was 20.5 weeks. Five of eight pregnant women were transfused. Out of the five transfused fetus, three survived. The overall survival rate was 60% *(Table 1)*.

Table 1.

	Gestational age (weeks)	Ultrasound indirect signs of anemia	MCA-PSV	Hemogram Pre- IBT	Hemogram final Post IBT	Number of IBT	Final outcome
Case 1	21 + 2	Hydrops, IUGR *	>1,5 MoM	1,1 gr/dl	-	1	Severe pancytopenia Fetal Death
Case 2	20+6	Hydrops Cardiomegaly, pericardial effusion	<1,5 MoM	3,2 gr/dl	-	2	Fetal death at 25 weeks
Case 3	21+1		>1,5 MoM	4 gr/dl	13,8 gr/dl	4	Eutocic birth
Case 4	21+1		>1,5 MoM	2,8 gr/dl	11 gr/dl	3	Instrumented birth
Case 5	21+2	Hydrops	>1,5 MoM	****		Expectant management	Fetal death at 23 +2 weeks
Case 6	20+5	Hydrops, cardiomegaly	>1,5 MoM	8 gr/dl	12 gr/dl	1	Eutocic birth
Case 7	12+5	Hydrops	Not done	****	-	-	Spontaneous abortion
Case 8	25	Nuchal oedema, pericardial effusion	< 1,5 MoM	-	-	Expectant management	Eutocic birth

** IUGR: Intrauterine growth restriction.*

Summary/Conclusions: The development of an acute PV19 infection during pregnancy can cause prenatal complications ranging from early pregnancy loss to nonimmune hydrops and intrauterine death. Measurements of MCA-PSV represent a non invasive procedure to diagnose fetal anemia, thus leading to an early correction of anemia trough IBT and reducing prenatal mortality.

Platelet disorders

P399

Abstract withdrawn.

P400

DETERMINING EFFECTS OF PLATELET INHIBITION ON VASO-OCCLUSIVE EVENTS (DOVE) TRIAL: A DOUBLE-BLIND, PLACEBO-CONTROLLED, STUDY OF PRASUGREL IN PAEDIATRIC PATIENTS WITH SICKLE CELL ANAEMIA

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Background: Sickle cell anaemia (SCA) is an inherited red cell disorder characterised by haemolytic anaemia, recurrent painful vaso-occlusive crises and end organ damage. There are few treatment options. Platelets, activated by ADP released through haemolysis, contribute to vaso-occlusion by forming multi-cellular aggregates and promoting inflammation. Prasugrel is an oral, third-generation thienopyridine inhibitor of ADP-mediated platelet activation and aggregation. Prior studies showing a reduction in markers of platelet activation, as well as the rate and intensity of pain suggested a potential therapeutic benefit of prasugrel in SCA.
Aims: To assess the efficacy of prasugrel in children with SCA, as measured by reduction in the rate of vaso-occlusive crisis (VOC).
Methods: The DOVE trial was a phase 3 double-blind, placebo-controlled, parallel-group, multinational trial of children with SCA, ages 2 to <18 years, who were randomly assigned to receive oral prasugrel or placebo, dose titrated to achieve a pre-specified reduction of platelet reactivity, for 9 to 24 months. The primary endpoint was the rate of VOC, a composite of painful crisis or acute chest syndrome (ACS). Secondary endpoints included electronic diary-documented rate and intensity of sickle cell related pain, analgesic use and school attendance; rates of sickle cell related RBC transfusion; and rates of hospitalisation for VOC, painful crisis and ACS.

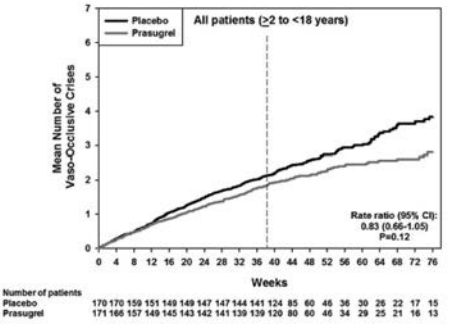

Figure 1.

Results: A total of 341 patients with SCA were randomized at 51 sites in 13 countries across the Americas, Europe, Asia, and Africa. Of these, 308 (90.3%) had HbSS and 153 (45%) were receiving stable dose hydroxyurea (HU); 275 patients completed the 9 month visit, of which 266 continued beyond 9 months and 9 discontinued prior to locking the database. A total of 736 VOC events occurred in the study: 328 among 115 (67.3%) patients in the prasugrel group and 408 among 123 (72.4%) patients in the placebo group. The rate of VOC trended lower in the prasugrel compared to the placebo groups but did not reach

significance: 2.30 per person-year and 2.77 per person-year, respectively (rate ratio, 0.83; 95% CI, 0.66-1.05; P=0.12) (Figure). There were no significant differences between groups in the secondary diary-reported outcomes. Pre-specified subgroup analyses identified a modest treatment effect in two subgroups; the oldest age (≥12 to <18 years) group (0.72; 95% CI, 0.51-1.02; P=0.06) and patients not on concomitant HU (0.74; 95%CI 0.54-1.01; P=0.06). Safety endpoints, including frequency of bleeding events requiring medical intervention, treatment emergent adverse events, both haemorrhagic and non-haemorrhagic, and discontinuations due to study drug did not significantly differ between groups.
Summary/Conclusions: In children with SCA, prasugrel did not significantly reduce the rate of the primary composite endpoint of VOC at the levels of platelet inhibition attained in this study. Prasugrel treatment had no impact on secondary outcomes. The overall adverse event rate, including haemorrhagic and non-haemorrhagic events, did not differ between the treatment groups. A trend towards an effect of prasugrel on VOC in the ≥12 to <18 year age group and in patients who were not receiving HU suggest that anti-platelet agents may have a therapeutic role as part of a multi-drug approach toward the complex pathophysiology of SCA. The DOVE study is the first multinational interventional trial in children with SCA and provides valuable guidance for future multi-continental SCA clinical trials. (Funded by Daiichi Sankyo, Ltd and Eli Lilly and Company; DOVE ClinicalTrials.gov number, NCT01794000).

P401

ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA: A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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Background: Romiplostim could be a treatment option for symptomatic children with ITP.
Aims: This was a phase 3, double-blind study designed to evaluate weekly romiplostim or placebo (randomized 2:1) given for 24 weeks to children with ITP for ≥6 months.
Methods: Dose was adjusted weekly from 1-10 μg/kg to target platelet counts of 50-200×10^9/L. Platelet response was defined as platelet counts ≥50×10^9/L any week of weeks 2-25 (excluding 4 weeks after rescue medication). The primary endpoint, durable platelet response, was defined as ≥6 weekly responses during the final 8 weeks, weeks 18-25. Overall platelet response was defined as ≥4 weekly responses weeks 2-25.

Table 1.

		Romiplostim (N = 42)	Placebo (N = 20)	
Durable platelet response, all patients		22/42 (52%)	2/20 (10%)	p<0.002
By baseline age	1 to <6 years	3/8 (38%)	1/4 (25%)	
	6 to <12 years	10/18 (56%)	1/9 (11%)	
	12 to <18 years	9/16 (56%)	0/7 (0%)	
Overall platelet response, all patients		30/42 (71%)	4/20 (20%)	p=0.0002
By baseline age	1 to <6 years	5/8 (63%)	2/4 (50%)	
	6 to <12 years	15/18 (83%)	1/9 (11%)	
	12 to <18 years	10/16 (63%)	1/7 (14%)	

Results: Patients (n=62) had median (min-max) age 9.5 (3-17) years, ITP duration 2.1(0.5-10.7) years, and baseline platelet counts 18 (1-94)×10^9/L and 44% were male; 66% were Caucasian, 13% African American, and 8% Asian. For the primary endpoint, measured in the final 8 weeks after 16 weeks of dose titration, the proportions of patients with a durable platelet response were romiplostim: 52% and placebo: 10% (p<0.002). The proportions of patients with overall platelet response were romiplostim: 71% and placebo: 20% (p=0.0002), and with any platelet response were romiplostim: 81% and placebo: 55% (p=0.03). For romiplostim, the median (min-max) time to first platelet response was 4.5 (1-20) weeks and the median average weekly dose was 3.9 (0.9-8.1) μg/kg. Median platelet counts with romiplostim were ≥50×10^9/L from week 8 on. When the number of bleeding events was divided by exposure time, duration-adjusted rates per 100 patient-weeks of any bleeding (30.9 *vs* 47.3, p<0.0001), CTCAE grade ≥2 bleeding (5.9 *vs* 17.9, p=0.0006), and composite bleeding episodes, ie CTCAE grade ≥2 bleeding and/or rescue medication use (8.1 *vs* 18.4, p <0.0001), were all significantly lower with romiplostim than placebo. No patients withdrew due to adverse events (AE). Incidence rates of serious AEs (SAEs) were romiplostim: 23.8% (10 patients) and placebo: 5.3% (1 patient). SAEs with romiplostim consisted of 2 cases each of contusion, epistaxis, and headache, and 1 case each of petechiae, thrombocytosis, nephrotic syndrome, nausea, vomiting, bronchiolitis, fever, urinary tract infection, and seizure. Only one headache SAE and the thrombocytosis SAE (same patient) were considered treatment-related by the investigator. There were no thrombotic events, no malignancies observed, and no findings indicating bone marrow dysplasia or fibrosis in the only bone marrow biopsy performed (in a placebo-treated patient).

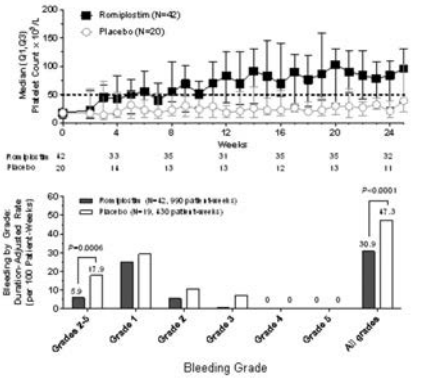

Figure 1.

Summary/Conclusions: In children with symptomatic ITP of ≥6 months duration, romiplostim was effective in inducing durable and overall platelet responses and reducing bleeding. There were no new safety signals. Romiplostim may be a treatment option for children with symptomatic chronic ITP.

P402

NEW MUTATION C.-140C>G IN 5'UTR OF ANKRD26 GENE IN PATIENTS WITH MILD FORM OF INHERITED THROMBOCYTOPENIA ASSOCIATED WITH MALIGNANCIES

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Background: The identification of ANKRD26 as the gene responsible for a mild form of inherited thrombocytopenia stimulated revealed that this disorder exposes subjects to the risk of haematological neoplasm. A similar pattern is present in subject with platelet disorder due to mutations in RUNX1 and ETV6 related familial thrombocytopenia. A recent study about paediatric inherited thrombocytopenia show a presence in the 5' UTR of ANKRD26 gene of a new mutation c.-140C>G, not previously described in the Italian population
Aims: Screening of 5'UTR of ANKRD26 gene in patients with mild form of inherited thrombocytopenia
Methods: We studied 50 patients with mild form of inherited thrombocytopenia lacking a definite genetic characterization followed at the 1st Clinical Medicine of the University City Padua Hospital. Molecular analysis in the 5'-UTR of the ANKRD26 was carried out to investigate the presence of the gene mutations.
Results: We identified 6 patients belonging three family trees and three sporadic cases with single nucleotide substitutions in the 5'-UTR region of the ANKRD26 gene: one family presented with c. -128C>G mutation, one family presented with c. -140C>G mutation, one family presented with c. -140C>G and c. -134G>A mutations, three sporadic patients presented with c. -140C>G mutation. The clinical features of the patients are mild thrombocytopenia (>90×109/l), normal mean platelet volume (MPV), autosomal dominant transmission and absence of haemorrhagic symptoms. One patient was first diagnosed as immune thrombocytopenia. Cases of malignancies registered in the subjects with mutations are breast cancer, prostate cancer, kidney cancer and acute myeloid leukaemia.
Summary/Conclusions: Thrombocytopenia with 5'UTR ANKRD26 gene mutation must be considered in case of a mild thrombocytopenia with a low bleeding tendency, associated with autosomal dominant transmission, normal platelet size and history of malignancies. This newly described pathology is very probably under diagnosed. The new mutation c. -140C>G in the 5'-UTR region of the ANKRD26 gene, recently described by Boutroux *et al.* in 2015, must be investigated in patients with these clinical features.

P403

MYCHOFENOLATE MOFETILE FOR THE TREATMENT OF CHILDREN WITH IMMUNE THROMBOCYTOPENIA AND EVANS SYNDROME. A RESTROSPECTIVE STUDY OF THE ITALIAN ASSOCIATION OF PEDIATRIC HEAMATOLOGY/ONCOLOGY GROUP

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Background: The treatment of chronic/relapsing immune thrombocytopenia purpura (ITP) is not well established due to the lack of evidence-based data, and is particularly challenging in children who are more at risk of severe side-effects secondary to prolonged steroid therapies. Mychofenolatemofetile (MMF) is an immunosuppressor which reduces T and B-cells proliferation by inhibiting the inosine monophosphate dehydrogenase. It has been shown to be safe and effective in some series of patients with ITP, and in few cases of Evans Syndrome secondary to ALPS. In the largest reported cohort of 46 adults with ITP, 52% achieved a response, which was complete in 33%. However, data from larger group of children and details on timing of response still have to be investigated.
Aims: The aim of this study is to evaluate the outcome, timing of response and toxicity of children treated with MMF for ITP.
Methods: We collected data of children undergoing MMF treatment for ITP, with or without involvement of other cell lineages, in 8 Italian Centers. Patients showing other signs of autoimmunity or with ITP secondary to an ALPS-related disorder, defined as the presence of at least one absolute or primary additional criterion for ALPS, were included in the study. Patients with ALPS were excluded. Complete response (CR) and partial response (PR) were defined as a platelet count >/= $100x10^9$/Land >$30x10^9$/L and at least 2 fold increase of the baseline count, respectively.
Results: 56 children with primary ITP (37, 66%) or secondary to an ALPS-like disorder (19, 34%), were treated with MMF. 16/56 (28%) patients had also an Evans syndrome (ES) due to the association of leukopenia (6), autoimmune haemolytic anemia (5) or both (5). All patients but 2 who were treated as first line therapy, received MMF as second (35), third (15) or fourth (2) line treatment. 35/54 (65%) evaluable patients responded to the treatment and achieved a CR and PR in 25 (46%) and 10 (18%) cases, respectively. PR and CR were obtained after 20 (range 7-137) and 37 (range 7-192) days of treatment, respectively. 14/19 (73%), and 21/35 (60%) evaluable children with signs of autoimmunity/ALPS-like syndromeand primitive disease achieved a response, respectively. Patients with ES and with monolinear thrombocytopenia responded in13/16 (81%) and in 22/38 (58%) cases, respectively. Six out of 37 (16%) responding children relapsed at median of 283 days (range 189-1036) from the response. Four patients (7%) reported mild toxicity consisting of nausea/vomit (2), asthenia (1), skin hyperpigmentation (1) that didn't require the interruption of the treatment. Median duration of treatment and follow-up were 7 months (range 1-42,8) and 12.7 months (range 1-92), respectively.
Summary/Conclusions: To the best of our knowledge this is the largest cohort of patients treated with MMF for ITP or Evans Syndrome confirming its safe profile and showing that children seem to have a better overall response compared to what has been reported in adults, in particular a higher rate of CR. It also shows, for the first time, that MMF represents a valid option also for children with ES other than ALPS, who showed a better, although not statistically significant, response compared to the ones with monolinear thrombocytopenia. Thanks to the relatively short time of response, MMF can be considered as second-line option before the use of more aggressive and expensive treatments as Rituximab or splenectomy. Clinical trials are needed to confirm these results.

P404

SAFETY AND EFFICACY OF ELTROMBOPAG IN SPLENECTOMIZED AND NONSPLENECTOMIZED PATIENTS WITH IMMUNE THROMBOCYTOPENIA: RESULTS FROM THE EXTEND STUDY

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Background: Although splenectomy remains an appropriate treatment for ITP, it is associated with adverse events (AEs). The most recent guidelines for ITP indicate that splenectomy should be designated a second-line treatment and delayed for 6-12 months. Eltrombopag is an oral thrombopoietin receptor agonist (TPO-RA) approved in Europe for treatment of splenectomized adult chronic ITP patients who are refractory to other treatments, and as a second-line treatment for adult nonsplenectomized patients in whom surgery is contraindicated. The EXTEND study assessed long-term safety and efficacy of eltrombopag in ITP patients previously enrolled in 6-week or 6-month placebo-controlled trials.
Aims: To compare the safety and efficacy of long-term eltrombopag treatment in patients with or without splenectomy in the EXTEND trial.
Methods: Eltrombopag was started at 50 mg weekly and titrated to maintain platelet counts 50–200 Gi/L.
Results: Among patients enrolled in EXTEND (N=302), 115 splenectomized (38%) and 187 nonsplenectomized (62%) patients had similar characteristics at baseline, except that more splenectomized patients were receiving ITP medications at baseline (47% *vs* 25%) and more had a history of bleeding (23% *vs* 13%). Greater than half of both groups received eltrombopag for ≥24 months. After eltrombopag treatment, a greater proportion of nonsplenectomized patients achieved platelets ≥50 Gi/L than splenectomized ones, and was consistent by age group (Table). Nonsplenectomized patients experienced proportionately fewer serious bleeding AEs (Table). Rates of any bleeding AE were experienced by 49 (26%) and 35 (30%) nonsplenectomized and splenectomized groups, respectively. Events occurring in ≥4% of patients in the splenectomized and nonsplenectomized groups, respectively, included mouth hemorrhage (4% and 1%), epistaxis (14% and 6%), petechiae (8% and 2%), ecchymosis (2% and 4%), contusion (3% and 4%), and hematuria (2% and 4%). In each group, 13% had a sustained reduction or permanently stopped at least one concomitant ITP medication without having received any on-treatment rescue medication. Splenectomized patients had higher rates of Grade 1-4 World Health Organization bleeding at baseline (64% *vs* 52%), but rates declined in both groups over time.

Table 1. Results of the EXTEND eltrombopag study by baseline splenectomy status.

	Not Splenectomized (n=187; 62%)	Splenectomized (n=115; 38%)	Total (N=302)
Platelet count ≥50 Gi/L, n (%)			
Post-baseline	167 (89)	92 (80)	258 (85)
>50% of assessments	126 (67)	59 (51)	185 (61)
>75% of assessments	90 (48)	36 (31)	126 (42)
Platelet count ≥50 Gi/L by age group (years), n/N (%)			
18 to 49	76/87 (87)	46/62 (74)	122/149 (82)
50 to 64	58/62 (94)	36/41 (88)	94/103 (91)
≥65	33/38 (87)	10/12 (83)	43/50 (86)
Bleeding adverse events, n (%)*	(n=187)	(n=115)	(N=302)
Any event	49 (26)	35 (30)	84 (28)
Serious AEs	7 (4)	11 (10)	18 (6)
Discontinuations	1 (<1)	2 (2)	3 (<1)

*Number of patients with an event.

Summary/Conclusions: Long-term treatment with eltrombopag increased platelets in both nonsplenectomized and splenectomized patients, with numerically higher response rates in the former. Rates of AEs were similar to those in patients with splenectomy.
Funding: This study (NCT00351468) is/remains sponsored by GlaxoSmithKline; however, as of March 2, 2015, eltrombopag is an asset of Novartis AG.

P405

CHARACTERIZATION OF PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP) ENTERING REMISSION IN A ROMIPLOSTIM BONE MARROW STUDY

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Background: The thrombopoietin receptor agonist romiplostim is approved for use in adults with chronic ITP. In this study, patients with ITP (N=169) had bone marrow biopsies performed at baseline and after 1, 2, or 3 years of romiplostim; 24 patients discontinued romiplostim and entered remission.
Aims: To examine remission in patients with ITP receiving romiplostim in a bone marrow study.
Methods: Patients with ITP entering the bone marrow study had a platelet count <50x109/L and ≥1 prior ITP therapy. Romiplostim, received weekly for up to 3 years, was adjusted from 1 10 µg/kg to target platelet counts of 50-200x109/L; doses were reduced for platelet counts >200x109/L for 2 consecutive weeks and no romiplostim was given for platelet counts >400x109/L. A post hoc analysis was conducted of those who entered remission, ie platelet counts ≥50×109/L for ≥6 months with no ITP therapy, including romiplostim.
Results: The median years since ITP diagnosis for those who did (N=24) and did not (N=145) enter remission (1.66 years *vs* 5.16 years) had overlapping ranges [not significant (NS)], as did the median average weekly dose (1.1µg/kg *vs* 3.5µg/kg, NS, included zero doses prior to last non-zero dose) (Table 1). Adverse events (AEs) occurred at similar rates. A *post hoc* analysis examining the association between remission and baseline factors including age, gender, platelet count, prior splenectomy, ITP duration, and number of prior treatments indicated that ITP duration ≤1 year could be a potential predictor for remission

(hazard ratio 2.46, 95% CI: 1.04, 5.79, p=0.04); however, this association could be due to multiple comparisons (ie, type I error). Median time of onset for remission was 52 weeks (range, 6-124 weeks) and median duration of remission during the study was 88 weeks (range, 29-154 weeks); 21 of the 24 patients were still in remission at the last observation on study.

Table 1.

Characteristic	Remission (N=24)	No Remission (N=145)
Women, n (%)	12 (50)	102 (70)
Age, y, median (Q1, Q3)	45.5 (31.0, 58.0)	51.0 (37.0, 64.0)
Years since ITP diagnosis, median (Q1, Q3)	1.66 (0.46, 7.75)	5.16 (1.62, 12.84)
Prior splenectomy, n (%)	7 (29.2)	53 (36.6)
Platelet count at screening, $\times 10^9$/L, median (Q1, Q3)	20.9 (7.5, 35.0)	23.0 (11.0, 35.0)
Time to first platelet response, n, weeks, median (Q1, Q3)	24, 3.5 (2.0, 14.0)	131, 2.0 (2.0, 6.0)
Treatment duration, weeks, median (Q1, Q3)	62.5 (14.1, 82.1)	155.7 (66.0, 156.0)
Average weekly dose, µg/kg, median (Q1, Q3)	1.1 (1.0, 2.2)	3.5 (1.9, 7.2)
Splenectomy on study, n (%)	0	3 (2.1)
Any treatment-related AE, n (%)	9 (37.5)	51 (35.2)
Serious AE / Treatment-related serious AE, n (%)	8 (33.3) / 0	48 (33.1) / 6 (4.1)
Fatal AE, n (%)	0	7 (4.8)
Bone marrow changes (increased reticulin ≥2 grades or collagen), n (%)	0	9 (6.2)
On-study bleeding AE, n (%)	15 (62.5)	84 (57.9)
On-study grade ≥2 bleeding AE, n (%)	4 (16.7)	34 (23.5)
On-study serious bleeding AE, n (%)	1 (4.2)	13 (9.0)

Summary/Conclusions: In this *post hoc* analysis, 14% (24/169) of patients in a romiplostim ITP bone marrow study were able to enter remission following standard dosing rules; more studies are needed to confirm whether shorter ITP duration is a predictor of remission.

P406

THE IMMUNOMODULATORY EFFECTS OF RETINIC ACID IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder in which platelets are destroyed by macrophages. Macrophages can polarize into pro-inflammatory M1 or anti-inflammatory M2 phenotypes in response to different environmental stimulation. Previous study showed that all-trans retinic acid (ATRA) combined with IL-4 induced M2 differentiation in mice. However, the effect of ATRA on macrophage polarization in ITP patients has not yet been reported.
Aims: To investigate the immunomodulatory effects of retinic acid in adult ITP patients.
Methods: Human spleens were obtained from 5 ITP patients and 5 patients with traumatic spleen rupture. The M1/M2 ratio of splenocytes was evaluated by immunofluorescence. Peripheral blood mononuclear cells (PBMCs) were isolated from 18 untreated ITP patients and 12 steroid-resistant ITP patients who were responsive to the ATRA therapy (10mg once, 3 times per day; median time to response, 63 days). Human monocyte-derived macrophages were induced and polarized into M1 or M2 by LPS plus IFN-γ or IL-4 respectively, in the presence or absence of ATRA. The phenotypes of M1 or M2 were analyzed by flow cytometry. The concentration of IL-10 and IL-12 in the culture supernatant was assayed by ELISA. CFSE-labeled $CD4^+$ or $CD8^+$ T cells were co-cultured with allogeneic M1, M2, ATRA-M1 or ATRA-M2. The proliferation of T cells and the proportion of $CD4^+CD49b^+LAG3^+$ regulatory T (Tr1) cells were analyzed by flow cytometry. The secretion of IL-12, IL-10, IL-1β, IFN-γ, IL-2, IL-4, IL-5, IL-6, TNF- a, GM-CSF and IL-18 in the co-culture supernatants were detected with ProcartaPlex assays. The expression of transcription factor cAMP response element-binding protein 1 (CREB1) was measured by RT-PCR.
Results: Our data demonstrated that there was a preferred polarization of macrophages towards M1 in the spleens of ITP patients compared with controls. In monocyte-derived macrophages induced from the 18 untreated ITP patients, the expression of M1 markers (CD80, CD86, TNF-α and IL-12) was dramatically increased and the expression of M2 markers (IL-10 and CD209) decreased compared to those from healthy controls. Macrophages induced from ITP patients displayed a higher ability to promote both $CD4^+$ T cell and $CD8^+$ T cell proliferation and a lower ability to induce Tr1 cells than those from controls. In both M1 and M2 macrophages, ATRA effectively down-regulated the expression of CD80, CCR7 and IL-12 while increased the expression of CD209 and IL-10. ATRA also inhibited the expression of CD86 and HLA-DR in M2 macrophages. ATRA-modulated macrophages (both M1 and M2) showed suppressive function on T cell proliferation compared with the untreated macrophages. Moreover, ATRA-modulated M2 macrophages expanded Tr1 cells but no effect was observed on M1. The concentration of IL-12, IL-2, TNF-α, IFN-γ and IL-1β was decreased while IL-4 and IL-10 increased in the co-culture supernatants with ATRA-modulated macrophages and T cells compared with unmodulated systems, indicating that ATRA-modulated macrophages could affect Th1/Th2 polarization by promoting Th2 responses. ATRA modulation was associated with the elevated expression of CREB1 in macrophages. *In vivo* ATRA therapy could correct the M1 deviation of macrophages and induce a M2 phenotype in the 12 steroid-resistant ITP patients.
Summary/Conclusions: Our findings demonstrate that an aberrant macrophage polarization is involved in the pathogenesis of ITP, while ATRA could correct this imbalance, thus being able to be used as a potential therapy for ITP.

P407

STUDIES ON PLATELET FUNCTION IN A GROUP OF CLL AND MCL PATIENTS TREATED WITH IBRUTINIB - PRELIMINARY RESULTS

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Background: Ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK) has a significant activity in chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). Although the treatment is well tolerated, some clinical studies reported mucocutaneous bleeding complications. Such complications may be caused by platelet or vessel abnormalities.
Aims: The aim of the study was to evaluate platelet function before and during the ibrutinib therapy.
Methods: We examined 24 patients: 21 with CLL and three with MCL (11 females and 13 males, median age 68 (48-83).The majority of patients received 420 mg Ibrutinib. Two patients received the dose of 140 mg because of atrial fibrillation. We performed platelet aggregation studies in whole blood samples using Multiplate technology, in which platelets were activated by: adenosine diphosphate (ADPtest), arachidonic acid (ASPItest), collagen (COLtest), TRAP-6 (TRAPtest). Platelet function was evaluated three times: before Ibrutinib treatment, after 1 or 2-4 months. The control group (CG) consisted of 20 healthy persons: 6 males and 14 females, median age 44(26-59).
Results: We detected statistically significant impaired platelet function [p<0.0001] in all the patients in COLtest in all points compared to the control group [CCL patients COLtest 1 Median (M) 35.41,6-75; COLtest2 M18.89, 4-35; COLtest 3 M:19.73,7-37; CG M 70.5, 49-107]. What is more, we observed exacerbation of this platelet defects during the treatment. COLtest after 2-4 months was statistically significantly diminished compared to the value before the treatment [COLtest 1: M: 35.41,6-75; COLtest 3 M: 19.73, 7-37, p=0,016]. We also detected statistically significant [p<0.0001] impaired platelet function in ADP test in all points compared to the control group (CLL patients: ADP 1median(M) 30.45; 0-74; ADP2 M: 35.11; 1-80; ADP3 M: 38.91; 6-87, CG:M: 84.5; 54-102]. In other two tests: ASPItest and TRAPtest, platelet function was statistically significantly impaired compared to the CG in all three points but a very interesting tendency appeared. The value of the third point was raised indicating some improvement in arachidonic acid and TRAP induced platelet aggregation. This tendency was the most pronounced in ASPItest but not statistically significant. We observed bleeding complications in five patients: in two patients there occurred nose bleeding, in three patients skin petechiae was reported. Platelet function in these patients was impaired, all test values were lower than in the patients without bleeding complication. However, this tendency did not achieve statistical significance. In three out of five patients lower platelet count (75-85G/l) was detected just before Ibrutinib therapy. In one patient because of atrial fibrillation a new oral anticoagulant-pradaxa was introduced causing aggravation of the skin petechiae. After diminishing the dose of pradaxa the skin changes disappeared.
Summary/Conclusions: We detected impaired platelet aggregation measured by Multiplate technology in all the patients. The impairment of collagen induced platelet aggregation was exacerbated during ibrutinib treatment. On the other hand, arachidonic acid and TRAP induced platelet aggregation tended to improve during the treatment. Only 21% of ibrutinib patients presented some mild bleeding complications.

P408

CLINICAL CHARACTERISTICS AND TREATMENT PATTERN OF NEWLY DIAGNOSED PRIMARY IMMUNE THROMBOCYTOPENIA CASES IN THE UNITED KINGDOM IMMUNE THROMBOCYTOPENIA (ADULT) REGISTRY

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Background: Primary immune thrombocytopenia purpura (ITP) is a rare autoimmune bleeding disorder. In a platelet depleted state, ITP patients present with bleeds or are at risk to bleeding. Treatment decisions may vary according to the clinical profile at presentation and patients' choice. This study sought to understand how newly diagnosed ITP patients are treated with newer treatment options becoming available.
Aims: To describe the demographic and clinical characteristics of newly diagnosed ITP patients and how these influence treatment choice.
Methods: The UK ITP Registry is a collaborative effort of 70 centres throughout the UK, coordinated at the Royal London Hospital (RLH), to permit adequate data collection on a rare disease. In this study, data collected on adults (≥18 years) up to July 2015 were analysed using various statistical techniques.
Results: A cohort of 1618 ITP patients [median age at diagnosis 49.3 (IQR 31.1, 64.2) years; 58.2% female; 76.2% European) contributed data for this

study. Age distribution at diagnosis resembled an 'M-shaped' pattern with peaks at 18 to 30 years (18%) and 50 to 70 years (17%) of age. The RLH (12.2%) and Castle Hill Hospital (6.2%) had highest recruitment. The most prevalent history of comorbidities before ITP were hypertension (16.7%), depression/anxiety (5.0%), diabetes (4.4%) osteoarthritis (4.1%) and arterial thromboembolism (4.0%). The cohort's median platelet count around diagnosis was 23.0 x10^9/L ([IQR 7-61 years]; 56.1% had platelet count <30 x10^9/L). Of those who received treatment within 3 months of ITP diagnosis, 96.9% had 1st line treatment [including prednisolone (86.1%) IVIg (34.3%), Anti-D (2.5%)], 19.8% had 2nd line treatment [including rituximab (6.3%); splenectomy (1.8%) and others (<1% to 4%)], and 11.9% had blood product transfusion [(platelet (8.8%), red blood cells (4.7%)]. Throughout the entire cohort history, common treatments were prednisolone (70.3%), IVIg (39.2%), splenectomy (12.8%), romiplostim (9.24%), Anti-D (6.5%) and eltrombopag (6.1%), whereas 19.5% had no treatment. For those with platelet counts <50 x10^9/L and bleed within 3 months of diagnosis, there was a significant association with use of both 1st and 2nd line treatment. A similar pattern of treatment was found for platelet counts ≥50 x10^9/L but to a lesser extent. Bleed from the respiratory system (*e.g.* haemoptysis), gastrointestinal system (exc. oral) and obstetric/gynaecological-related ones were associated with receiving transfusion (including platelet). The median time from ITP diagnosis to having a splenectomy was 1.4 (IQR 0.5-3.9) years; 77.1% were diagnosed below the age of 50. Fifteen individuals had this procedure within 3 months of diagnosis, of which 60% were before 2010. Over the last 2 decades there has been a decrease in the overall number of splenectomies carried out [and in relation to the year that the splenectomised patients were diagnosed (1990-99: 30.1%; 2000-09: 12.7%)], and for those diagnosed within the last five years, 4.4% had this procedure. The same time periods saw increased use of newer therapeutic agents (figure).

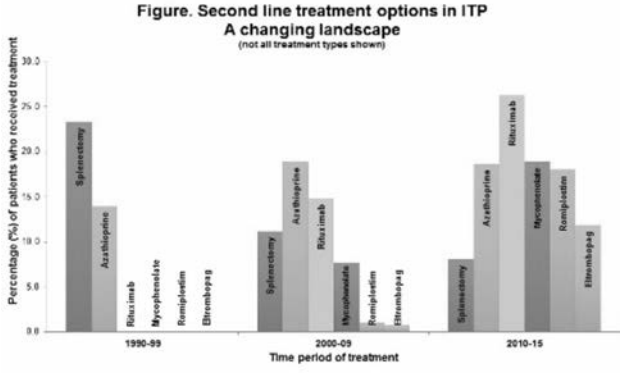

Figure 1.

Summary/Conclusions: Patients with low platelet and bleeding within the 1st three months of ITP diagnosis were likely to receive both 1st and 2nd line treatment options. Notable, is the decline of splenectomy as 2nd line treatment, while there are different drug options available. It would be important to increase the cohort size, including expanding internationally, to obtain more treatment data on higher number of patients who received 2nd line treatment options and long-term follow up data.

Coagulation - Clinical Research

P409

EFFICACY AND SAFETY OF RIVAROXABAN FOR NON-VALVULAR ATRIAL FIBRILLATION IN PATIENTS WITH SEVERE RENAL IMPAIRMENT

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Background: Patients with atrial fibrillation and severe renal impairment (CrCL <30ml/min) were excluded from the ROCKET-AF study and, indeed, most of the seminal phase 3 DOAC studies in atrial fibrillation. However, the summary of product characteristics states that rivaroxaban may be used at a reduced dose of 15mg OD for anti-coagulation management of atrial fibrillation in patients with severe renal impairment (CrCl 15-29 ml/min). This recommendation was solely based on pharmacokinetic analyses and has not been validated in a clinical study.
Aims: Assess safety and efficacy of rivaroxaban (15mg OD) in patients with atrial fibrillation and severe renal impairment (CrCl 15 – 29 ml/min). The primary safety point is occurrence of bleeding complications.
Methods: Retrospective cohort analysis of 30 patients with non-valvular atrial fibrillation and severe renal impairment who were commenced on rivaroxaban (15mg OD) in our anti-coagulation clinic between October 2012 and March 2015. Medical notes were reviewed and general practitioners were contacted by telephone or fax. Information collected included age, weight, CHA2DS2-Vasc and HAS-BLED scores, baseline blood investigations (CrCl, FBC, LFT and clotting screen) and where available blood investigations at the time of the bleeding event.
Results: A total of 30 patients were retrospectively followed up for a minimum of 10 months. The majority of patients were female (83.3%) with a median age and weight of 89.5 years and 54 kilograms respectively. Median CHA2DS2-Vasc score was 5, giving an estimated annual stroke risk of 6.7%, and approximately 76% of patients had a HAS-BLED score ≤2. Creatinine clearance was calculated using the Cockcroft-Gault formula, and the median CrCl was 26.39 ml/min. During the follow up period, 11 patients died (37%), one of whom died of massive gastrointestinal bleeding after being switched from rivaroxaban to warfarin due to worsening renal function. The cause of death for one patient could not be obtained, however there were no documented deaths due to rivaroxaban as a primary or contributing cause. A total of 6 rivaroxaban related bleeding events were identified with two events occurring in one patient. Bleeding sites included nasal (epistaxis), gastro-intestinal and genitourinary tracts but there were no incidents of intracranial haemorrhage or major bleeding as defined by the International Society on Thrombosis and Haemostasis. A patient who discontinued rivaroxaban due to haematuria later developed acute limb ischemia requiring embolectomy. Although compliance to treatment could not be assessed, a further patient developed an ischemic stroke whilst on rivaroxaban.
Summary/Conclusions: Clinically relevant bleeding events occurred in approximately 17% of our small cohort of very elderly patients with severe renal impairment receiving rivaroxaban (15mg) as anti-coagulant therapy for non-valvular atrial fibrillation. The event rate is almost identical to that in the Rocket-AF study (16.7%) and there were no major bleeding events. Our findings indicate that the use of rivaroxaban in this group of patients is feasible and relatively safe.

P410

ORAL DIRECT ANTICOAGULANTS IN THE TREATMENT OF NONVALVULAR ATRIAL FIBRILLATION. RESULTS OF THE DAILY CLINICAL PRACTICE

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Background: Atrial fibrillation (AF) is the most common arrhythmia. It leads to significant morbidity and mortality. The new oral anticoagulants (NOAC) represent an improvement compared with standard treatment (vitamin K antagonists (AVK)) in the prevention of thromboembolic complications in patients with non-valvular AF.
Aims: The aim of this study is to analyse the clinical characteristics of the patients (p) anticoagulated with NOAC and compared these with those taking AVK, as well as to assess their effectiveness and safety. The primary study outcome was the composite of stroke, systemic embolism, pulmonary embolism, myocardial infarction or death (MACE). The primary safety outcome was major haemorrhage.
Methods: We studied 688 p with a diagnosis of NVAF between November-2011 and November-2014. We made a prospective analysis, with a median follow-up of 14 months.
Results: A total of 688 p were included with a mean age of 73.4±7.9 in the AVK group *vs* 73.9±8.3 in the NOAC group (p=0.432). In the NOAC group,

hypertension was significantly more common (82.3% *vs* 92.2%, p<0.001), with more frequent history of heart failure (15.3% *vs* 30.4%, p<0.001), and more history of stroke or transient ischemic attack (10% *vs* 15.9%, p=0.022). Mean scores for thromboembolic and bleeding risk indices are shown in Table 1.

Table 1.

SCORE	ACENOCOUMAROL	NOAC	p
CHADS2	1.9±1.0	2.3±1.1	P<0.001
CHA2DS2VASc	3.5±1.3	3.9±1.5	P<0.001
HASBLED	1.3±0.7	1.4±0.7	P=0.086

The primary outcome occurred in 12 p receiving AVK (2.9%) and 11 p receiving NOAC (4.1%) (p=0.413) (HR acenocoumarol *vs* NOAC 1.737; IC 95%: 0.760-3.969, p=0.190). MACE were more common in patients with poor INR control (58.3% *vs* 41.8%, p=0.037). In the univariate analysis, the factor associated with MACE in the AVK group was the poor INR control (4.009 (1.266-12.696), p=0.018), showing the sex female category a strong trend to be a protective factor (0.228 (0.050-1.043), p=0.057). In the NOAC group, valvulopathy ≥moderate (3.840 (1.166-12.652), p=0.027) and renal insufficiency (7.197 (1.743-29-772), p=0.006) were significantly associated with MACE. The rate of major bleeding was 3.51% with AVK, as compared with 0.6% per year in the group that received NOAC (17 events -4.1% >vs. 2 events, 0.7%, p=0.009) (figure 1; HR acenocoumarol *vs* NOAC 0.252; IC 95%: 0.058-1.101, p=0.067). In the univariate analysis, the factors associated with bleeding in the AVK group were age ≥75 years (3.187 (1.028-9.882), p=0.045) and HASBLED score (2.106 (1.072-4.136), p=0.031). The rate of intracranial bleeding was 1.02% with AVK compared with 0.34% per year in NOAC group (5 events – 1.2% *vs* 1 event – 0.4%, p=0.248). There were no significantly differences in the rates of gastrointestinal bleeding (1.02% with AVK *vs* 0.94% per year, p=0.902) neither minor bleeding (4.88% with AVK *vs* 3.51% per year, p=0.309). There was a significantly higher rate of discontinuation with AVK (17.14% *vs* 6.68% per year, p<0.001).

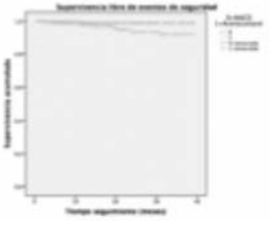

Figure 1.

Summary/Conclusions: Patients with NVAF anticoagulated with NOAC have higher embolic risk than those that receive acenocoumarol. No differences were found in efficiency. Patients anticoagulated with NOAC show a trend to lower bleeding risk. In the multivariate analysis, the predictors of events in the acenocoumarol group were the male category, poor INR control, ≥75 years, anemia and HASBLED score. The predictors of events in the NOAC group were valvulopathy≥moderate, renal insufficiency and anaemia.

P411

SWITCH FROM VKA, LMWH OR FONDAPARINUX TO DOACS IN THE CLINICAL PRACTICE

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Background: New oral anti-coagulant drugs (NOACs) are largely used both in AF (Atrial Fibrillation) and in VTE (Venous Thromboembolism) and, due to their important advantages over older drugs, many patients have been switched from VKA (Vitamin K antagonists), LMWH (Low Molecular Weight Heparin) or Fondaparinux to NOACs. Current recommendations by scientific society suggest switching from VKAs to NOACs according to the INR test result. Regarding how to switch from Fondaparinux or LMWH to NOACs the suggested wash out period is 24 hours.
Aims: Evaluate safety and efficacy of switching anticoagulant therapy from old to new anticoagulant drugs without monitoring the INR. Safety outcomes were major and minor bleeding rate. Effectiveness outcomes were VTE events. We also evaluate the tolerability of the new treatment.
Methods: We switched patients, affected by AF or DVT, from VKAs or LMWH or Fondaparinux to NOACs regardless from the INR. We follow current recommendations for switching from LMWH and Fondaparinux; we applied to the VKAs-DOACs switch the same schedule prescribed by current guidelines for bridging therapy from VKAs to LMWH. Therefore, we began the NOACs treatment after 24 hours since the last previous anticoagulant intake, if the patient has been switched from LMWH, Fondaparinux or Acenocumarol. We began the NOACs treatment after 48 hours if Warfarin was the patient's therapy. We checked the platelet count and the Creatinine Clearance collecting these laboratory measurements within 30 days before the end of VKA treatment. We collected outcomes measures until 30 days from the switch. Data are presented as mean±standard deviation (SD).
Results: We enrolled in the present study 603 patients: 458 have been switched to NOACs from VKAs, 101 from LMWH and 44 patients from Fondaparinux. 179 patients were affected by AF and 424 by VTE. Thirty days after the switching 99% of the patients were still on NOACs. Three patients complained about nausea, dyspepsia and abdominal pain. Two of them came back to VKAs and one patient change Rivaroxaban with Apixaban, without further side effects. Two patients reported minor bleedings: gingival hemorrhage and hematuria. One of them remained on NOACs treatment and another switch to acetylsalicylic acid. There were no VTE recurrence and no bleedings in the whole cohort.

Table 1.

Population	n	Age (y)	ClCr (ml/min)	CHA2DS2-VASc	HAS-BLED	Major Bleeding	Minor Bleeding	TEV recurrences
AF (from VKA)	179	75.3±7.4	69±29	4.5	3	0	0	0
VTE	424	62.5±15.3	84.4±31.3	/	/	0	2(0.0047%)	0
p value		<0.00001	<0.00001				0.85	
from VKA	279	62.7±15,1	83.8±31	/	/	0	0	0
from LMWH	101	63.1±14.2	86.2 ± 32	/	/	0	1(0.001%)	0
from Fondaparinux	44	58.8 ±18.3	83.3 ± 26.6	/	/	0	1(0.022%)	0
p value		0.25	0.81	/	/		0.37	

Summary/Conclusions: Safety and efficacy of switching anticoagulant therapy in our center are really satisfying compared to the studies that closely monitored INR to adjust anticoagulant therapy switching, suggesting that monitoring INR may not be routinely needed for the switch. Further studies are needed to confirm these findings.

P412

HIGH HEMOGLOBIN CONCENTRATIONS AND RISK OF THROMBOSIS: CAUSALITY OR CONFOUNDING?

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Background: Patients with polycythemia vera are known to have an increased risk for thrombotic events. This effect has also been observed among those with high as well as low concentrations of hemoglobin (Hgb) in the general population. However many of the earlier studies lack sufficient clinical data to account for underlying disease or causes of high Hgb.
Aims: To assess whether high concentrations of Hgb is an independent risk factor for thrombotic events.
Methods: Hgb measurements and baseline characteristics were obtained from participants in the Reykjavik-AGES study at enrollment in 2002. The Reykjavik-AGES study, a nationwide screening study of 5755 elderly individuals, includes thorough medical history, physical examination, and blood measurements. Lifetime incidents of thrombotic events were recorded up to 2015 in the Icelandic National Health Service and linked to the participants of the study through the National Registry. Primary outcomes of arterial and venous thrombosis were considered separately 10 years before and after enrollment. Hgb measurements at enrollment were used to determine exposure, both as a continuous variable in steps of 10g/L and stratified into five strata (<130, 130-144,145-159,160-175 and >175g/L for men and <120, 120-134,135-149,150-159, >160g/L for women). Men with Hgb concentrations of 120-144g/L and women with Hgb concentration of 120-134g/L were used as reference. Cox proportional hazard regression was used for the statistical analyses and adjusted for confounders (gender, age, body mass index (BMI), diabetes mellitus, smoking, hypertension, and statin use) in three different models.
Results: Analysis of Hgb concentration as a continuous variable in steps of 10 g/L revealed increased risk of arterial and venous thrombosis with increasing Hgb concentration (hazard ratio (HR) 1.06 95% confidence interval (CI) [1.04-1.09] p<0.001 and HR 1.08 95% CI [1.05-1.106] p<0.001). Adjustment for confounders revealed a reverse effect (HR 0.92 95% CI [0.89-0.94] p<0.001 and HR 0.89 95% CI [0.87-0.92] p<0.001). After excluding anemic patients (Hgb <130g/L for men and <120g/L for women) there was however, no association (Table 1). Crude analysis, using stratified Hgb levels, revealed increased risk of arterial and venous thrombosis associated with high Hgb (Hgb: 160-175g/L for men and 150-159g/L for women. HR 1.10 95% CI [1.02-1.20] p=0.02 and HR 1.17 95% CI [1.07-1.29] p<0.001). After adjusting for gender, age, and BMI, this association was no longer evident. There was however marginally increased risk of venous thrombosis in those with slightly higher Hgb concentrations than the reference group (Hgb: 145-

159 g/L for men and 135-149 g/L for women. HR 0.91 CI [0.85-0.98] p=0.01). Anemic participants had a decreased risk of both arterial and venous thrombosis (HR 0.76 CI [0.70-0.83] p<0.001 and HR 0.74 CI [0.68-0.81] p<0.001) (Table).

Table 1.

	Crude analysis HR [95% CI]	Model 1 HR [95% CI]	Model 2 HR [95% CI]	Model 3 HR [95% CI]
Arterial thrombosis				
Continuous Hgb (Steps of 10 g/L)				
All participants (n=5755)	1.06 [1.04-1.09] ***	0.91 [0.89-0.93] ***	0.92 [0.89-0.94] ***	0.91 [0.88-0.93] ***
Excl. Anemia (n=4834)	1.14 [1.10-1.18] ***	0.98 [0.94-1.01]	0.99 [0.95-1.03]	0.97 [0.93-1.01]
Stratified Hgb (Male/Female Hgb g/L)				
<129/<120 (n=921)	0.9 [0.83-0.98] *	0.75 [0.69-0.81] ***	0.76 [0.70-0.83] ***	0.74 [0.68-0.81] ***
130-144/120-134 (n=2784)	1	1	1	1
145-159/135-149 (n=1934)	1.11 [1.03-1.18] **	1.00 [0.93-1.07]	1.00 [0.94-1.08]	0.98 [0.91-1.04]
160-175/150-159 (n=202)	1.10 [1.02-1.20]*	0.99 [0.91-1.08]	1.00 [0.92-1.09]	0.99 [0.91-1.07]
>175/ >160(n=14)	0.95 [0.76-1.18]	0.95 [0.76-1.18]	0.97 [0.76-1.22]	0.93 [0.74-1.17]
Venous thrombosis				
Continuous Hgb (Steps of 10 g/L)				
All participants (n=5755)	1.08 [1.05-1.11] ***	0.90 [0.87-0.92] ***	0.89 [0.87-0.92] ***	0.89 [0.86-0.91] ***
Excl. Anemia (n=4834)	1.13 [1.09-1.17] ***	1.00 [0.95-1.04]	0.99 [0.95-1.04]	0.97 [0.93-1.01]
Stratified Hgb (Male/Female Hgb g/L)				
<129/<120 (n=921)	1.03 [0.95-1.12]	0.74 [0.68-0.81] ***	0.74 [0.68-0.81] ***	0.74 [0.68-0.81] ***
130-144/120-134 (n=2784)	1	1	1	1
145-159/135-149 (n=1934)	1.10 [1.02-1.18] *	0.97 [0.90-1.05]	0.97 [0.89-1.04]	0.91 [0.85-0.98] *
160-175/150-159 (n=202)	1.17 [1.07-1.28] ***	1.04 [0.95-1.14]	1.03 [0.94-1.12]	1.04 [0.95-1.13]
>175/>160 (n=14)	0.95 [0.74-1.22]	0.92 [0.72-1.18]	0.89 [0.68-1.17]	0.85 [0.65-1.12]

*: <0.05, **: <0.01, ***: <0.001, Hgb: hemoglobin
Model 1: Adjusted for gender, age and BMI
Model 2: Adjusted for gender, age, BMI, diabetes, pack years, hypertension and statin use
Model 3: Censored at diagnosis of malignant neoplasm and adjusted for gender, age, BMI, diabetes, pack years, hypertension and statin use

Summary/Conclusions: In this large population based cross sectional cohort study, including detailed clinical information on elderly individuals, we observed that high concentrations of Hgb were not associated with risk of thrombosis after adjusting for confounding factors. Furthermore, we found that low concentration of Hgb was associated with a lowered risk of thrombosis. This is contradictory to many of the previous studies done with less extensive clinical data and suggests that previous studies expressed association by confounding rather than causality.

P413

HEMOSTATIC BIOMARKERS BASED PREDICTIVE MODEL FOR METASTATIC DISEASE, PROGRESSION AND MORTALITY IN CANCER PATIENTS

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Background: Hemostatic factors have been implicated as effectors in key processes of tumor biology. Exploratory clinical studies of plasma hemostatic components as potential tumor biomarkers with putative predictive and prognostic potential could provide useful information for monitoring of cancer patients and justifying novel anti-tumor strategies.
Aims: To determine the predictive utility of TF-dependent microparticle activity (MPTF), tissue factor antigen (TFAg), angiopoietin-2 (ANG-2) and soluble urokinase activator receptor (suPAR) for metastatic disease at onset, disease progression and death in cancer patients undergoing chemotherapy.
Methods: MPTF, TFAg, ANG-2 and suPAR were measured in 128 patients with various cancer types and in 82 healthy controls. MPTF was determined with the Zymuphen MPTF kit; TFAg, ANG-2 and suPAR were measured by ELISA. Conventional coagulation parameters: thrombin time, prothrombin time, fibrinogen and mean platelet volume were determined and VTE score calculated for all patients as well. Patients were followed up prospectively for two years (2013-2015) from chemotherapy initiation until metastatic progression and/or death. Predictive value of the tested variables, including age and sex, was analyzed by retrograde logistic regression. The study has been IRB approved and written informed consent was obtained from all participants.
Results: MPTF activity was significantly decreased in cancer patients compared to controls, while TFAg levels, ANG-2 and suPAR were significantly elevated, p<0.0001. There was no significant association of MPTF, TFAg, ANG-2 with any of the VTE risk groups, however suPAR levels correlated significantly with VTE score (ρ=0.235, p<0.05). Significant predictor variables were determined by logistic regression and the final model included VTE score, MPTF, TFAg, ANG-2 and suPAR. The model tested significant regarding its ability to determine presence of metastatic disease at onset (χ=16.779, p=0.01), disease progression (χ=14.347, p=0.026) and death (χ=17.282, p=0.008). Elevated levels of ANG-2 are associated with increased probability of metastatic disease (OR_{ANG-2}=15.795, p=0.041), TFAg levels are associated with higher probability of disease progression (OR_{TFAg}=1.224, p=0.03), VTE score is associated with mortality (OR=13.488, p=0.041).
Summary/Conclusions: The probability of primary metastatic disease, progression of disese and death in cancer patients increases with higher VTE score, elevated levels of TFAg, ANG-2, suPAR and decreased MPTF procoagulant activity.

P414

ANNEXIN A5 M2 HAPLOTYPE: TO BE OR NOT TO BE, THAT'S THE QUESTION!

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Background: Hereditary thrombophilias are known to impair placental function and cause adverse pregnancy outcomes (APO) such as recurrent pregnancy loss, pre-term labour, intrauterine growth restriction or small for gestacional age newborns. Mutations in blood coagulation factors II and V, alterations in protein C and its co-factor protein S are commonly accepted as hereditary thrombophilias. Furthermore alterations resulting in reduced fibrinolysis have also been associated with APOs. More recently conflicting data on the possible involvement of M2 Annexin A5 (ANXA5) haplotype have been published, with evidence that supports its involvement in embryonic-induced anticoagulation. So far consensus on its role in APO has not been achieved.
Aims: Assess the presence of M2 ANXA5 haplotype in a population of women with a previous history of APO.
Methods: Retrospective case-control study including women referred to the Pregnancy and Thrombophilia Consultation in our Center, between January 2012 and May 2015, with a previous history of APO including recurrent miscarriages (early and late), fetal death, pre-eclampsia and HELLP syndrome. Detailed previous medical history was reviewed. Patients with hereditary thrombophilias, antiphospholipid syndrome or autoimmune diseases were excluded. The genotyping for the presence or absence of carriage of the M2 ANXA5 promoter haplotype was assessed. The possible relationship between M2 and APO was evaluated by comparing our case-group with two independent control groups. Odds ratio (OR) with 95% confidence intervals (CI) and chi-squared tests were calculated. Statistical significance was defined as p<0.05.
Results: The case group included 110 women with a previous history of unexplained APO. They had a mean (range) age of 33.2 years (17-41), median gravidity of 2 (0-7) and median parity of 1 (0-2). Forty-nine women had experienced a total of 108 pregnancy losses (median 2, range 1-5), 91% in the first trimester. Cardiovascular (CV) risk factors were distributed as follows: smoking habit 12.7% (n=14), dyslipidemia 10.9% (n=12), hypertension 2.7% (n=3), only 1 had previous history of diabetes. Mean body mass index (BMI) was 24.62±3.98 kg/m^2. All but one were Caucasians. Eight patients had prior history of thrombotic events (75% venous events). Presence of M2 ANXA5 promoter haplotype was observed in 30% (n=33) of our case group individuals. When comparing our case group with a general control population we found statistically significant association between the presence of M2 haplotype and the presence of unexplained APO (OR 2.35, 95% CI 1.47-3.77; p<0.001). When comparing with a group of fertile female controls we also found a significant association (OR 4.80, 95% CI 2.86-8.05; p<0.0001). No other differences in age, BMI, CV risk factors were identified when comparing carriers of M2 *versus* non-carriers. No association between carriage status for M2 haplotype and subtype of APO was identified.
Summary/Conclusions: A higher frequency of the M2 ANXA5 haplotype was found in our case group when compared with two independent control populations, suggesting a possible relationship between this specific genotype and unexplained APO. Genotyping ANXA5 M2 status seems to be a well oriented approach in the diagnostic of a subset of women with unexplained APO.

P415

IRON METABOLISM IN PATIENTS WITH PORTAL THROMBOSES

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Background: Portal thrombosis (of portal, splenic or superior mesenteric vein) is the most common cause of prehepatic portal hypertension. Bleedings from esophageal and/or gastric varices are the frequent complication of prehepatic portal hypertension. Posthemorrhagic anemia is diagnosed in 58% of patients with prehepatic portal hypertension and is usually considered to be an indication for iron therapy.
Aims: To study iron metabolism in patients with prehepatic portal hypertension due to portal thrombosis and recurrent bleedings from esophageal and/or gastric varices.
Methods: The study group included 70 patients (31 males and 39 females aged from 22 to 73 years, median age 45 years) with prehepatic portal hypertension and portal thromboses diagnosed by abdominal Doppler ultrasonography or computed tomography. Thirty seven patients (53%) of the study group

had recurrent bleedings from esophageal and/or gastric varices. Patients were divided into 2 groups based on the results of hematological examination (blood count and bone marrow morphology): 43 patients with chronic myeloproliferative disorders and 27 patients with cytopenias associated with other disorders (antiphospholipid syndrome, paroxysmal nocturnal hemoglobinuria, inherited thrombophilia). The following parameters were studied to characterize iron metabolism: ferritin, transferrin, iron concentration and total iron binding capacity. In patients previously treated with iron parameters of iron metabolism were investigated not less than 2 weeks after termination of iron therapy.
Results: Anemia was revealed in 34 (48%) patients of the study group. Only 4 (12%) patients with anemia had laboratory signs of iron deficiency. Other 30 (88%) patients with anemia had laboratory signs of functional iron deficiency, which is a key feature of anemia of chronic disease. All patients of the study group were divided in 2 groups: 1) patients who received iron therapy in the past (34 patients) 2) patients who never received iron treatment (36 patients). Frequency of new thrombotic events (*i.e.*, pulmonary embolism, mesenteric thromboses, arterial thromboses, thromboses of jugular vein, myocardial infarction, transient ischemic attack) or portal rethromboses was 35% in the first group of patients and 6% - in the second group. Frequency of recurrent bleedings from esophageal and/or gastric varices was 79% in the first group of patients and 28% - in the second group.
Summary/Conclusions: 88% of pts with prehepatic portal hypertension due to portal thrombosis had anemia of chronic disease. Iron therapy in such patients was associated with higher incidence of new thrombotic events or portal rethromboses and recurrent bleedings.

P416

PERIPHERALLY INSERTED CENTRAL VENOUS CATHETER-RELATED VENOUS THROMBOSIS IN PATIENTS WITH HEMATOLOGICAL DISEASES

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Background: The use of peripherally inserted central venous catheters (PICCs) has been markedly increased during the last decade due to their advantages over conventional central venous catheters, such as a reduced risk of complications during insertion and durable venous access. These characteristics make PICC suitable for use in patients with hematological malignancies. Catheter-related deep venous thrombosis (DVT) has been described as the late complication of PICC. The incidence of PICC-related DVT in patients with hematological disorders is however unknown.
Aims: The aim of this study was to establish the risk factors and evaluate the incidence of the PICC-related DVT in patients with hematological disorders.
Methods: We performed a retrospective cohort study evaluating the thrombotic complication of PICCs placed in a single center between 2011 and 2015 to the patients with hematological malignancies or aplastic anemia with the need of the prolonged venous access. Symptomatic catheter-related DVT was determined by Doppler ultrasound. The following information was retrieved for each patient: type of disease, therapy applied through PICC, serum creatinine level, hemoglobin (Hb) level, white blood counts (WBC) and platelet (Plt) number at the time of PICC placement, and position of the tip of the catheter determined by the chest x-ray. All data were retrieved from the medical records.
Results: A total of 182 patients (104 men/78 women, median age of 50,2 years (range 17-82)) with hematological disorders were included, with 201 catheters and total of 27056 catheter-days. 163 patients had one, and 19 patients had two catheters inserted. Hematological disorders included 53 acute myeloid leukemias, 35 acute lymphoblastic leukemias, 63 malignant lymphomas, 9 multiple myelomas, 9 aplastic anemias and 13 patients in the miscellaneous group consisting of chronic myeloid leukemia, chronic lymphoid leukemia, chronic myelomomocyctic leukemia and osteomyelofibrosis. Indication for the PICC placement included necessity for venous access due to the need for a long-term chemotherapy (129 catheters) and supportive treatment in case of difficult venous access (72 catheters). The incidence of PICC-related venous thrombosis was 11,9% (0,88/1000 PICC days). The median duration form insertion to diagnosis of catheter-related thrombosis was 17 days (range 1-241), with 45% of the events described within the first 7 days from the PICC insertion. WBC count and catheter malposition were found as significant risk factors for PICC-related DVT (p=0,007 and 0,008, respectively) in univariate analysis and only catheter malposition in multivariant analysis (p=0,002, OR 4,37, CI 95% 1,69-11,33).
Summary/Conclusions: The study found catheter-related DVT as important complication of PICC in patients with hematological malignances. Also, catheter malpositioning was identified as s significant risk factor for developing thrombosis. Moreover, our study speaks in favor of the hypothesis suggesting that thrombogenicity of PICC could be related to endothelial injury and phlebitis following the insertion of the catheter, since almost 50% of thrombosis happened within the first seven days from the catheter placement. This study highlights the need of vigilant evaluation of thrombosis risk previous to PICC insertion in patients with malignant hematological diseases and careful monitoring of the patient during the first week of the catheter dwelling. Also, better clinical outcomes could be achieved by recognition of the catheter malposition, and the correction of its position.

P417

EQUATIONS FOR ESTIMATING GFR: COMPARING RENAL FUNCTION CATEGORISATION IN THE CATCH STUDY

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Background: The CATCH study was a randomized, multicenter study (NCT01130025) of 900 patients comparing tinzaparin 175 IU/kg once daily with dose-adjusted warfarin for 6 months in patients with active cancer and acute, symptomatic venous thromboembolism. Only patients with very severe renal impairment at baseline (estimated glomerular filtration rate [eGFR] below 20 mL/min) were excluded from the study. Over time, the recommendations for estimating renal function have changed, reflecting that improved tools for screening for renal impairment have been developed and validated in broader patient populations. These tools have further standardized the estimation of renal function, as they are based on results from isotope dilution mass spectrometry (IDMS) standardized assaying of serum creatinine.
Aims: To compare three different validated equations for estimating renal function in a population of patients with cancer-associated thrombosis, older age and multiple ethnicities.
Methods: eGFR was evaluated in a patient population with cancer-associated thrombosis. Creatinine was determined at baseline, and monthly during the 6-month study. All samples were analyzed centrally using a validated (IDMS) assay. Renal function was estimated using three different equations: the Modification of Diet in Renal Disease (MDRD) study equation, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and the Cockcroft-Gault (CG) equation, using SI units only.
Results: During the CATCH study, 5238 samples were taken from which eGFR could be calculated in 896 patients. Mean age was 59 years; 40% were male; 55% had metastatic cancer at baseline; 53% received anticancer treatment at any time during the study. The racial distribution was: 50% White, 34% Asian, 2% Black, 14% Multiple or Other. The equations provided largely consistent findings when eGFR estimations were below 60mL/min, with an overall agreement between MDRD and CKD-EPI of 97%, between MDRD and CG: 88%, and between CKD-EPI and CG: 89%. The CKD-EPI and MDRD equations had similar results in eGFR range 60-90 mL/min, but whereas MDRD had eGFR estimations ranging up to 350 mL/min, CKD-EPI yielded no eGFR estimations beyond 200mL/min. When MDRD and CKD-EPI were compared with CG, the eGFR above 60 mL/min varied to a much higher degree (Fig. 1). The MDRD/CG comparison covered ranges up to 350–400 mL/min, whereas the CKD-EPI/CG comparison tapered off, limited by CKD-EPI not yielding any values above 200mL/min. Categorisation of severe renal impairment (eGFR below 30 mL/min) differed in only four samples (0.1%) when comparing MDRD and CKD-EPI, whereas when these were compared with CG, 63 and 64 samples (1.2%), respectively, were discrepant.

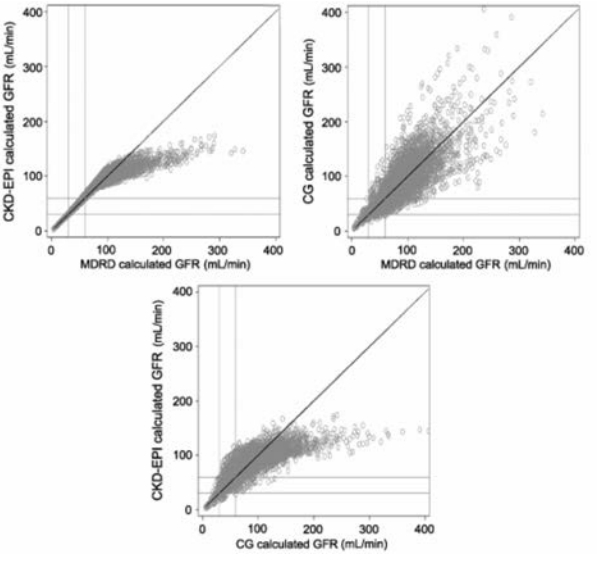

Figure 1. Scatter plots to show the comparsion of equations for estimating renal function, including x=y reference line.

Summary/Conclusions: Overall, there was a strong agreement between MDRD and CKD-EPI for estimating renal function when eGFR estimations were below 60 mL/min. Estimating the eGFR using CG yielded results that diverged to a greater degree from both MDRD and CKD-EPI. CKD-EPI had an added limitation of yielding no eGFR estimations above 200mL/min.

LB418

IMPACT OF CAPLACIZUMAB TREATMENT ON MORTALITY AND MAJOR THROMBOEMBOLIC EVENTS IN ACQUIRED TTP: PHASE II TITAN STUDY RESULTS

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Background: Acquired thrombotic thrombocytopenic purpura (TTP) is a potentially life-threatening thrombotic microangiopathy in which aggregation of platelets to von Willebrand Factor (vWF) leads to profound thrombocytopenia, hemolytic anemia, and systemic microvascular thrombosis. In spite of treatment with plasma exchange and immunosuppression, the current standard of care, mortality from an episode of acquired TTP is approximately 20% and patients remain at risk for thrombotic complications until remission is achieved[1]. Caplacizumab is an anti-vWF Nanobody® in development for the treatment of acquired TTP. The efficacy of caplacizumab in conjunction with standard of care in reducing the time to confirmed platelet count normalization was demonstrated in the Phase II TITAN study of patients with acquired TTP[2].
Aims: To report the results of a post-hoc analysis which assessed the impact of treatment with caplacizumab on the incidence of major thromboembolic events during the study drug treatment period and the incidence of TTP-related mortality during this Phase II study.
Methods: The occurrence of treatment-emergent major thromboembolic adverse events was investigated in the Phase II clinical study database, using the Standardized MedDRA Query (SMQ) for 'embolic and thrombotic events'. The proportion of subjects with at least one of these events together with the total number of events were summarized and reported per treatment group. Transient episodes were not considered major thromboembolic events and were, therefore, not included in this analysis. TTP-related mortality during the study was evaluated based on adverse events reporting, with relatedness to TTP as judged by the Investigator.
Results: The safety population consisted of 35 caplacizumab-treated and 37 placebo-treated subjects. Four major thromboembolic events were reported in 4 subjects in the caplacizumab group (1 pulmonary embolism and 3 TTP exacerbations). In the placebo group, 20 major thromboembolic events were reported in 14 subjects (2 acute myocardial infarctions, 1 ischemic and 1 hemorrhagic stroke, 1 pulmonary embolism, 1 deep vein thrombosis, 1 venous thrombosis and 13 TTP exacerbations). Two TTP-related deaths occurred during the study, both in the placebo treatment group (causes of death: refractory TTP and cerebral hemorrhage). In total, 11.4% of caplacizumab-treated subjects *versus* 43.2% of placebo-treated subjects experienced one or more thromboembolic events or died (Table 1).

Table 1. Treatment-emergent major thromboembolic events during the treatment period and overall TTP-related mortality in the Safety Population of the Phase II TITAN study.

	Caplacizumab (N=35)			Placebo (N=37)		
	# Events	# Subjects	% of subjects	# Events	#Subjects	% of subjects
Embolic and thrombotic events (SMQ)						
Acute myocardial infarction	0	0	0	2	2	(5.4%)
Deep vein thrombosis	0	0	0	1	1	(2.7%)
Venous thrombosis	0	0	0	1	1	(2.7%)
Pulmonary embolism	1	1	(2.9%)	1	1	(2.7%)
Ischemic stroke	0	0	0	1	1	(2.7%)
Hemorrhagic stroke	0	0	0	1	1	(2.7%)
Thrombotic thrombocytopenic purpura [1]	3 [2]	3 [2]	(8.6%)	13	11	(29.7%)
TTP-related mortality						
Deaths related to TTP	0	0	0	2	2	(5.4%)
TOTAL	4	4 [3]	(11.4%)	22	16 [3]	(43.2%)

[1] this preferred term consisted of recurrences of TTP during the treatment period, defined in the protocol as exacerbations of TTP
[2] one adverse event reported as 'Thrombocytopenia' was not considered in this analysis, as this event was reported as part of the presenting disease
[3] a subject may have experienced more than one event

Summary/Conclusions: Compared to subjects who received placebo and standard of care treatment, a lower proportion of those treated with caplacizumab and standard of care had one or more major thromboembolic adverse events or died. The results of this post-hoc analysis suggest that treatment with caplacizumab has the potential to reduce the significant morbidity and mortality associated with acquired TTP. A phase III confirmatory study is ongoing and will include a prospectively defined assessment of this clinically meaningful endpoint.

References

1. Peigne V, *et al.* Intensive Care Med. 2012;38(11):1810-1817;
2. Peyvandi F, *et al.* N Engl J Med. 2016;374(6):511-522.

Quality of life, palliative care, ethics and health economics 1

P418

A PROSPECTIVE EVALUATION OF THE IMPACT OF TRANSFUSION DEPENDENCY ON QUALITY OF LIFE AND SYMPTOM BURDEN IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Red blood cell (RBC) transfusion support is standard of care in the majority of patients with myelodysplastic syndromes (MDS). Despite that, there is a dearth of information regarding its impact on patient quality of life (QoL).
Aims: The main objective of this study was to investigate the burden of transfusion dependency, by comparing patterns of QoL over time between transfusion dependent (TD) *versus* transfusion independent (TI) patients.
Methods: Analysis is based on 280 newly diagnosed IPSS intermediate-2 or high risk (*i.e.*, higher risk) MDS patients who were consecutively enrolled in an international prospective cohort observational study. Quality of life was assessed at baseline (*i.e.*, before treatment) with the well validated EORTC QLQ-C30 questionnaire and thereafter at week 4 and week 8 after the first treatment start. Transfusion dependency at baseline was defined as having received at least one red blood cell transfusion every 8 weeks over a period of 4 months (Malcovati L, et al, *J Clin Oncol*. 2007; 25: 3503-10). Differences were assessed at baseline between TD and TI by Fisher exact and Wilcoxon-Mann-Whitney tests as appropriate. Trajectories over time of QoL outcomes were estimated for TD and TI patients by a linear mixed model with an unstructured covariance structure which accounted for treatment received (hypomethylating agents and intensive chemotherapy *vs* lower intensity therapies). An overall F test was used to test the significance of possible differences of estimated QoL patterns over time between TD and TI groups. No adjustment was performed for multiple testing thus statistical significance was set as α=0.05.
Results: Median age was of 71 years and there were 176 men (63%) and 104 (37%) women. Mean Hb level for the overall population was 9.3 g/dl (range 4.1-15.8). There were 224 patients (68%) considered as TI and 56 (32%) considered as TD. At baseline, there were no statistically significant differences between TD and TI patients with regard to age, gender, IPSS risk score (int.-2 *vs* high risk) and comorbidity. With regard to functional limitations at baseline, worse statistically significant differences for TD patients were found for physical functioning (P=0.003), role functioning (P=0.013), social functioning (P=0.039) as well as for global QoL (P=0.012). Greater symptom severity for TD patients were found for fatigue (P=0.020), pain (P=0.034) and dyspnea (P=0.008). After baseline assessment, treatment with hypomethylating agents was the most common one being received by 165 patients (59%). Investigation of QoL trajectories over time between groups, since treatment start, revealed that patterns of QoL changes was different between groups regardless of type of therapy. QoL of the overall population generally deteriorated during the first two months of therapy. However, the magnitude of this change was worse in TD patients. This latter group, compared to TI patients, deteriorated to a greater extent with regard to physical functioning (P=0.019) and role functioning (P=0.009). The same pattern was observed for fatigue (P=0.019) and pain (P=0.014).
Summary/Conclusions: This study indicates that transfusion dependency not only impairs QoL of MDS patients at baseline, but also limits recover over time compared to patients who are not transfusion dependent at the start of therapy. Future longer follow-up studies are needed.

P419

EFFECT OF LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE TREATMENT UNTIL PROGRESSION ON HEALTH-RELATED QUALITY OF LIFE OVER TIME IN TRANSPLANT-INELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: The FIRST trial established a progression-free survival and overall survival benefit for transplant-ineligible patients with newly diagnosed multiple myeloma (NDMM) treated with lenalidomide plus low-dose dexamethasone (Rd) until disease progression (Rd continuous) *vs* melphalan, prednisone, and thalidomide (Benboubker et al, *NEJM*, 2014). Furthermore, treatment with Rd improved health-related quality of life (HRQoL) over the first 18 months of treatment (Delforge et al, *Haematologica*, 2015). HRQoL data were not collected past 18 months.
Aims: The aim of this analysis was to examine the effect of Rd continuous on HRQoL beyond 18 months by identifying predictors of change in HRQoL scores using data from the FIRST trial.
Methods: Univariate linear mixed-effects regression analyses (n=535) identified variables collected until progression as predictors of 7 preselected HRQoL domains of interest: global QoL, physical functioning, fatigue, and pain (EORTC QLQ-C30), disease symptoms and side effects of treatment (EORTC QLQ-MY20), and health utility (EQ-5D). Variables with univariate $P<.1$ were combined into multivariate models for each HRQoL domain; variables with $P<.1$ were retained for final models. Modeled HRQoL domain score changes were validated against observed score changes prior to 18 months and extended to extrapolate HRQoL changes from baseline based on observed predictor values beyond 18 months for patients still receiving Rd at 24, 30, 36, 42, and 48 months.
Results: Key time-varying determinants of final mixed models of HRQoL domains included: eastern Cooperative Oncology Group performance status: all domains; ongoing hospitalization: global QoL, fatigue, side effects of treatment, health utility; serum albumin: global QoL, physical functioning, fatigue, pain, side effects of treatment, health utility; red blood cell transfusion within 30 days: fatigue, pain, disease symptoms; ongoing adverse events: global QoL; ongoing grade ≥3 pain: physical functioning, fatigue, pain, disease symptoms, side effects of treatment, health utility; ongoing grade ≥2 fatigue: fatigue; ongoing grade 3/4 anemia: physical functioning, side effects of treatment. Nearly all observed changes in HRQoL scores from baseline fell within the 95% confidence interval of the final predictive models, including the final EQ-5D health utility model (Figure 1). Exceptions were month 1 of the global QoL and fatigue models and month 3 of the disease symptoms model.

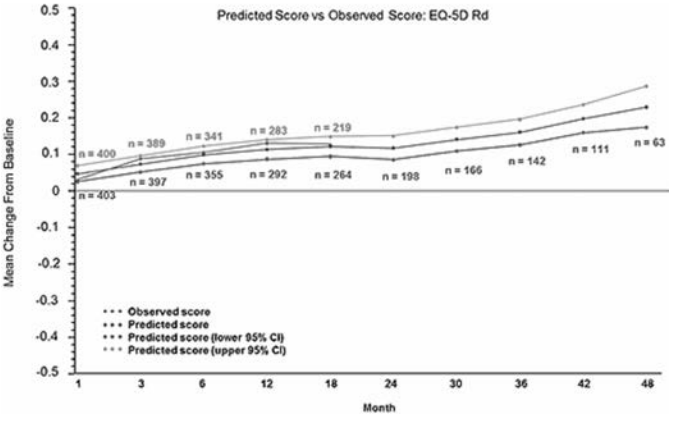

Figure 1.

Summary/Conclusions: Our results suggest that the gains in HRQoL over the first 18 months in Rd-treated patients with NDMM are maintained for patients on treatment up to 48 months. The analysis is exploratory in nature; further research is needed to better define the impact of continuous therapy on HRQoL compared to a fixed duration of therapy followed by a period of observation.

P420

HEALTHCARE COSTS AMONG NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS UNDERGOING FRONTLINE STEM CELL TRANSPLANTATION IN THE U.S. COMPARISON OF OUTPATIENT *VERSUS* INPATIENT-BASED CARE

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Background: The use of stem cell transplantation (SCT) in the U.S. in patients (pts) with MM has increased from 5.1% to 25.1% between 1994 and 2005 (McCarthy 2013), with inpatient (inpt) costs increasing from $684 million in 2004 to $1.3 billion in 2007 for SCT procedures overall (Strangers 2009). However, improved supportive care has enabled a shift of SCT to the outpatient (outpt) setting. Prior research in small cohorts suggests that this shift may produce substantial cost savings (Rizzo 1999; Frey 2002) but these studies were based on single-institutional experiences and focused on short-term costs only.
Aims: The goal of this study was to compare short- and long-term costs of outpt- *vs* inpt-based SCT among MM pts in a large, national, U.S. healthcare claims database receiving SCT within 300 days post-initiation of initial MM-directed therapy (frontline SCT).
Methods: Adult pts with newly diagnosed MM were identified between 1/2008 and 3/2015 from among commercially insured and Medicare Advantage beneficiaries. Pts with continuous enrollment (CE) from 12 months (mos) prior to the first observed claim for MM were included. Pts with frontline SCT were identified and stratified according to SCT setting (inpt *vs* outpt). Short- and long-term costs were estimated from 14 days prior to SCT through 100 days and 12 mos after SCT, respectively, among pts with CE (including pts who died). Total healthcare costs (payer + patient paid) during each time period were calculated. Baseline characteristics and costs were compared using univariate analysis (chi-square test for categorical and t-test for continuous variables). Generalized linear models compared 100-day and 12-mos costs for the outpt *vs* inpt groups.
Results: Of 607 MM pts with frontline SCT, 97% received autologous SCT; 67% (n=407) underwent SCT during an inpt stay. Compared to pts receiving outpt SCT, the inpt SCT group was older (median age: 58 *vs* 60 years old, P=.02), but baseline Charlson Comorbidity index score and gender distributions were similar across cohorts. In the inpt SCT group, initial hospitalization length of stay was 17 days (IQR: 3). Post-SCT hospitalizations in the outpt SCT cohort were more common than either rehospitalizations within 100 days (50% *vs* 20%, p<0.001, n=536) or within 12 months (58% *vs* 40%, p<0.001, n=390) in the inpt SCT cohort. Overall, the mean 100-day and 12-mos costs were $133,215 and $228,497, respectively. Total mean 100-day costs were higher among the inpt *vs* outpt SCT group (adjusted: $139,269 *vs* $121,313, p=0.004). Mean difference in 12-mos costs remained higher among inpt *vs* outpt SCT (adjusted: $238,184 *vs* $211,734, p=0.059) but did not reach statistical significance (Table 1).

Table 1. Costs associated with frontline SCT among MM patients.

		Overall (N=607)	Inpatient Transplant (N=407)	Outpatient Transplant (N=200)	p-value
Costs (2014 US$)					
Total unadjusted cost					
Within 100 days[a]	mean (SD)	133,215 (69,316)	139,007 (71,811)	121,760 (62,731)	0.004
	median	120,397	125,587	113,149	
	IQR	56,968	56,195	50,495	
Within 12-months[b]	mean (SD)	228,497 (143,649)	235,750 (154,168)	215,102 (121,256)	0.146
	median	202,496	207,808	196,812	
	IQR	115,193	110,492	121,062	
Total adjusted cost[c]					
Within 100 days[a]	predicted mean	-	139,269	121,313	0.004
Within 12-months[b]	predicted mean	-	238,184	211,734	0.059
Inpatient unadjusted cost					
Within 100 days[a]	mean (SD)	75,138 (67,933)	103,188 (62,485)	19,663 (37,286)	<0.001
	median	79,288	93,966	0.00	
	IQR	91,921	43,975	24,488	
Within 12-months[b]	mean (SD)	89,783 (109,152)	123,708 (117,445)	27,133 (49,247)	<0.001
	median	51,965	102,567	4,584	
	IQR	103,225	58,456	31,085	
Outpatient unadjusted cost					
Within 100 days[a]	mean (SD)	49,049 (47,738)	29,532 (27,584)	87,650 (55,194)	<0.001
	median	32,577	22,334	87,142	
	IQR	63,376	29,537	56,522	
Within 12-months[b]	mean (SD)	91,129 (84,014)	66,534 (65,087)	136,548 (95,581)	<0.001
	median	69,571	52,203	117,690	
	IQR	80,347	53,357	68,901	

IQR: interquartile range; SD: standard deviation;
[a]14 days prior through 100 days post SCT
[b]14 days prior through 365 days post SCT
[c]adjusted for age, CCI, gender, days from diagnosis to transplant, transplant year

Summary/Conclusions: Total short-term costs were lower in pts undergoing outpt SCT compared with the inpt SCT group. A trend towards lower long-term total costs was noted in the outpt *vs* inpt SCT groups at 12 months. However, in both time frames, the initial cost savings associated with outpt SCT were partially offset by higher hospitalization rates both in the first 100 days and the first 12 months after transplant. Future work will examine cost drivers, including post-transplant complications.

References

1. McCarthy *et al. Biol BMT* 2013; 19: 1116-23;
2. Strangers *et al.* AHRQ 2009 [HCUP Statisitcal Brief 82];
3. Rizzo *et al. JCO* 1999; 17: 2811-18;
4. Frey *et al. BMT* 2002; 30: 741-48.

P421

COST-EFFECTIVENESS OF OFATUMUMAB FOR MAINTENANCE IN RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is incurable disease and patients eventually relapse after lines of therapies. Ofatumumab (OFA) is the first therapy approved by the Food Drug Agency as extended treatment in relapsed CLL. PROLONG was phase III trial where 474 subjects in remission after second- or third-line therapy for CLL were randomized 1:1 to OFA or observation (OBS). OFA showed lengthened progression free survival (PFS) by 14.2 months (median 29.4 *vs* 15.2; hazard ratio [HR] 0.49, p<0.0001) and longer time to next treatment (median 38.0 *vs* 31.1 months; HR 0.623, p=0.005). The estimated median overall survival (OS) was not reached in either arm (HR 0.85; p=0.517). In addition to clinical efficacy, economic evaluation of novel health care technologies is important for decision making in both Europe and North America.
Aims: To evaluate the cost-effectiveness of OFA compared to OBS as maintenance treatment in patients with relapsed CLL who are in remission after induction therapy.
Methods: A partitioned survival model, commonly adopted in economic evaluations based on randomized clinical trials in oncology, was developed with three health states: progression free, post-progression, and death. Parametric survival functions for PFS, OS, and time to treatment discontinuation were developed based on the PROLONG individual patient data. Model fit was assessed by Akaike Information Criteria and Cox-Snell residual plots. Resource use was modelled adopting a Canadian public health care system perspective. Costs (2015 CAD) included drug acquisition and administration, medical care for adverse events, and pre- and post-progression routine care including follow up therapies (a variety of mono and combo therapies) based on the PROLONG data. Utilities were sourced from the literature, and used to calculate quality-adjusted life years (QALYs) based on modeled PFS, OS, and adverse events (grade 3 or 4 events occurring in at least 2% of the PROLONG patients). Cost-effectiveness was evaluated as the incremental cost per QALY gained, and uncertainty was explored through deterministic and probabilistic sensitivity analyses.
Results: Lognormal functions were selected to model PFS and OS. The former included a treatment effect (acceleration factor) that was applied for two years (whilst patients remained progression free and on-therapy), consistent with the trial; due to the immaturity of the OS data and the availability of effective salvage treatments following relapse, no impact of OFA on OS was assumed. Over patients' modelled lifetime, OFA provided an additional 1.26 progression free life years. After applying utilities and discounting (at 5% per annum), this translated into 0.44 QALYs gained. OFA was associated with approximately an additional cost of CAD $30,000 over patients' lifetime. Incremental cost/QALY was CAD $68,600 (95% confidence interval 49,900-107,600), with a 96% probability of being cost-effective at a threshold of 100,000 CAD/QALY. Deterministic sensitivity analyses suggested OFA's cost-effectiveness was most sensitive to shorter time horizons and quality of life weights for post-progression, but was largely insensitive to alternative scenarios regarding PFS and OS functions, including direct use of Kaplan-Meier survival estimates.
Summary/Conclusions: In the PROLONG trial OFA demonstrated substantial gains in terms of PFS and time to next therapy. OFA may represent a cost-effective addition to the management of relapsed CLL patients who have responded to induction therapy.

P422

PATIENT PREFERENCES - SURVEY OF HODGKIN LYMPHOMA SURVIVORS ON TREATMENT-ASSOCIATED BURDEN AND SIDE EFFECTS

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Background: Hodgkin lymphoma (HL) survivors often report impaired quality

of life due to side effects such as organ failure, fatigue or second malignancies. Patient's experiences, needs and worries related to therapy-associated and social burden are mostly unclear.
Aims: To evaluate the relevance of efficacy outcomes and side effects from the patient's point of view, we created a questionnaire for long-term HL survivors. In addition, the impact of treatment efficacy was put into perspective with long-term side effects of treatment.
Methods: The questionnaire was developed by an interdisciplinary team of physicians, psychologists and statisticians. It consists of six blocks of questions, each containing multiple aspects to be rated on four-point Likert scales. We sent the questionnaire to randomly selected 150 male and 150 female patients of each of our 5th generation trials (HD13, HD14, HD15) with no documented refractory disease or relapse (rrHL) (N=900) and to all candidates with documented rrHL (N=249).
Results: 52% of eligible patients returned the questionnaire (N=484 without rrHL, N=97 with rrHL) with a median follow up from study entry of 107 months. Chemotherapy was a substantial burden for 76% of the participants, independent of stage, treatment and study. Patients in early favorable (HD13) and unfavorable (HD14) stages, received standard radiotherapy (RT) with 30 Gy and for 64% of them, this was a great or very great burden. In advanced stages (HD15), RT was only performed in patients with PET-positive residual tissue after chemotherapy. 26% of them rated RT as great or very great burden. Fatigue was the most frequent side effect, reported by 87% during and 41% after treatment, and considered as a great or very great burden by 75% of the affected patients. Other acute side effects frequently considered as a burden were related to social aspects such as strain for the family and loss in productivity. Most long-term side effects were significantly more frequent in patients with rrHL. Socioeconomics aspects such as financial problems are considered a very great burden. At the time of first diagnosis, >50% of participants had concerns about acute and long-term side effects, relapse and death due to HL. However, concern about relapse was mentioned most frequently. At the time of survey, 25% of patients without rrHL still worried much or very much about side effects of therapy and death from HL and 40% worried much or very much about long-term effects and relapse. In participants with rrHL, concern of side effects or death from HL was still present in 35%, whereas the percentage of patients worrying about long-term effects or another relapse increased to about 60%. Cure of HL was rated as the most important treatment goal by 72% of patients. Retrospectively, only 9 participants (2%) would have chosen a slightly less effective therapy to avoid side effects. Overall, >80% of patients felt well informed about their disease and therapy but 35% felt uninformed regarding potential late effects.
Summary/Conclusions: HL patients frequently consider chemotherapy as a great burden independent of duration and intensity of the administered regimen. HL survivors are more concerned about relapsing than dying from HL, which could be interpreted as fear of another treatment. The results suggest that avoidance of relapse should be the primary therapy goal, and cure is more relevant than possible side effects. Our results imply that progression-free survival is a very important patient-relevant outcome in clinical studies in HL.

P423

UNDERESTIMATED IMPACT OF MILD COGNITIVE IMPAIRMENT IN "CLINICALLY FIT" OLDER PATIENTS WITH MALIGNANT HEMOPATHIES ADMITTED TO RECEIVE CHEMOTHERAPY

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Background: We reported recently that neither the G8 screening tool nor the CGA total score (≥2 impairments) seem to predict chemotherapy completion and overall survival (OS) among *clinically fit* older patients with malignant hemopathies (1). Regarding survival, we found in a multivariate analysis that only mild cognitive impairment (MCI) (MMSE<27 and/or MoCA<26) in the CGA had a predictive value for one-year OS (1). However, this study did not investigate the relationship between MCI and other specific, clinical and biological parameters of the CGA.
Aims: 1) To determine the predictive value of MCI, clinical and biological parameters in terms of chemotherapy completion and two-year OS. 2) To further investigate whether MCI is correlated with specific clinical and biological parameters and two-year OS.
Methods: CGA were proposed to 117 consecutive patients (65-89yrs) with malignant hemopathies assessed by their physicians as *clinically fit* to receive chemotherapy, meaning no geriatric syndromes and/or irreversible comorbidities significantly impairing their daily function. Biological parameters, chemotherapy completion and causes of deaths were extracted from medical records.
Results: One hundred and two patients were evaluable. Eighty-six percent had a favorable prognosis (CLL, Lymphoma or Multiple Myeloma) and 69% received a full dose of chemotherapy at the time of treatment initiation. Non-completion of chemotherapy (38%; n=39) was predicted only by polypharmacy (drugs ≥5) at the time of treatment initiation (OR=2.83, 95%CI: 1.16-6.91; p=0.023). After adjusting for established prognostic factors, two-year OS (54%, n=52) was predicted by disease recurrence (HR=4.28, 95%CI: 1.42-12.88; p=0.010); MCI (MoCA <26) (HR=4.83, 95%CI: 1.34-17.41; p=0.016), low albumin (HR=1.11, 95%CI: 1.01-1.22; p=0.040) and elevated potassium level (HR=0.32, 95%CI: 0.11-0.89; p=0.030). MCI was associated with a reduced-dose of chemotherapy at the time of treatment initiation (p=0.007), higher age (p=0.025), loss of functional autonomy (p=0.002), a higher number of comorbidities (p=0.036).
Summary/Conclusions: In our selected population of clinically fit patient with malignant hemopathies 1) polypharmacy predicted the non-completion of chemotherapy 2) in addition to classical prognostic factor (advanced disease), MCI, poor nutritional status and severe ionic impairment appear to be powerful prognostic factors. The presence of MCI in *clinically fit* older patients with malignant hemopathies may be a marker of an unsuspected vulnerability. Moreover, MCI seems to be a risk factor for under-treatment of *clinically fit* older patients with malignant hemopathies. Prospective trials are required to further determine whether better identification and management of MCI in *clinically fit* older patients with malignant hemopathies could improve survival.

Reference

1. Dubruille S *et al.* Identification of clinical parameters predictive of one-year survival using two geriatric tools in *clinically fit* older patients with haematological malignancies: major impact of cognition. J Geriatr Oncol, 2015, 6, 362-369.

P424

WHAT DO PATIENT EXPERIENCE DATA REVEAL ABOUT THE DIAGNOSTIC JOURNEY FOR MYELOMA PATIENTS? FINDINGS FROM SECONDARY ANALYSIS OF NATIONAL CANCER PATIENT SURVEY DATA

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Background: Despite improvements in long-term survival myeloma remains one of the most difficult cancers to diagnose. In 2010 34% of myeloma (MM) patients in England were diagnosed through an emergency route, one of the highest rates of all cancers. While 78% of MM patients are now surviving >1 year after diagnosis this drops to 51% for patients diagnosed as an emergency. Dealing with a diagnosis of a rare and complex cancer can be a major challenge for patients. It is critical that MM patients receive clear and accurate information during testing and subsequent diagnosis. To date there is little evidence derived directly from MM patients to assess the current state of the myeloma diagnostic journey. Such evidence is crucial for developing strategies for policy makers, professionals and patient groups to improve patient experiences and outcomes.
Aims: The study aimed to explore patient experiences of the myeloma diagnostic journey from symptom presentation to receiving a diagnosis and compare MM patient experiences during diagnosis with the all cancer (AC) average.
Methods: Myeloma UK obtained data from the National Cancer Patient Experience Survey (NCPES) 2014 to conduct secondary analysis of MM patients' reported experiences of diagnosis. The NCPES is the largest NHS patient experience survey of its kind, covering a comprehensive journey through care. In 2014, 70,141 cancer patients responded across England, a response rate of 64%, including 4,023 MM patients. The large sample size provides a reliable basis from which to draw conclusions about MM patients' experiences of diagnosis. We compared MM patient responses to questions covering diagnosis with those for AC patients using +/-5% to indicate practical significance.
Results: Our comparative analysis with the cancer average found MM patients reported significantly worse diagnosis-related experiences than AC patients, from length of time in primary care before referral to receiving information about their tests and diagnosis. The data showed MM patients made significantly more visits to their GP with symptoms than the AC average. Of those MM patients who visited their GP with symptoms before their diagnosis 20% made ≥5 visits before being referred to hospital compared to just 9% of AC patients. Only 31% of MM patients were referred after just one visit compared to 54% of AC patients. There were also significant differences in how long it took patients to see a hospital doctor after first suspecting something might be wrong, with 28% of MM patients reporting a wait of ≥3 months before seeing a hospital doctor compared to 19% of AC patients. More MM patients (37%) said that their health got worse during this time than AC patients (20%). In relation to information provision, MM patients scored significantly worse than the AC average on receiving easy-to-understand written information before their diagnostic test(s) (MM 80%, AC 87%) and their test results being explained in a way they completely understood (MM 72%, AC 78%). Only 52% of MM patients said they understood the explanation of their subsequent diagnosis compared to 73% of AC patients.
Summary/Conclusions: The analysis confirms that diagnosis is a particular challenge in myeloma, right through from initial symptom presentation, testing and receiving information following diagnosis. These data emphasise a critical need to increase understanding of the barriers to early diagnosis in myeloma and identify ways to tackle them. Follow-up qualitative research with patients would be useful to explore their experiences of symptom presentation in primary care in more depth. Myeloma UK will raise awareness of these findings and

use them to further develop our Myeloma Diagnosis Pathway and other educational resources for general practitioners. Through targeted information provision and initiatives such as our Clinical Service Excellence Programme we will continue to work with primary and secondary care professionals to ensure every myeloma patient is given quality information at critical time-points through their diagnosis journey.

P425

STREAMLINING OF THE CHEMOTHERAPY SERVICE WITH FOCUS ON DRUG SUPPLY AND PATIENT SAFETY

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Background: In times of increasingly complex requirements for preeminent patient (pt) care accompanied by high pt turnover, organisational, safety, quality and financial aspects play a pivotal role. In an interdepartmental, cross functional cooperation, we are investigating the feasibility and benefits of implementing dose-banding in chemotherapy (CTx) at our university center. The dose-banding concept very elegantly and efficiently allows the preproduction of frequently used doses for CTx substances with adequate stability data, offering potential to substantially improve the process of drug supply for pts. As part of the CTx service at Freiburg university medical center, CTx prescriptions are generated via an e-database (Chemo-AS) by the physicians and clinically checked by a surveillance team: the Clinical Cancer Research Group (CCRG) in close cooperation with pharmacy. Any detected error is instantly reported, corrected and recorded electronically. This system has now been established for over 10 years resulting in an interception of 99.9% of the CTx related errors (Lohfert award 2015).
Aims: a) Identify CTx substances suitable for dose banding from a practical and stability point of view and to implement dose banding into clinical practice. b) Gain insight into causes and potential consequences of CTx prescribing errors. c) Design a next generation software with improved safety features.
Methods: Analysis of local prescribing practice via the pharmacy database (Zenzy) applying the banding model described by Zavery et al *(Clin Pharm 2011; 3:116-18)*. In order to simulate the worst case, microbiological stability tests were performed using liquid media instead of CTx: samples were stored for 3 months and incubated at different time points. In an additional container integrity test, the stored infusion bags and syringes were inserted into a highly contaminated broth for 1 hour, incubated and analysed for sterility. Physical and chemical stability tests (colour, particles, pH-changes) for a maximum storage period of 3 months of the respective CTx preparations at relevant concentrations were performed. A quantitative assay was conducted by HPLC analysis. We also performed a detailed analysis of 406 CTx prescribing errors prevented by CCRG initiative using SPSS. The data had been collected prospectively over 2 years (2013/2014) via Chemo-AS by the CCRG. In an interdisciplinary approach, in cooperation with IT specialists, the causes of the errors were analysed for effective future preventability.

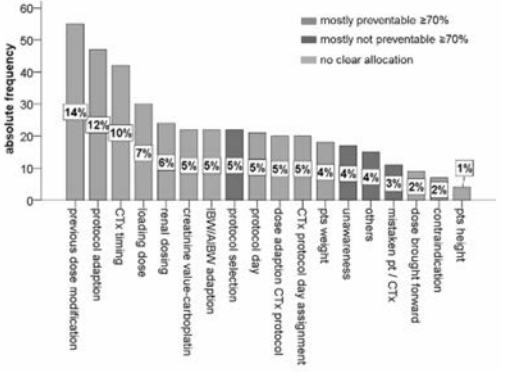

Figure 1. Analysis of CTx error preventability software engineering.

Results: Gemcitabine, 5-FU bolus and carboplatin were identified as most suitable CTx preparations for dose banding by statistical analysis. Microbiological stability could be demonstrated over the complete storage period and all preparations passed the container integrity test. For the selected substances physical and chemical stability over 3 months was shown. In line with international guidelines, we determined a gemcitabine content of ≥95% at 12 weeks storage. The 406 prescribing errors of 18.823 CTx orders were affecting 375 (2%) orders, 303 (12,4%) tumor pts and 508 (1,3%) CTxs. Errors were divided into categories of potential consequences: overdose 203 (51%), underdose 59 (15%), incorrect length of cycle/CTx timing 56 (14%), wrong CTx 40 (11%), CTx not ordered 38 (10%) (with 10 errors additive in consequence). 256 CTxs were affected by the 203 overdosed CTx orders: 110 (43%) were overdosed ≤25%, 66 (26%) with 26-50%, 63 (25%) with 51-100% and 17 (7%) >100%. Of all errors analysed, 61% were identified as avoidable by specific software improvements. Results of the assessment of error preventability by program engineering are shown in Figure 1.
Summary/Conclusions: This analysis shows that dose banding is feasible for gemcitabine and possibly other CTx preparations taking into account statistical and stability aspects. Our "error analysis" was very useful for pinpointing areas, where CTx error reduction by software engineering is possible. However as, according to our analysis, 30-40% of errors are not electronically avoidable, the CCRG, ward pharmacists or other surveillance teams remain indispensable. Dose banding, together with an upgraded CTx prescribing software tool, bear high potential for moving towards standardisation and considerable improvement of the CTx service at Freiburg university hospital. This contributes to a very safe therapy and reduces waiting time for pts as well as ensuring support for doctors and pharmacy staff. Moreover, our new CTx software will be available to other external hospitals, resulting in shared benefit for a large number of pts and healthcare staff.

P426

INTERIM QUALITY OF LIFE RESULTS WITH VENETOCLAX (ABT-199/GDC-0199) MONOTHERAPY IN PATIENTS WITH RELAPSED/REFRACTORY DEL(17P) CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is associated with reduced health-related quality of life (HRQoL) with progressive severe fatigue being a particularly relevant burden. Additionally, treatment can further reduce HRQoL, based on a recent study from Netherlands (Holtzer-Goor KM *et al.* QLR 2015). Recently, deep and durable responses to the oral BCL-2 inhibitor venetoclax (VEN) were achieved in patients with relapsed/refractory (R/R) CLL with deletion of chromosome 17p [del(17p)] in a phase 2 study (NCT01889186, Stilgenbauer S *et al.* ASH 2015).
Aims: To evaluate changes in HRQoL in patients with VEN monotherapy treatment based on interim results from this study.
Methods: In this single-arm trial, patients ≥18 years of age with R/R del(17p) CLL received VEN monotherapy. Patient-reported HRQoL measures were an exploratory endpoint for this study and included the EORTC-QLQ-C30 and EORTC-QLQ-CLL16. The mean changes from Baseline (BL) to visit Weeks 4, 12, 24, 36, and 48 were reported. Clinical relevance was based on minimum important difference (MID) of values from BL at different assessment points. The lower bound of 5–10 point changes, previously considered a "little" change for EORTC-QLQ-C30 (Osoba D *et al.* JCO 1998 and Osoba D *et al.* QLR 1994) was used for MID acceptance for this and EORTC-QLQ-CLL16.

Table 1.

Table 1. Clinically relevant changes in EORTC-QLQ-C30 and EORTC-QLQ-CLL16 parameters with VEN monotherapy treatment.

EORTC-QLQ-C30 parameter (N)	BL Mean	Visit Mean	Mean change from BL (95% CI)
Global Health Status			
Week 4 (70)	55.2	64.4	9.2 (4.3, 14.1)
Week 24 (73)	58.6	67.9	9.4 (3.3, 15.5)
Emotional functioning			
Week 4 (73)	72.0	81.2	9.2 (5.4, 12.9)
Week 24 (76)	74.6	82.4	7.9 (3.6, 12.1)
Role functioning			
Week 4 (70)	64.3	73.6	9.3 (3.7, 14.8)
Week 24 (74)	68.0	79.1	11.0 (3.8, 18.2)
Social functioning			
Week 4 (73)	64.6	71.5	6.8 (0.5, 13.2)
Week 24 (76)	69.1	80.9	11.8 (5.6, 18.1)
Fatigue			
Week 4 (74)	42.4	35.7	-6.7 (-11.7, -1.7)
Week 24 (77)	37.1	30.6	-6.5 (-11.6, -1.3)
EORTC-QLQ-CLL16 parameter (N)	**BL Mean**	**Visit Mean**	**Mean change from BL (95% CI)**
Future health			
Week 4 (73)	56.6	39.7	-16.9 (-23.4, -10.4)
Week 24 (73)	54.8	32.9	-21.9 (-30.1, -13.7)
Fatigue			
Week 4 (75)	34.0	24.0	-10.0 (-16.1, -3.9)
Week 24 (77)	29.7	21.2	-8.4 (-14.3, -2.6)

Results: Clinically relevant gains in global health status and role, social, and emotional functioning of the EORTC-QLQ-C30 occurred from Week 4 to Week 24 (Table 1). Furthermore, early and sustained improvements in fatigue were shown in both EORTC-QLQ-C30 and EORTC-QLQ-CLL16 (Table 1). Associated with these positive HRQoL results was considered as a large effect (>20 points) in patient EORTC-QLQ-CLL16 future health views at Week 24 (Table 1) (Osoba D *et al.* JCO 1998 and Osoba D *et al.* QLR 1994). These clinically relevant improvements were seen in patients having generally lower BL HRQoL than patients in a recent Netherlands study using similar measures.
Summary/Conclusions: Understanding the effect of therapy on HRQoL and patient well-being is an important factor when making treatment choices. These interim results showed that in this very symptomatic and difficult to treat patient population, VEN improved HRQoL and patients' view of their future health.

SIMULTANEOUS SESSIONS II

Innovative therapies in CLL

S427

PRELIMINARY SAFETY DATA FROM THE PHASE 3B GREEN STUDY OF OBINUTUZUMAB (G) ALONE OR COMBINED WITH CHEMOTHERAPY FOR PREVIOUSLY UNTREATED OR RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: GREEN (NCT01905943) is an ongoing phase 3b, open-label trial of G alone or with chemotherapy in pts with previously untreated (1L) or relapsed/refractory (R/R) CLL; the primary outcome is safety.
Aims: To assess the frequency, type and severity of AEs in all pts in GREEN, based on a 6-month safety review (data cut-off for analysis, 26 August 2015).
Methods: Enrolled pts are aged ≥18 yrs with documented CLL, ECOG PS 0-2 and adequate hematologic function. Pts receive G 1000mg IV, alone or with chemotherapy (at investigator's discretion), on days 1 (dose split over 2 days [25+975mg or 100+900mg]), 8 and 15 of cycle (C)1, and day 1 of C2-6 (six 28-day cycles). Chemotherapy options were: fludarabine and cyclophosphamide (FC) for fit pts only (CIRS ≤6 and CrCl ≥70mL/min); chlorambucil (Clb) for unfit pts only (CIRS >6 and/or CrCl <70mL/min), or bendamustine (B) for any pt. Pts refractory to previous G monotherapy had to receive G with chemotherapy. All pts gave informed consent.
Results: In 825 pts analyzed (1L, 485; R/R, 340), median age was 66.0 (33-90) yrs, 63.4% were male, 434 (52.6%) were at risk of tumor lysis syndrome (TLS; tumor burden ≥10cm or ≥5cm but <10cm with lymphocytes ≥25x10^9/L) and 41.1%/33.5% were Binet stage B/C. Median observation time was 12.7 (0.1-22.1) months and median exposure time was 20.3 (0.1-33.1) weeks. AEs, grade ≥3 AEs and SAEs occurred in 96.1%, 72.0% and 43.1% of 1L pts, and 96.5%, 73.8% and 46.5% of R/R pts, respectively (results by treatment group in Table 1).

Table 1.

N (%)	Total (n=825)	G alone (n=106)	G-FC (n=159)	G-Clb (n=97)	G-B (n=463)
Any AE	794 (96.2)	98 (92.5)	157 (98.7)	97 (100)	442 (95.5)
TLS	51 (6.2)	6 (5.7)	7 (4.4)	3 (3.1)	35 (7.6)
Grade ≥3 AEs	600 (72.7)	66 (62.3)	133 (83.6)	63 (64.9)	338 (73.0)
Neutropenia	369 (44.7)	26 (24.5)	93 (58.5)	41 (42.3)	209 (45.1)
TCP	131 (15.9)	11 (10.4)	34 (21.4)	19 (19.6)	67 (14.5)
Grade ≥3 infections	123 (14.9)	17 (16.0)	17 (10.7)	16 (16.5)	73 (15.8)
Grade ≥3 IRRs	152 (18.4)	25 (23.6)	33 (20.8)	19 (19.6)	75 (16.2)
Any SAE	367 (44.5)	44 (41.5)	61 (38.4)	39 (40.2)	223 (48.2)
Any fatal AE	32 (3.9%)	4 (3.8%)	4 (2.5%)	3 (3.1%)	21 (4.5%)

The most common grade ≥3 AEs were neutropenia (44.7%), thrombocytopenia (TCP; 15.9%), anemia (9.0%), febrile neutropenia (6.9%), and leukopenia (6.2%). AEs were considered related to G treatment in 83.0% pts, most commonly neutropenia (36.0%), TCP (23.0%), pyrexia (21.7%), nausea (17.5%), chills (15.3%) and anemia (10.4%). AEs of particular interest (any grade) were: cardiac events, 8.7%; hemorrhagic events, 6.2%; TCP, 32.4%; and secondary malignancies, 4.1%. Other AEs of interest (any grade) were: infusion-related reactions (IRRs, *i.e.* events occurring during or within 24h of G infusion and treatment-related), 63.3% (most common: pyrexia, 17.7%; chills, 14.8%; nausea, 12.5%; causing G discontinuation, 2.8%); infections, 45.2% (most common: pneumonia, 7.0%; bronchitis, 5.0%; URTI, 5.0%); neutropenia, 58.4% and TLS, 6.2%. In the G-B arm, TLS was more common in unfit pts (23; 10.0%) than fit pts (12; 5.2%). AEs caused G discontinuation in 111 (13.5%) pts. In 43 pts who died (1L fit, 9 [3.4%]; 1L unfit, 10 [4.5%]; R/R, 24 [7.1%]), primary causes were AEs (n=32) and disease progression (n=11); 15 were related to underlying cancer. One pt in the G-B arm (1L unfit) died of TLS before data cut-off.
Summary/Conclusions: Preliminary safety data from GREEN are in line with the known safety profile of G in similar populations.

S428

QUANTITATIVE MRD IS PROGNOSTIC FOR PROGRESSION-FREE & OVERALL SURVIVAL IN ELDERLY PATIENTS RECEIVING CHLORAMBUCIL ALONE OR WITH OBINUTUZUMAB/RITUXIMAB: A PROSPECTIVE ANALYSIS OF THE GCLLSG CLL11 STUDY

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Background: The CLL11 study (NCT01010061) is an ongoing open-label, randomized, three-arm study in patients (pts) with previously untreated chronic lymphocytic leukemia (CLL), comparing the efficacy and safety of obinutuzumab (GA101; GAZYVA/GAZYVARO; G) plus chlorambucil (Clb; G-Clb) with rituximab (R) plus Clb (R-Clb) or Clb alone.
Aims: To determine the prognostic value of minimal residual disease (MRD) quantification with respect to clinical risk factors, treatment regimen, and MRD-source tissue in an advanced-age CLL population.
Methods: In total, 781 patients with treatment-naïve CLL, a median age of 73 years and a Cumulative Illness Rating Scale total score of >6 were included and randomized to receive Clb, G-Clb or R-Clb. Peripheral blood (PB) samples were taken at repeated timepoints during and up to 12 months (mo) after treatment. Bone marrow (BM) aspirate was taken 3 mo after end of treatment (EOT) to confirm complete response (CR). MRD was analyzed by quantitative immunoglobulin allele-specific real-time PCR. MRD results at EOT (PB or BM) were available in 73% of pts. Outcome was analyzed according to known MRD risk groups, *i.e.* MRD-positive (+ve; ≥1%), MRD intermediate (int; <1% and ≥0.01%) and MRD-negative (-ve; <0.01%), and other known risk factors.
Results: At EOT, combination treatment with either G-Clb or R-Clb achieved higher MRD negativity rates in PB and BM compared with Clb alone (PB: G-Clb 35.8% *vs* R-Clb 3.3% *vs* Clb 0%; BM: G-Clb 18.2% *vs* R-Clb 2.6% *vs* Clb 0%). Due to the absence of MRD negativity in the Clb arm, all further MRD analyses were restricted to the comparison of G-Clb *vs* R-Clb. When measured in PB, MRD risk groups significantly correlated with progression-free survival (PFS; median PFS: MRD-ve 49.3 mo; MRDint 23.9 mo; MRD+ve 13.9 mo; p<0.001) and overall survival (OS; median OS not reached in any group; p<0.001; see Figure 1 for KM curves). When MRD was measured in PB, three-year OS rates were: MRD-ve 94.3%; MRDint 87.3%; and MRD+ve 69.5%. When comparing G-Clb with R-Clb, median PFS in the MRD-ve group was not significantly different (p=0.15). Results were similar for MRD measured in PB after 3 cycles of treatment (interim staging [IST]; median PFS: MRD-ve not reached; MRDint 27.3 mo; MRD+ve 15.2 mo; p<0.001). A total of 53/417 (12.7%) pts converted from PB MRD+ve or MRDint at IST to MRD-ve at EOT across the G-Clb/R-Clb arms. In multivariate analysis (including various baseline markers), MRD in PB at EOT was an independent prognostic factor for PFS (HR 5.29, 95% CI 3.48-8.04, p<0.001) and OS (HR 3.04, 95% CI 1.53-6.03, p=0.002), with the highest weight in the model. When analyzing MRD in PB at EOT in conjunction with clinical remission status, MRD-ve/CR and MRD-ve/PR pts had similar PFS (median PFS 49.3 mo *vs* 56.4 mo, HR 1.74, 95% CI 0.79-3.86, p=0.17), whereas MRD+ve/CR pts had a worse outcome than MRD-ve/CR pts (median PFS 32.7 mo *vs* 49.3 mo, HR 3.6, 95% CI 1.64-7.99, p=0.001).

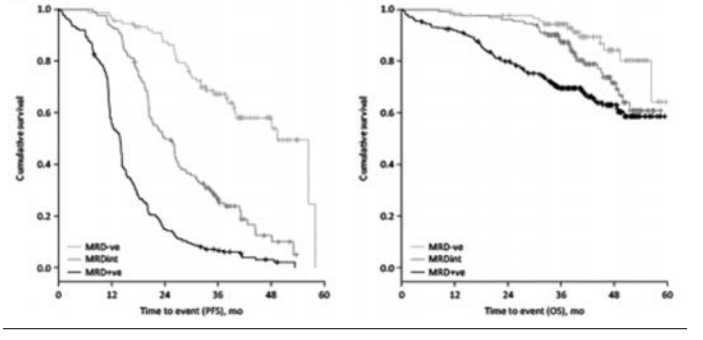

Figure 1. Kaplan Meier curves for PFS (A) and OS (B) according to MRD risk groups in PB at EOT.

Summary/Conclusions: Data presented are consistent with the CLL8 study and confirm that, when measured in PB or BM, MRD is prognostic in elderly

pts with comorbidities, with G-Clb achieving a much higher rate of MRD negativity than R-Clb and Clb alone. In this cohort, pts achieving MRD-ve status at IST had a similar outcome to pts with later conversion (at EOT). Although MRD measurement in BM seems to be more sensitive, MRD measurement in PB, at least in this trial, is sufficient to identify MRD based risk groups.

S429

EVALUATION OF 243 PATIENTS WITH DELETION 17P CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH IBRUTINIB: A CROSS-STUDY ANALYSIS OF TREATMENT OUTCOMES

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Background: Patients (pts) with deletion 17p (del17p) chronic lymphocytic leukemia (CLL) have aggressive disease and poor outcomes with chemoimmunotherapy. Ibrutinib (ibr), a once-daily oral inhibitor of Bruton's tyrosine kinase, is approved for pts with CLL after ≥1 prior therapy and for pts with del17p CLL in the US (including first-line) and first-line with del17p or *TP53* mutation unsuitable for chemoimmunotherapy in the EU. Ibr trials that enrolled pts with del17p CLL include a phase 2 study in treatment-naïve (TN) or relapsed/refractory (R/R) CLL (PCYC-1102) with extension (PCYC-1103), phase 3 RESONATE study of pts with R/R CLL (PCYC-1112), and phase 2 RESONATE-17 study in pts with R/R CLL (PCYC-1117).
Aims: This study evaluated efficacy and safety outcomes in an integrated analysis of pts with del17p CLL/SLL across 3 ibr clinical trials, including outcomes with complex karyotype (CK) in 1 longstanding extension trial.
Methods: All pts gave informed consent. Del17p was determined by FISH in a central laboratory (PCYC-1102/1117) or by local laboratory (PCYC-1112). Pts received once-daily oral ibr 420 (n=232) or 840 mg (n=11) until progressive disease (PD) or unacceptable toxicity. Sustained hematologic improvement over baseline was defined as ≥56 days without transfusion support and included platelet (PLT) or absolute neutrophil count (ANC) increase ≥50% and/or hemoglobin (Hgb) increase ≥2 g/dL. In PCYC-1102, CK was defined as ≥3 unrelated chromosomal abnormalities by stimulated cytogenetics as assessed by a reference laboratory.
Results: 243 CLL pts with del17p (241 R/R and 2 TN) were evaluated, with median age 65 y (37% ≥70 y). At baseline, 63% had Rai stage III-IV disease, and 53% had bulky disease ≥5 cm; cytopenias were observed in 68%. With median 2 prior therapies (range, 0-12), del11q was observed in 18% of 239 pts and unmutated *IGHV* in 80% of 201 pts. The median time on study was 28 mos; 66% received ibr for >2 y. ORR (including PR-L) was 84%; estimated median PFS was 32 mo (95% CI: 28, 40), and estimated 30-mo landmark OS was 67% (95% CI: 59, 74) (Figure 1).

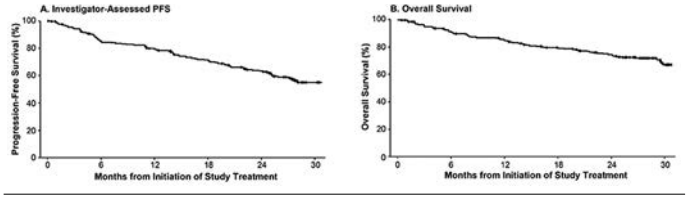

Figure 1. PFS (A) and OS (B) in ibrutinib-treated Del17p Patients.

Sustained hematological improvement in Hgb, PLT, and ANC occurred in 61%, 68%, and 68% of pts with baseline cytopenias, respectively. For pts with PD, Richter transformation tended to occur earlier at a median of 253 d (range, 31-786) *vs* CLL progression at median 594 d (range, 26-1572). Of 36 pts with del17p on PCYC-1102/1103, median time on study was 42 mo; baseline CK was observed in 22 pts. Median number of prior therapies was 2.5 *vs* 4 in pts without *vs* with CK, respectively. ORR was 80% in del17p pts without CK and 82% with CK. Estimated median PFS was 52 mo for pts without CK and 25 mo in those with CK. Estimated median OS was not reached for pts without CK and 32 mo with CK. Across all 243 pts with del17p, grade (Gr) ≥3 adverse events occurring in ≥5% of pts over the >2 y median treatment period were neutropenia (Gr 3/4, 5/14%), pneumonia (Gr 3/4/5, 9/1/2%), hypertension (Gr 3/4, 11/0%), thrombocytopenia (Gr 3/4, 5/5%), and anemia (Gr 3/4, 7/1%). Gr 3/4 atrial fibrillation occurred in 2/1% of pts. Gr ≥3 hemorrhage occurred in 6/1% (Gr 3/4). 110 pts (45%) remain on study treatment.
Summary/Conclusions: In this analysis of 243 pts, the estimated median PFS and 30-mo OS for ibr surpass those of other therapies for del17p CLL. Results from pts with central karyotype information suggest those without CK experience more favorable PFS/OS outcomes. These results provide further evidence of ibr's robust clinical activity and survival outcomes in difficult-to-treat CLL populations.

S430

IBRUTINIB PLUS BENDAMUSTINE AND RITUXIMAB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA (CLL/SLL): 2-YEAR FOLLOW-UP INCLUDING MRD FROM THE HELIOS STUDY

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Background: The international, double-blind, placebo-controlled, phase 3 HELIOS study evaluated ibrutinib (ibr)+bendamustine and rituximab (BR) *vs* placebo (plb)+BR in patients with previously treated CLL/SLL. At 1st analysis (median follow-up, 17.0 months), progression-free survival (PFS) was significantly improved for ibr+BR *vs* plb+BR (HR [95% CI], 0.203 [0.15-0.28]; $p<0.0001$). Prior studies have shown deepening responses with continued once-daily ibr treatment; thus, long-term follow-up assessing durability and depth of response is important, as is evaluating safety and tolerability over time.
Aims: The 1st HELIOS study results were reported with a median follow-up of 17 months. 2-year data are now available with additional safety follow-up. Because minimal residual disease negativity (MRD-ve) represents a lower disease burden and an endpoint that can potentially affect patient outcomes, we focus on these data to better understand the benefit of ibr over time in previously treated CLL patients.
Methods: 578 patients received BR (≤6 cycles) and were randomized 1:1 to ibr (420 mg/d) or plb (N=289 per arm). Patients with del17p (≥20% of cells) were excluded. The primary endpoint was independent review committee (IRC)-assessed PFS. Key secondary endpoints were investigator (INV)-assessed PFS, overall survival (OS), overall response rate (ORR; IRC and INV), and rate of MRD-ve response. With continued follow-up, we report INV-assessed clinical endpoints. At 1st analysis, overall concordance between IRC and INV assessments for progressive disease (PD) was 90% in the ibr group and 85% in the plb group.
Results: Median follow-up is now 25.4 months. Ibr+BR continues to show improvement in PFS *vs* plb+BR (INV-assessed median, not reached *vs* 14.2 months; HR [95% CI], 0.199 [0.15-0.26]; p<0.0001; 2-yr rate, 74.8 *vs* 20.9%). Median PFS2 (randomization to PD corresponding to the next line of treatment, or death) is unreached in both arms but PFS2 was significantly longer for patients assigned to ibr+BR *vs* plb+BR, despite crossover (HR [95% CI], 0.62 [0.42-0.92]; p=0.016). Median OS is still unreached in either arm (HR [95% CI], 0.67 [0.44-1.02]; p=0.058; 2-yr rate, 86.2 *vs* 81.5%); 142 patients (49.1%) in the plb+BR arm with confirmed PD have crossed over to receive ibr. The updated INV-assessed best ORR (at any time point) is 87.2% for ibr+BR *vs* 66.1% for plb+BR (p<0.0001); updated rates of complete response (CR)/ CR with incomplete bone marrow recovery (CR/CRi) are 33.9 *vs* 7.2% (rates at 1st analysis, 21.4 *vs* 5.9%). Rates of MRD-ve response for the intent-to-treat population are 18.0% (52/289) for ibr+BR *vs* 4.8% (14/289) for plb+BR (p<0.0001) (rates at 1st analysis, 12.8 *vs* 4.8%). Among patients who were evaluated for MRD status post-BR, ibr showed a more sustained PFS over plb whether or not MRD-ve response was achieved, though patients in the ibr arm who achieved MRD negativity had a longer PFS than those who did not (Figure 1). There were no new or unexpected safety signals reported in this update.
Summary/Conclusions: Ibr+BR continues to demonstrate superiority *vs* plb+BR, with significantly longer PFS and higher ORR. Moreover, responses continue to deepen with continuous ibr therapy, with rates of CR/CRi and MRD-ve response increasing over time. Patients on the ibr arm demonstrate a more sustained PFS at each MRD level (<0.01% and ≥10%) than those on the plb

arm. Ibr maintains the same degree of safety as previously observed in CLL patients. These 2-year follow-up data further confirm the important role of ibr in patients with previously treated CLL.

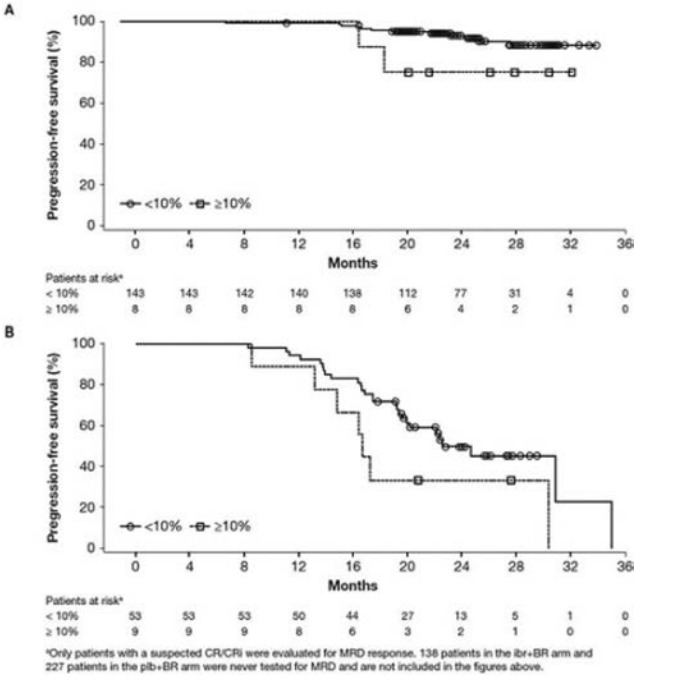

Figure 1. PFS by MRD level for (A) ibr+BR and (B) plb+BR.

S431

ACALABRUTINIB, A SECOND-GENERATION BRUTON TYROSINE KINASE (BTK) INHIBITOR, IN PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Btk is a kinase involved in B-cell receptor signaling and a critical therapeutic target in CLL. Acalabrutinib—an irreversible, selective Btk inhibitor has demonstrated clinical efficacy in relapsed CLL (Byrd NEJM 2015).
Aims: Here, we present preliminary results from an ongoing Phase 1-2 study of acalabrutinib monotherapy in patients (pts) with previously untreated CLL.
Methods: Pts with previously untreated CLL who met IWCLL 2008 criteria for treatment were eligible, irrespective of any cytopenias. Pts received oral acalabrutinib at 100 mg BID (n=37) or 200 mg QD (n=37). Pts had a median age of 64 (48-85) years, bulky lymph nodes ≥5 cm (47%) and unmutated IGHV gene (57%, 38/67). CLL responses were assessed per modified IWCLL criteria.
Results: Results are presented through 07Dec2015 for the first 74 treated pts, including 72 evaluable for response. Median time on study (N=74) was 11 (1-15) months. Acalabrutinib was well tolerated with 97% (72/74) of pts continuing on study drug. Most AEs were Grade (Gr) ≤2. The most common Gr 1-2 AEs (≥15% pts) were headache (42%), diarrhea (35%), arthralgia (22%), contusion (18%), nausea (18%) and increased weight (18%). Gr 3-4 AEs that occurred in ≥2 pts were syncope (n=2, both Gr 3) and hypertension (n=2, both Gr 3). One Gr 5 event (pneumonia) has occurred. One Gr 3 upper GI bleed due to a gastric ulcer and aspirin use has occurred. No atrial fibrillation was reported. Clinical activity was observed with both dose schedules; Btk occupancy was highest with BID dosing (98% *vs* 93% predose at Day 28). All pts had a rapid reduction in lymphadenopathy. Treatment-related lymphocytosis occurred in 53% (39/74) of pts and resolved in 97% (38/39) of these pts. In general, lymphocytosis peaked at a median of 1 week and resolved by a median of 7 (3-15) weeks. Best ORR was 96% (PR=86%, PR+L=10%, SD=4%, PD=0%). Median time to response was 2 (2-8) months. No CLL progression or Richter's transformation have occurred.
Summary/Conclusions: In pts with previously untreated CLL, a favorable safety profile and high response rates that appear durable were observed with acalabrutinib therapy. Based on these results, a Phase 3 trial has commenced (NCT02475681).

Chronic myeloid leukemia - Clinical

S432

EFFICACY AND SAFETY OF IMATINIB GENERICS; A REPORT FROM POLISH (PALG) IMATINIB GENERICS REGISTRY

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Background: Imatinib generics could substantially decrease costs of CML therapy, however there is a lack of data regarding the efficacy and safety of its use in larger populations of CML patients.
Aims: The aim of the study was to evaluate the efficacy and safety of imatinib generics in patients suffering from chronic phase CML treated *de novo* with generics (…*de novo*" patients), and in the group of patients switched from glivec to imatinib generics (…switched" patients) during a one-year therapy period.
Methods: In …*de novo*" group the rate of BCR/ABL1 reduction to <10% at 3 months and to <1% at 6 months of therapy, the rate of optimal response, and failure according to current ELN guidelines, the rate of CCyR,MMR,MR4, and MR4,5 achieved at 12 months of therapy, and the rate of patients switched to second generation TKI have been assessed. In the …switched" group the rate of sustained, improved and worsened molecular response, the rate of CCyR, MMR, MR4, and MR4,5 loss have been estimated. To assess the safety of imatinib generics in both groups the rate of hematologic (3rd or 4th grade), and of non-hematologic adverse events (all grades according to CTCAE criteria) have been evaluated. In current report the results of one-year, "real-life" observation started on 03.APR.2014 in 501 patients treated in 12 Polish Hematology Centers are presented. Polish Adult Leukemia Group imatinib generics registry records approximately 900 patients, in the current report only patients who completed 12-month observation (all patients with available RQ-PCR result at 12 month), with MMR achieved before start of therapy with generics, and treated for more than 12 months with generics (…switched" patients) have been analyzed.
Results: Forty patients started *de novo* treatment with generics (24 Nibix, 16 Meaxin), and 461 patients were switched from Glivec to imatinib generics (343 to Nibix, 118 to Meaxin). Early molecular response (BCR/ABL1 <10% at 3 months) was achieved in 75%, and the reduction of BCR/ABL1 to <1% at 6 months in 68% of …*de novo*" patients. Optimal response (MMR at 12 months) was achieved in 75% of …*de novo*" patients. Two patient from this group were switched to 2GTKI due to simultaneously occurred resistance and non-hematological toxicity. Hematologic toxicity (grade 3 or 4) was observed in 2 patients (therapy was not changed), non-hematologic toxicity occurred in 17 patients (2 patients were switched to 2GTKI). In the …switched" group the molecular response under therapy with generics was sustained, improved and worsened in 47.0%, 27.3%, and 25.5% of patients respectively. MMR was lost in 2.4%, CCyR in 0.4% and MR4.5 in 4.5% of patients switched to imatinib generics. During a one-year observation 4.5% of patients were switched to 2GTKI; 3,8% for intolerance (non-haematologic toxicity only), 0.8% for resistance, and 0.6% for intolerance+resistance.
Summary/Conclusions: This is the first report on "real life" imatinib generics effectiveness and safety in a big cohort of CML patients. Two tested generics of imatinib (Meaxin and Nibix) seem to be not less effective as Glivec in therapy of patients with CML CP, safety profile of both generics is acceptable – no increased switching rate between 1st and 2GTKI was noted.

S433

GLUCOSE AND LIPID METABOLISM ABNORMALITIES DURING NILOTINIB TREATMENT AND COMPARISON WITH IMATINIB AND DASATINIB THERAPY – RESULTS FROM ENIGMA 2 STUDY

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Background: Our pilot study on 10 CML patients demonstrated fast development of hyperinsulinaemia, peripheral insulin resistance, hypoadiponectinaemia and hypercholesterolemia during nilotinib (NILO) therapy.
Aims: The aim of this multicenter ENIGMA 2 study was to confirm these results and to compare data obtained during NILO therapy with control groups of patients treated with imatinib (IMA) and dasatinib (DASA).
Methods: Patients received intensive laboratory workup before the start of tyrosine kinase inhibitor (TKI), after 3 and 12 months of therapy. This included oral glucose tolerance test, fasting insulin, glucose, adiponectin and serum lipid concentration. Patients with diabetes mellitus, TKI treatment interruption for >2 weeks (start-month 3) or >4 weeks (month 4-12) or TKI dose reduction for >25% were excluded.
Results: Between 2/2011-11/2015 87 CML patients in 6 centers initiated TKI therapy – 48 NILO (1st line - 25; 2nd line - 23), 24 with IMA (all 1st line) and 15 with DASA (1st line - 5; 2nd line - 10). After 3 months of NILO treatment patients evolved significant hyperinsulinaemia and hyperglycaemia (presented in 75% of patients) as result of fast development of peripheral insulin resistance. This was proved by significant increase in HOMA-2 index during this period (appeared in 73% of patients). Moreover, total and LDL cholesterol concentration significantly increased already after this short NILO treatment (developed in 92% and 87% of patients, respectively). All these abnormalities were significant also after 12 months of therapy (Table 1). A separate analysis of patients treated with NILO either 1st or 2nd line confirmed these metabolic changes after 3 months of treatment. On the contrary – none of these abnormalities were detected in the control group of patients treated with IMA and DASA, including any change in insulin resistance measured by HOMA-2 index.
Interestingly, previously described decrease in adiponectin (major insulin sensitizer) concentration during NILO administration was confirmed subsequently when all NILO treated patients were analyzed together. However, in separate analysis decrease in adiponectin was proved only in patients with the 2nd line NILO therapy (median concentration at baseline *vs* month 3 – 14.3 *vs* 7.1 mg/l, p<0.001; at baseline *vs* month 12 – 14.3 *vs* 10.5 mg/l, p<0.001), but not in the 1st line NILO patients (median concentration at baseline *vs* month 3 – 7.3 *vs* 7.3 mg/l, p=0.875; at baseline *vs* month 12 – 7.3 *vs* 7.8 mg/l, p=0.917). While in the control IMA group (but not DASA) adiponectin concentration was significantly and persistently increased. Thus hypoadiponectineamia observed in the whole NILO group was caused by spontaneous decrease in adiponectin after the stop of IMA in the subgroup of the 2nd line NILO-treated patients.

Table 1.

	Start median (range)	N	Month 3 median (range)	N	p (compare to start)	Month 12 median (range)	N	p (compare to start)
NILOTINIB - ALL LINES (N = 48)								
Fasting glucose [mmol/l]	5.3 (2.1-6.7)	48	5.7 (4.5-8.3)	48	**<0.001**	5.7 (4.5-7.8)	38	**<0.001**
Fasting insulin [mU/l]	8.4 (2.4-27.1)	48	11.3 (2.5-32.7)	48	**<0.001**	9.8 (3.8-38.0)	35	**0.003**
HOMA2 - IR	1.09 (0.31-3.42)	48	1.50 (0.32-4.20)	48	**<0.001**	1.35 (0.51-4.98)	35	**0.001**
Fasting adiponectin [mg/l]	10.3 (2.1-50.0)	48	7.3 (2.2-38.4)	46	**<0.001**	8.4 (1.9-21.2)	38	**0.003**
Total cholesterol [mmol/l]	4.9 (2.5-6.7)	48	5.6 (3.4-7.9)	47	**<0.001**	5.7 (3.9-8.7)	37	**<0.001**
LDL cholesterol [mmol/l]	2.7 (1.1-4.9)	48	3.4 (1.6-5.7)	46	**<0.001**	3.5 (1.7-5.4)	35	**<0.001**
IMATINIB - 1st LINE (N=24)								
Fasting glucose [mmol/l]	5.1 (4.1-7.7)	24	5.6 (4.2-6.4)	24	*0.052*	5.6 (4.5-6.4)	18	*0.193*
Fasting insulin [mU/l]	7.6 (2.6-26.0)	24	6.7 (2.4-37.8)	24	*0.700*	7.8 (2.8-49.0)	18	*0.317*
HOMA2 - IR	1.01 (0.34-3.48)	24	0.90 (0.30-4.67)	24	*0.530*	1.04 (0.36-6.17)	18	*0.257*
Fasting adiponectin [mg/l]	10.4 (1.1-25.7)	24	24.3 (6.0-50.0)	22	**<0.001**	22.3 (9.5-65.6)	18	**<0.001**
Total cholesterol [mmol/l]	5.3 (3.3-7.1)	24	4.5 (2.6-7.1)	24	**0.006**	5.0 (2.9-6.6)	18	*0.071*
LDL cholesterol [mmol/l]	3.1 (1.7-4.7)	23	2.5 (1.2-4.7)	24	**0.004**	2.7 (1.3-4.3)	16	*0.061*
DASATINIB - ALL LINES (N=15)								
Fasting glucose [mmol/l]	5.6 (4.5-6.8)	15	5.2 (3.3-6.7)	15	*0.065*	5.4 (4.3-5.9)	12	**0.041***
Fasting insulin [mU/l]	7.9 (2.5-50.7)	15	8.8 (2.6-27.7)	15	*1.000*	8.3 (2.4-12.0)	11	*1.000*
HOMA2 - IR	1.00 (0.35-6.29)	15	1.21 (0.30-3.60)	15	*1.000*	1.10 (0.31-1.61)	11	*0.966*
Fasting adiponectin [mg/l]	13.9 (2.7-50.0)	14	11.4 (1.3-50.0)	14	*0.733*	15.7 (6.8-27.2)	11	*0.577*
Total cholesterol [mmol/l]	4.4 (3.0-5.6)	15	4.6 (2.5-7.2)	15	**0.048***	4.3 (1.7-7.2)	10	*0.241*
LDL cholesterol [mmol/l]	2.5 (1.5-3.9)	15	2.4 (1.0-5.0)	15	*0.182*	2.4 (1.5-4.7)	9	*0.078*

* Possible role of limited number of patients

Summary/Conclusions: We proved fast and persisting development of peripheral insulin resistance during NILO therapy as underlying cause of glucose and lipid metabolism impairment during NILO treatment. This was not proved for patients treated with IMA and DASA. IMA administration significantly increases serum adiponectin (major insulin sensitizer) concentration, which spontaneously declines after discontinuation of IMA and start of 2nd line NILO therapy.
Supported by the CELL – the Czech Leukemia Study Group – for life.

S434

THE EUTOS LONG-TERM SURVIVAL SCORE IS PREDICTIVE FOR RESPONSE AND OUTCOME IN CML PATIENTS TREATED FRONTLINE WITH NILOTINIB-BASED REGIMENS

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Background: The outcome of BCR-ABL+ chronic myeloid leukemia (CML) has been significantly improved by the introduction of tyrosine kinase inhibitors (TKIs) and, actually, many patients die of reasons unrelated to CML. A new scoring system, the EUTOS long-term survival (ELTS) score, based on the analysis of a large cohort of CML patients treated frontline with IM and aimed to discriminate the probability of dying of CML, has been recently published by the European LeukemiaNet (ELN). The relevant variables included were: age, peripheral blasts, spleen size and platelet count (Pfirrmann *et al.* Leukemia 16). Nilotinib (NIL) is a 2nd generation TKI approved as frontline therapy of CML in many countries.
Aims: To investigate the prognostic value of the ELTS score in a cohort of CML patients in early chronic phase treated with NIL-based regimens as first-line therapy.
Methods: Three hundred and forty-five adult patients were included. The patients were enrolled in three multicenter studies (ClinicalTrials.gov NCT00481052, NCT00769327 and NCT01535391) conducted by the GIMEMA CML WP, or treated at the University Hospital of Bologna, Italy. The initial treatment was NIL 300 mg BID or NIL 400 mg BID. The intention-to-treat population of each study was analyzed. Definitions: risk scores were defined according to Sokal, EUTOS and ELTs formulations; major molecular response (MMR): BCR-ABL1^{IS} ratio <0.1%; MR$^{4.0}$: BCR-ABL1^{IS} ratio <0.01% with >10,000 ABL1 copies; progression: transformation to advanced phases according to ELN criteria; leukemia-related death (LRD): death after progression.
Results: The median age was 53 years (range 18-86). The patient distribution according the different scoring systems was as follows: 41% low, 40% intermediate and 19% high Sokal score, 94% low and 6% high EUTOS score, 59% low, 30% intermediate and 10% high ELTS score, respectively. The median follow-up was 59 months (range: 24-82 months). The cumulative incidence of MMR and MR$^{4.0}$ was 83% and 69%, respectively; the 6-year progression-free survival (PFS), overall survival (OS) and cumulative incidence of LRD were 91%, 92% and 4%, respectively. All the 3 scores were associated with significantly different probabilities of MMR (cumulative incidence of MMR according to ELTS score: 90%, 77% and 61% in low, intermediate and high risk patients, respectively; p<0.001), but only the ELTS score was able to predict the achievement of MR$^{4.0}$ (cumulative incidence of MR$^{4.0}$: 75%, 63% and 53% in low, intermediate and high ELTS score patients, respectively; p=0.013). Interestingly, both the Sokal score and the ELTS score, but not the EUTOS score, predicted the OS (p=0.021 and p=0.037, respectively), while only the ELTS score predicted a significantly different PFS (p=0.038); the 6-year cumulative incidence of progression was 2%, 7% and 6% in patients with low, intermediate and high ELTS score, respectively. Probably due to the low number of deaths related to CML, all the 3 scores failed to predict LRD, but comparing patients with low ELTS score *versus* patients with intermediate and high ELTS risk the difference became significant.
Summary/Conclusions: In a cohort of CML patients treated with NIL-based regimens as frontline therapy, the prognostic predictive ability of ELTS score resulted superior to Sokal and EUTOS score.

S435

HIGH GENE EXPRESSION OF HIST1H2AG AND HIST1H4A REDUCES IMATINIB UPTAKE INTO CML CELLS AND PREDICTS POOR RESPONSE TO FRONTLINE IMATINIB THERAPY

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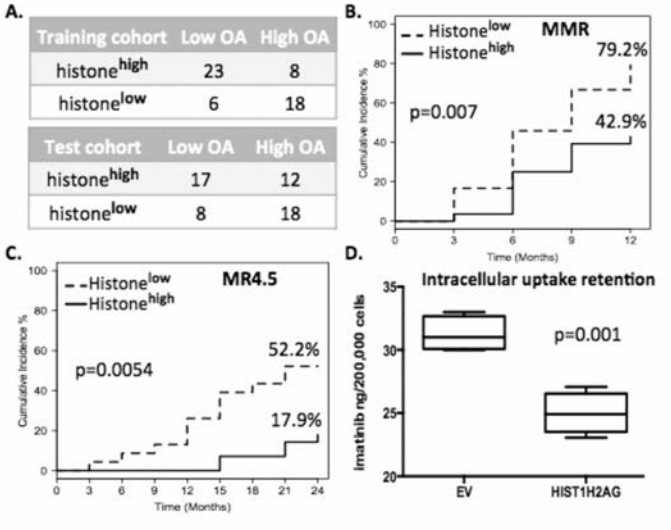
Background: Imatinib (IM), the first generation tyrosine kinase inhibitor (TKI), remains standard frontline treatment for newly diagnosed patients with chronic-phase chronic myeloid leukaemia (CP-CML). None of the second generation TKIs have shown an overall survival advantage over IM. We have previously demonstrated that the OCT-1 activity (OA) assay, (which measures the cellular uptake of IM at diagnosis), is highly predictive of subsequent treatment outcomes. To derive an assay more readily adaptable for other labs, and to understand the biological basis of this functional assay we performed gene-expression profiling (GEP) of low and high OA patients.
Aims: To identify a set of genes measured at diagnosis that predicts patients who have high IM uptake (high OA) and favourable clinical outcome.
Methods: RNA was isolated from total white cells collected from patients on TOPS, TIDEL-I and TIDEL-II trials. The OA associated genes were identified based on a pilot microarray study (n=14). Subsequently the identified genes were validated by qRT-PCR in 110 diagnostic CP-CML samples also obtained from these trials. Patients were divided into low and high gene expression groups using the recursive partitioning and regression trees (rpart) with 10-fold cross validation in the training cohort (n=55), and validation in an independent cohort (n=55).
Results: Previous studies have associated low expression of histone genes with higher rates of complete cytogenetic response (CCyR) by 12 months in IM treated patients. We have measured the expression levels by qRT-PCR of 7 histone genes that demonstrated significant association with OA based on our GEP. Importantly, we identified that low expression of 2 histone genes (*HIST1H2AG* and *HIST1H4A* based on ΔCt 11.7 and 13.0 cutoff respectively) was significantly associated with high OA in the training cohort, and this was also validated in the independent patient test cohort by both Student's t-test and rpart statistical methods (Figure 1A). Significantly, in the validation cohort, patients with low expression of these 2 histone genes (histone^{low}) demonstrated superior rates of major molecular response (MMR: 79% *vs* 43%, p=0.007) by 12 months, and superior rates of MR$^{4.5}$ (52% *vs* 18%, p=0.005) by 24 months compared to patients with high expression of these genes (histone^{high}; Figure 1B-C). There were 26 patients in the validation cohort with histone^{low} based on the cut-offs above, and of these 18 were high OA patients and 8 low OA patients. Although 8/26 (31%) patients were low OA, 7 of these 8 patients achieved MMR by 12 months. There were 6 patients with blast crisis (BC) progression and/or mutations that classified as low OA, and all 6 were in the histone^{high} group. The histone^{high} group is significantly associated with high blast counts in our study compared to histone^{low} group (p=0.01), consistent with a previous study that demonstrated BC patients have higher histone GEP compared to CP patients. Furthermore, overexpression of *HIST1H2AG* using lentiviral transduction significantly reduced the IM cellular uptake in K562 cells compared to empty vector control (p=0.001, Figure 1D), supporting our clinical data observation.
Summary/Conclusions: Histone gene expression likely plays a role in regulating the uptake of imatinib, and possibly BC progression. Patients with low expression of *HIST1H2AG* and *HIST1H4A* are highly likely to achieve good molecular responses and excellent clinical outcomes on imatinib.



(A.) Patients were divided into low and high *HIST1H2AG* and *HIST1H4A* gene expression group using the classification tree method in the training cohort (n=55). Histone^{low} patient group was significantly associated with high OA (p=0.0003). This result was validated in an independent test cohort (p=0.038). **(B-C.)** In the test cohort, histone^{low} patient group (dotted line) demonstrated superior rates of MMR and MR4.5 compared to histone^{high} patient group (solid line). **(D.)** Overexpression of *HIST1H2AG* gene significantly reduced cellular uptake of imatinib compared to empty vector control (EV) in K562 cells.

Figure 1. Low *HIST1H2AG* and *HIST1H4A* gene expression predicts high OCT-1 activity (OA) and favorable clinical outcomes.

S436

ALLELES OF SNPS IN REGULATORY REGIONS OF SLC22A4 AND SLC22A5 GENES ARE SIGNIFICANTLY ASSOCIATED WITH STABLE MAJOR MOLECULAR RESPONSE TO IMATINIB FIRST LINE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: The bioavailability of imatinib (IM) in leukemic cells at a curative concentration is an important pharmacokinetic factor influencing the response to the treatment of chronic myeloid leukemia (CML) with IM. Pharmacogenetics represents a potential source of molecular markers as patients with inherited haplotype associated with the key genes encoding drug transporters, have genetic predisposition to respond optimally or non-optimally. IM transport was confirmed for transporters encoded by some genes from SLC and ABC families.
Aims: The aim of this study was to screen polymorphisms in the promoter regions of selected SLC and ABC genes and to identify SNPs associated with the response to the first line IM therapy of CML patients.
Methods: Using NGS we screened promoters of 15 SLC and 4 ABC genes on testing cohort of 83 CML patients with optimal (n=42) and non-optimal (n=41) response to IM. Patients were treated with 400mg/day of IM during the minimal follow-up of 24 months. SNPs confirmation in SLC22A4 and SLC22A5 promoters were performed by Sanger Sequencing in the validation cohort of 42 patients. Linkage disequilibrium analysis of genotyped SNPs on European population and proxy identification with the regulatory function in SLC22A4 and SLC22A5 non-coding regions were performed using LDlink1.1. The Fisher´s exact probability test and Chi-square test were used for allele frequency analysis. Cumulative achievement of stable major molecular response (MMR) was calculated using XLSTAT. Kaplan-Meier method was applied for event free survival calculation. Events were defined as loss of MMR, loss of cytogenetic response, TKI switch, BCR-ABL1 mutations, death, hematological relapse and progression.
Results: Among 1486 NGS evaluated sequences we identified 95 SNPs. In the merged cohort of patients from testing and validation cohorts (n=125) we revealed significant differences in the frequencies of rs460089-GC and rs460089-GG (SLC22A4) genotypes among rs2631365-TC (SLC22A5) genotype carriers associated with optimal and non-optimal response, respectively. We found that loci rs460089 and rs2631365 are in highly significant linkage disequilibrium with another 12 regulatory loci (R^2=0.98-1.0; P=0.0) located in introns of SLC22A4 and SLC22A5 encoding OCTN1 and OCTN2 IM transporters, respectively. Based on the genotypes association analysis with the response to IM we revealed that rs460089-GC genotype carriers had significantly higher probability to achieve stable MMR (P =0.001) and this was enhanced in rs460089-GC_rs2631365-TC genotypes carriers (Figure 1; P=0.000). Consequently, patients with rs460089-GC genotypes have higher probability to survive without event in contrast to patients with rs460089-GG (P=0.003), when the contrast was higher in patients with rs460089-GC_rs2631365-TC genotypes in comparison to rs460089-GG_rs2631365-TC (P<0.0001).

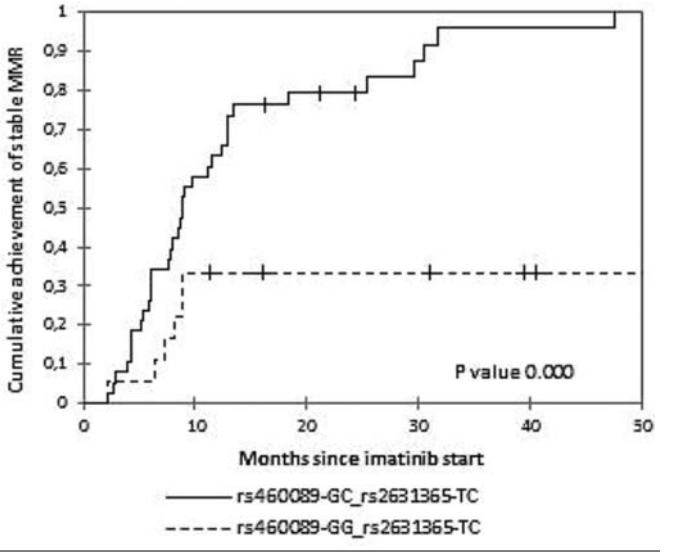

Figure 1.

Summary/Conclusions: We found that SNPs rs460089 and rs2631365 may represent important genetic markers for European population of CML patients by predicting response to the IM therapy at the time of diagnosis. Patients with rs460089-GC_rs2631365-TC genotype would likely respond optimally, in contrast, rs460089-GG_rs2631365-TC genotype represents a risk factor for IM failure and disease progression. This risk factor maybe associated with sublethal concentration of IM, which may lead to resistant clone development and disease progression. Patients with high-risk genotype rs460089-GG_rs2631365-TC may profit from therapy with tyrosine kinase inhibitors that are independent on OCTN1 and OCTN2 carriers.

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Follicular and Mantle Cell Lymphoma - Clinical

S437

15-YEAR FOLLOW-UP OF THE NORDIC MCL2-TRIAL: DESPITE LONG-TERM RESPONSES LATE RELAPSES STILL OCCUR

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Background: Until recently, Mantle Cell Lymphoma (MCL) was associated with an exceedingly poor prognosis with a median overall survival (OS) of 3-5 years (Herrmann, JCO, 2008). However, during the last 15 years the outcome has improved substantially by an intensified Ara-C-containing induction regimen, addition of rituximab and consolidation with high-dose therapy and autologous stem cell transplantation (ASCT). One such regimen was introduced by the MCL2 trial, conducted by the Nordic Lymphoma Group (NLG), and showed a projected 10-year OS and PFS of 58% and 43%, respectively, after a median follow-up time of 6.5 years (Geisler, BJH, 2012).

Aims: The MCL2 regimen is still the 1-st-line regimen of choice for younger patients in many centers worldwide. Here we present the updated results after a median follow-up of 11.4 years of the MCL2 trial.

Methods and Patients: Between 2000 and 2006, 160 untreated stage II-IV MCL patients younger than 66 years were enrolled in Denmark, Norway, Sweden and Finland. Patient characteristics have previously been described elsewhere (Geisler, Blood, 2008). Histological diagnoses were confirmed by a central pathology review board. An informed consent was obtained from all patients.

Treatment: Six alternating cycles of maxi-CHOP and high-dose Ara-C, with rituximab after cycle 3, followed by BEAM/BEAC and ASCT in responders (n=145). Patients who subsequently developed solely molecular relapse detected by clonal IGHV or t(11;14) PCR received 4 weekly cycles of rituximab (Andersen, JCO, 2009).

Results: With a median follow-up of 11.4 years, the median OS and PFS were 12.7 and 8.5 years, respectively (Figure 1A,B). The median response duration (RD) of the 145 patients who underwent ASCT was 12.4 years (Figure 1C). The MCL international prognostic index (MIPI) and the biological index (MIPI-B) significantly divided patients into three risk groups according to OS, PFS and RD (Figure 1D,E, data only shown for PFS). The median OS and PFS were 4.0 and 2.5 years in the MIPI high-risk group, 11.0 and 8.0 years in the intermediate risk group, and not reached and 12.8 years in low risk group, (Figure 1D). Interestingly, 17 patients relapsed after 5 years or more in CR, and 6 patients beyond 10 years, primarily from the intermediate and low risk groups. Meanwhile, the MIPI high risk group reached a plateau at 24% (n=9) after 7 years. We recently proposed an improved prognosticator including expression of micro-RNA-18b (MIPI-B-miR) based on the same patient cohort (Husby, Blood, 2016). In the present update, it remains highly significant and identifies a high-risk group of an exceedingly poor prognosis with OS and PFS of only 1.6 and 1.0 years, respectively (n=10) (Figure 1F), while still separating the low- and intermediate risk groups.

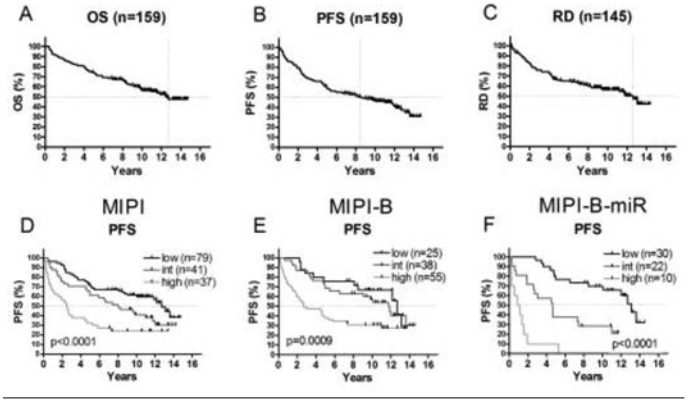

Figure 1.

Summary/Conclusions: After an extended median follow-up of 11.4 years, the outcome of the Nordic MCL2 trial is still good. However, a pattern of continuing relapse is observed, seemingly precluding cure. MIPI, MIPI-B and, in particular,

MIPI-B-miR remain valid prognosticators that clearly separate patients into risk groups with different outcomes. All risk groups might benefit from addition of novel agents, and such approaches are underway in large randomized trials.

S438

OVERALL SURVIVAL OUTCOMES IN PATIENTS WITH MANTLE-CELL LYMPHOMA (MCL) TREATED WITH IBRUTINIB IN A POOLED ANALYSIS OF 370 PATIENTS FROM 3 INTERNATIONAL OPEN-LABEL STUDIES

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Background: MCL is a rare, aggressive B-cell lymphoma with a poor prognosis. For patients who fail initial therapy, conventional chemotherapy achieves only short-term remissions. Ibrutinib is a first-in-class, once-daily, oral, covalent inhibitor of Bruton's tyrosine kinase shown to be highly active for patients with MCL. The current pooled analysis from 3 ibrutinib studies (PCYC-1104, MCL2001 [SPARK] and MCL3001 [RAY]) assessed the impact of baseline factors on overall survival (OS) in patients with relapsed/refractory (R/R) MCL.
Aims: To explore baseline factors impacting OS in patients with MCL receiving ibrutinib.
Methods: Patients with R/R MCL were enrolled across 3 studies to receive ibrutinib 560 mg orally once daily until progressive disease or unacceptable toxicity. Inclusion and exclusion criteria were similar in all 3 studies; however, patients in SPARK were required to have received both rituximab and bortezomib. Patient-level data from all 3 studies were combined into one database. Simple descriptive statistics were used, and exploratory analyses were conducted using Kaplan-Meier estimates for progression-free survival (PFS) and OS. Univariate and multivariate analyses were also conducted with hazard ratios to allow for comparisons of variables. All patients provided written informed consent.
Results: Overall, 370 patients were included in this analysis (PCYC-1104, n=111; SPARK, n=120; RAY, n=139); median age was 67.5 years, 94% had ECOG 0-1, 45% and 32% had intermediate and high-risk sMIPI, most patients had 1-3 prior lines of therapy (27%, 29%, 22% had 1, 2, 3 prior lines of therapy, respectively), 49% had bulky disease (>5 cm) and 88% had non-blastoid histology. Overall response rate (ORR) was 66% (20% CR; 46% PR), with a median DOR, PFS and OS of 18.6, 12.8 and 25.0 months, respectively. ORR (CR) for patients with 1, 2 and ≥3 prior lines of therapy was 77.8% (34%), 71% (24%) and 64% (16%). Of patients who achieved a CR, 70% were progression-free and 90% were alive at 2 years. Univariate analyses showed that patients with 1 *vs* >1 prior line of therapy had significantly longer OS, with longer OS also observed in those who were younger and had non-blastoid histology (Figure 1), non-bulky disease or better sMIPI score. Patients with blastoid and non-blastoid histology had similar ORR (55 *vs* 72%) and time to best response (2.2 *vs* 2.1 months); however, DOR (8.6 *vs* 18.8 months), PFS (5.1 *vs* 14.6 months) and OS (12.8 *vs* not reached) were significantly shorter in patients with blastoid histology. Multivariate analyses identified ECOG, sMIPI, bulky disease and blastoid histology as impacting OS, and sMIPI, bulky disease, blastoid histology and 1 prior line of therapy as impacting PFS.

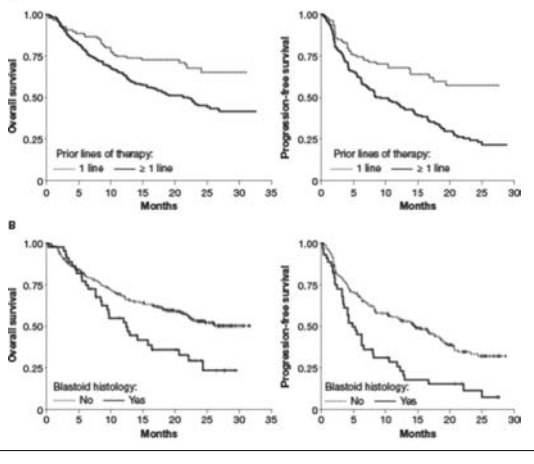

Figure 1. Kaplan-Meier Curves of OS and PFS by Baseline Factors: 1 *vs* >1 Prior Line of Therapy and Blastoid Histology.

Summary/Conclusions: Here we show that OS is significantly longer in ibrutinib-treated patients who are younger and who have fewer prior lines of therapy, better sMIPI scores, non-bulky disease and non-blastoid histology. While PFS and OS in patients with blastoid *vs* non-blastoid histology are shorter, these rates are higher than seen with other agents, indicating that ibrutinib is an effective agent to achieve a response and potentially provide a bridge to transplant. Multivariate analyses indicate that traditional poor prognostic factors adversely impact OS, suggesting that worsening OS in later lines of therapy is associated with disease characteristics rather than an impact of ibrutinib on postprogression survival. Data support the preferential use of ibrutinib after initial *vs* later relapse, as PFS and OS are longer in patients receiving 1 *vs* >1 prior line of therapy.

S439

SEQUENCE VARIANTS IN PATIENTS WITH PRIMARY AND ACQUIRED RESISTANCE TO IBRUTINIB IN THE PHASE 3 MCL3001 (RAY) TRIAL

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Background: The phase 3 MCL3001 (RAY) study compared the efficacy and safety of ibrutinib with that of temsirolimus in patients with relapsed or refractory mantle-cell lymphoma (MCL). Ibrutinib significantly prolonged progression-free survival (PFS) *versus* temsirolimus (14.6 *vs* 6.2 months; p <0.0001) and increased overall response rate (ORR) (72 *vs* 40%; p <0.0001). The current study explores underlying molecular mechanisms of resistance to ibrutinib in patients from the RAY study.
Aims: To identify specific mechanisms underlying ibrutinib resistance in MCL, and to correlate potential genetic signatures with patient response.
Methods: The clinical results of the RAY study are reported elsewhere (Dreyling 2015). Patients were grouped by best ORR+PFS: Durable Responder: complete response or partial response (PR)+PFS ≥4 months; Moderate Clinical Benefit: PR+PFS <4 months or stable disease; and Primary Resistant Disease. For mutational analysis, the Durable Responder group was compared with the Poor/Non-responders group (Moderate Clinical Benefit+Primary Resistant Disease combined) using Fisher's exact test for comparisons between the 2 groups. For primary resistance analysis, baseline variant differences between groups were compared. For acquired resistance analysis, initial samples and samples obtained at progression after response were compared. Deep sequencing was performed on DNA from tumor cells (Illumina HiSeq instrument) using a custom gene panel. Sequences were aligned to hg19 reference genome, variants were described using SAMtools, and germline filters were applied to identify possible somatic mutations. All patients provided written informed consent.
Results: Mutations associated with primary resistance to ibrutinib were identified in NF-kB signaling pathways, both canonical (*e.g.*, A20) and noncanonical (*e.g.*, BIRC2). Other mutations were found in epigenetic modifiers and in the EGFR family. To explore acquired resistance, 34 paired samples (11 ibrutinib; 23 temsirolimus) were analyzed. Two PLCg2 mutations were found in patients with durable PRs (18.5 and 8.5 months); a CARD11 mutation after PR was found in 1 patient (after 12 months). Mutations in epigenetic modifiers and alternate NF-kB or PI3K/mTOR pathways were found after a short treatment duration (<4 months). No primary or acquired Bruton's tyrosine kinase (BTK) C481S mutations were detected.
Summary/Conclusions: Mutations in the NF-kB pathway bypassing BTK appear to be a common mechanism of resistance in this population with MCL. The similarity between primary and acquired mutations, as well as the lack of BTK C481S mutations to date, may possibly be explained by the short follow-up and shorter response duration compared with chronic lymphocytic leukemia. Some mutations seen in the acquired setting (*e.g.*, CARD11, PLCg2) have been associated with ibrutinib resistance. Understanding both primary and acquired resistance patterns is key in order to improve outcomes and define the populations that benefit from ibrutinib treatment.

S440

OBINUTUZUMAB PLUS BENDAMUSTINE *VERSUS* BENDAMUSTINE ALONE IN PATIENTS WITH RITUXIMAB-REFRACTORY FOLLICULAR LYMPHOMA: RESULTS FROM THE GADOLIN STUDY

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Background: Limited treatment options are available for patients (pts) with rituximab-refractory (R-R) indolent non-Hodgkin lymphoma (iNHL). GADOLIN (NCT01059630) is an open-label, randomized, phase 3 trial comparing the efficacy and safety of obinutuzumab (GA101; GAZYVA/GAZYVARO; G) plus bendamustine (B; G-B) induction, followed by G maintenance, with B standard of care in this pt group. In the primary analysis (median observation time: 21 months [mo]) of iNHL pts, median Independent-Review Committee (IRC)-assessed progression-free survival (PFS) was longer in the G-B arm (194 pts; median not reached) than in the B arm (202 pts; 14.9 mo), with a 45% reduction in risk of progression or death (HR 0.55; 95% CI 0.40-0.74; p=0.0001). Safety profiles were comparable.
Aims: To evaluate efficacy and safety in the follicular lymphoma (FL) subset of GADOLIN pts considered in the primary analysis.
Methods: GADOLIN pts were aged ≥18 years with documented R-R iNHL and an Eastern Cooperative Oncology Group performance status of 0-2. Pts received either G 1000mg intravenously (IV) (days [D] 1, 8 and 15 of cycle (C) 1 and D1 of C2-6) plus B 90mg/m^2/day IV (D1 and 2 of C1-6) or B monotherapy (120mg/m^2/day IV D1 and 2 of each cycle for up to six cycles); each cycle was 28 days. Following induction, pts in the G-B arm without evidence of progression received G maintenance (1000mg IV every 2 mo for 2 years or until disease progression). Endpoints included IRC-assessed PFS (primary), investigator (INV)-assessed PFS, response and safety. All pts gave informed consent.
Results: 321 (81%) of 396 iNHL pts enrolled had FL (G-B, 155; B, 166). Baseline characteristics of the FL population were balanced between arms. Median number of prior therapies was 2. Most pts were refractory to their last prior rituximab (R)-containing regimen (G-B, 94%; B, 93%) and double-refractory to R and an alkylating agent (G-B, 77%; B, 80%). IRC-assessed end of induction (EOI) and best overall response were similar in the G-B and B arms (p>0.05 for comparison; Table 1). Median IRC-assessed PFS was not reached in the G-B arm and was 13.8 mo in the B arm (Figure 1), while median INV-assessed PFS was more than twice as long in the G-B arm than in the B arm (Table 1); in both instances, the advantage corresponded to a 52% reduction in risk of progression or death relative to B (Table 1). Survival data were immature at the time of analysis. Safety profiles were comparable. Of note, grade 3-5 adverse events (AEs) and grade 5 AEs (fatal outcome) occurred in 65.8% and 5.2% pts in the G-B arm and 58.9% and 6.1% pts in the B arm, respectively.

Table 1.

Parameter	FL subpopulation	
	G-B (n=155)	B (n=166)
Median observation time (range), mo	22.08 (0.4-48.5)	20.27 (0.0-50.0)
PFS (IRC)		
Pts with event, n (%)	54 (34.8)	90 (54.2)
Median (mo)	Not reached	13.8
HR [95% CI]; stratified*	0.48 [0.34-0.68]	
PFS (INV)		
Pts with event, n (%)	62 (40.0)	102 (61.4)
Median (mo)	29.2	13.7
HR [95% CI]; stratified*	0.48 [0.35-0.67]	
Response† (IRC)		
EOI response (%): overall‡/CR	70.5/9.4	62.6/13.5
Best response (%): overall‡/CR	79.7/15.7	77.0/19.3

**Stratification factors for FL population were refractory type (R vs R-chemo) and prior therapies (≤2 vs >2); †During treatment and within 12 mo after start of treatment; ‡Complete response (CR) or partial response.*

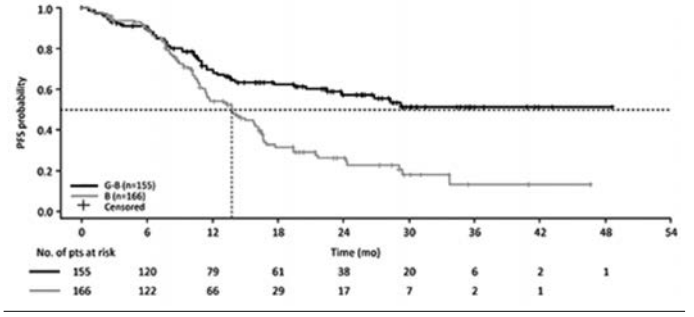

Figure 1. Kaplan Meier plot of IRC-assessed PFS in patients with FL.

Summary/Conclusions: In the FL subset of GADOLIN pts, PFS was significantly longer in the G-B arm compared with the B arm, corresponding to a 52% reduction in risk of progression or death. No unexpected safety signals were identified. G-B is an effective therapy for pts with R-R FL.

S441

ANALYSIS OF SECONDARY NEOPLASIAS AFTER HIGH DOSE THERAPY SUPPORTED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN FOLICULAR LYMPHOMA PATIENTS. A LONG TERM FOLLOW-UP ANALYSIS FROM THE GELTAMO REGISTRY.

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Background: High dose therapy supported by autologous stem cell transplantation (HDT/ASCT) has been a treatment frequently indicated in follicular lymphoma (FL) patients and has contributed to modify the natural history of the disease, however secondary neoplasia is one of the concerns after HDT/ASCT with a reported incidence very variable among studies due in part to cohort different in terms of age, pre-ASCT treatments, used of total body irradiation (TBI)-based conditioning regimen or length of follow-up rather than to the procedure itself.
Aims: To evaluate the cumulative incidence and characteristics of myelodysplastic syndromes and acute myeloid leukemia (sMDS/sAML) and solid tumors after HDT/ASCT in a very long-term follow-up analysis of FL patients.
Methods: A total of 655 FL patients reported to the Spanish GELTAMO registry and intensified with HDT/ASCT between 1989 and 2007 were analyzed. Baseline characteristics and therapeutic-related data are listed in the Table 1. Standardized Incidence Ratios (SIR) were calculated to assess the risk of a second malignancy by dividing the number of observed second malignancies with the number of expected sex matched incidence using the 2008 crudes rates in the Spanish population (*M.J Sánchez, Annals oncology 2010*).

Table 1. Main clinical Features at Diagnosis and Treatment Variables of the Series.

Characteristics		No. [a]	%
All patients		655	100
Median age, years (range)		47 (18-73)	
Sex: Male/ Female		330/ 325	50.4/ 49.6
FLIPI Score	Low	108	33
	Intermediate	120	36
	High	102	31
FLIPI 2 Score	Low	69	22
	Intermediate	118	38
	High	125	40
Disease Status at ASCT	CR	405	62
	PR	221	34
	Refractory disease	29	4
Anthracycline-containing first line therapy		460	76
Fludarabine-containing first line therapy		36	6
Only one therapy line before HDT/ASCT		183	28
Rituximab previous HDT, Yes/ No		184/ 436	30/ 70
Conditioning Regimen TBI based, Yes/ No		109/ 504	17/ 83
PBPC, Yes/ No		517/87	14.5/ 85.5

Abbreviations: BM: Bone Marrow, FLIPI: Follicular Lymphoma prognostic index, CR: Complete Response,PR: Partial Response, ASCT: Autologous Stem Cell Transplantation, HDT: High Dose Therapy, TBI: Total Body Irradiation, PBPC: Peripheral Blood Progenitor Cells.
a There are some missing data for several variables. No. of missing values can be directly derived for each variable by the equation: 655-(sum of available results)

Results: At a median follow-up of 12 years from HDT/ASCT and 14.2 years from diagnosis of FL the median OS were 21.3 years from HDT/ASCT and 22.6 years from the time of FL diagnosis. Of the 645 evaluable patients; 80 (12.5%) developed a second malignancy: solid tumors (38 cases; 47.5%; of them 5 were skin cancers), sMDS/sAML (34 cases; 42.5%), ALL (2 cases),

CML (1 case), Hodgkin lymphoma (1 case) and not specified (4 cases). The accumulated incidence at 5, 10 and 15 years was 1.8%, 3.5% and 4.9% for solid tumors and 2.6%, 4.3% and 5% for sMDS/sAML, respectively. sMDS/sAML and solid tumors were documented with a median time of occurrence since HDT/ASCT of 4.2 years (0.3-15.5) and 8.3 years (0.5-24.4), respectively. The SIR for second neoplasia was 2.8.Male sex (*P*=.1) and the use of BM as stem cell source (*P*=.1) tended to be associated with an increased number of second neoplasia. There were no differences according to the use of anthracycline, fludarabine or rituximab previously to HDT/ASCT, number of therapy lines before HDT/ASCT, time from diagnosis to HDT/ASCT, status of the disease at HDT/ASCT or conditioning regimen. Median OS for patients with second neoplasia is 11.8 years from the time of FL diagnosis, 9.4 years from ASCT [14.5 y. for solid tumors and 8 y. for sMDS/sAML (*P*=.01)] and 1.4 years from the time of diagnosis of second neoplasia [2.7 y. for solid tumors and 1.3 y. for sMDS/sAML (*P*=.01)], respectively.

Summary/Conclusions: Our results, from the longest follow-up study in FL including a large number of cases from the rituximab era, indicate that FL patients undergoing and ASCT are at an increased risk of developing a second malignancy, however, the incidence is not higher than that reported in other series without transplantation (*Rummel, Lancet 2016; Ardeshna, Lancet 2003*). Low percentage of patients conditioned with TBI and a considerable number of patients been transplanted soon during the disease, could explain these good results. Once a secondary neoplasia is diagnosed prognosis is dismal, and consequently a carefully selection of patients candidates to ASCT is necessary. For all that, we suggest that, given the favorable survival obtained by HDT/ASCT makes not evident to what extent incidence of secondary neoplasia will diminish the benefit of HDT/ASCT in FL.

Myelodysplastic syndromes - Biology

S442

DYNAMICS OF CLONAL EVOLUTION IN MYELODYSPLASTIC SYNDROMES

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Background: The development and progression of cancer are shaped by dynamic alterations of clonal architecture, characterization of which in terms of gene mutations is essential for the understanding of the pathogenesis of cancer. However, the clonal architecture in myelodysplastic syndromes (MDS) has been only inferred from the allelic burden of a limited number of driver mutations at a single time point. Comprehensive mutation profiling of serial samples has been performed only for a small number of patients.

Aims: We aimed to delineate the impact of clonal dynamics on disease phenotypes, progression to secondary acute myeloid leukemia (sAML), and clinical outcomes in a large cohort of fully genotyped MDS patients.

Methods: Clonal architecture and dynamics were investigated by whole exome sequencing (WES) and/or targeted sequencing of 779 patients with MDS and sAML, of whom 97 were analyzed longitudinally. Combined with published data sets (N=2,133), the data were also used to interrogate differential roles of driver mutations in disease progression.

Results: Higher-risk MDS was characterized by a higher number of mutations with increasing diversity and a larger clone size. The number of cases with heterogeneity was significantly higher in sAML compared to lower-risk MDS (*P*=0.03). In WES analysis of serial samples, evolution of a new dominant clone that swept out other subclones was common during disease progression and frequently accompanied by newly emerging subclones. Leukemic transformation was heralded by acquisition of new mutations in most cases without clone sweeping. To clarify the relationship between driver mutations and disease progression or phenotype, we compared frequencies of major driver mutations between disease subtypes within a large cohort of patients. In total, data from samples with lower- (n=1,157) and higher-risk (n=672) MDS, as well as sAML (n=304), were subjected to analysis of 27 driver genes mutated in more than 2% of the entire cohort. Mutations in genes designated as Type-1 (*FLT3, PTPN11, IDH1, CBL,* and *NRAS*) were significantly enriched in sAML compared to higher-risk MDS. When compared between higher- and lower-risk MDS, we observed a skewed enrichment of certain driver mutations in higher-risk MDS, designated here as Type-2 (*TP53*, *GATA2*, *RUNX1*, *IDH2*, *STAG2, ASXL1*, and *NPM1*). In longitudinal samples, Type-1 mutations were more likely to be newly acquired or to increase in clone size ($P=1.94\times10^{-4}$, trend test). In contrast, more Type-2 and other mutations decreased in their clone size or were even lost in the second sampling. Patients with Type-1 mutations (Group-I) had a significantly shorter time to progression to sAML compared to other patients (hazard ratio (HR)=5.7, 95% confidence interval (CI)=3.4−9.6; *P* <0.001). Time to sAML in Group-I patients was also significantly shorter than that in patients who had Type-2 mutations but lacked Type-1 mutations (Group-II) (HR=3.0, 95% CI=1.7−5.4; *P* <0.001). Despite the significant difference in the rate of progression to sAML between patients in Group-I and Group-II, both had a similar overall survival. Accordingly, in Group-II patients more deaths occurred before progression to sAML, compared to Group-I patients.

Summary/Conclusions: Dynamics of clonal architecture strongly correlates with distinct types of mutations, which are significantly associated with leukemia-free survival and non-leukemia death, suggesting that screening of these specific mutations might be useful for the prediction of clinical outcome.

S443

INTEGRATIVE GENOMICS IDENTIFIES THE MOLECULAR BASIS OF RESISTANCE TO AZACITIDINE THERAPY IN MYELODYSPLASTIC SYNDROMES

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B Young[15], R Ramakrishna[16], C Arthur[17], R Stark[18], P Crispin[19], J Curnow[20], P Warburton[21], F Roncolato[22], J Boultwood[13], K Lynch[23], SE Jacobsen[8,9], G Mufti[12], E Hellstrom-Lindberg[9], K MacKenzie[7], J Wong[1,2], P Campbell[3], J Pimanda[1,2,15]
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Background: Myelodysplastic Syndrome (MDS) and Chronic Myelomonocytic Leukaemia (CMML) are haematological disorders that develop in haematopoietic stem or progenitor cells (HSPCs) and are characterised by ineffective haematopoiesis. 5'-Azacitidine (AZA), a DNA demethylating agent, is the primary drug for the treatment of high-risk MDS and CMML and response is associated with improved survival benefits. However, only half of treated patients will ever respond to AZA and the molecular basis for poor response is currently unknown. Additionally, AZA response is rarely sustained and a substantial fraction of responders will eventually relapse.
Aims: We aimed to: 1.) understand the molecular basis for poor response to AZA, and 2.) characterise the *in vivo* effect of AZA therapy on dysplastic cells in responders, as a first step towards understanding eventual relapse.
Methods: We enrolled 18 high-risk MDS and CMML patients on a compassionate access program for AZA in Australia. Bone marrow was collected at seven different points – before treatment; through 6 cycles of treatment; and at up to two years after initiation – and we isolated high-purity CD34$^+$ HSPCs (Figure 1 A). 10 patients had a complete response while 8 were poorer responders. We performed RNA-seq to query the transcriptomes and deduced the clonal evolution in the bone marrow in response to AZA therapy by whole exome-sequencing and single-cell genotyping.

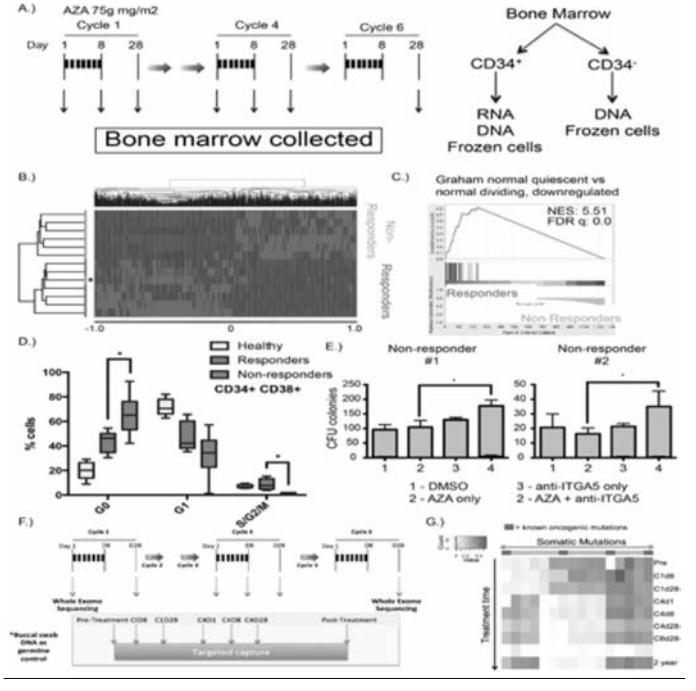

Figure 1.

Results: We hypothesised that primary AZA resistance would be driven by pre-existing molecular differences between responders and non-responders. Analysis of the pre-treatment RNA-seq data revealed differential gene expression between responders and non-responders (Figure 1 B). Pathway analyses of these genes indicated that cell cycle was relatively up-regulated in responders compared to non-responders (Figure 1 C). We validated these gene expression differences in independent patient cohorts. We then adapted a flow cytometry based assay, amenable to prospective use in a clinical diagnostic setting, to directly detect the increased quiescence of CD34$^+$ CD38$^+$ haematopoietic progenitors in unsorted bone marrows of non-responders (Figure 1 D). Finally, to reverse the quiescence of progenitor cells of non-responders, we developed a stromal co-culture drug testing platform and discovered that inhibiting integrin-linked signalling combinatorially with AZA improved the functionality of dysplastic cells (Figure 1 E). To trace the fate of dysplastic cells upon AZA therapy, we performed whole exome sequencing of all patients (Figure 1 F). Using the mutations as "molecular barcodes", we deduced the clonal architecture in each individual. We have discovered that although AZA alters the sub-clonal contribution to different lineages, founder clones are not eliminated and continue to drive hematopoiesis even in complete responders (Figure 1 G). Lastly, we have also discovered that AZA response is associated with an up-regulation of inflammation-associated pathways *in vivo*.
Summary/Conclusions: Our findings, across independent cohorts and relevant to both MDS and CMML, have immediate clinical utility not simply to prospectively identify AZA non-responders but also by suggesting combinatorial therapies that could improve response. Finally, elucidating the *in vivo* effects of AZA therapy lay the foundation for developing more durable treatments.

S444

HEDGEHOG SIGNALING PATHWAY INHIBITOR, PF-04449913 LIMITS THE SELF-RENEWAL OF MDS-DERIVED INDUCED POTENT STEM CELLS (IPSC): MOLECULAR MECHANISMS

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Background: Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders characterized by no efficient hematopoiesis and frequent progression to acute myeloid leukemia (AML). Even in low risk MDS, clonal hematopoiesis already dominates at diagnosis, and clones found in secondary AML originate from the MDS stage of disease, highlighting the need to specifically target the MDS-initiating clone. PF-0449913 is a potent and selective hedgehog pathway inhibitor that act by binding Smoothened (SMO) and blocking signal transduction. In xenograft models of human coloirectal and pancreatic cancer, treatment with PF-04449913 in combination with other anticancer agents reduced the tumor growth. Furthermore, PF-04449913 demonstrated preliminary antitumor activity in a phase I trial, when given as monotherapy in patients with several hematopoietic malignancy.
Aims: In the present study, we investigated the molecular mechanisms by which PF-04449913 regulate the self-renewal of MDS-derived iPS cells (iPSCs) *in vivo*.
Methods: We generated iPSCs from bone marrow mononuclear cells of two MDS patients (RAEB1 and RAEB2 by WHO clssification) with complex karyotypic abnormalities. Karyotyping analysis revealed that MDS-derived iPSCs have identical abnormalities to primary MDS cells. We also generated iPSCs from bone marrow mononuclear cells of normal volunteer as control. To investigate the effects of PF-04449913 on self-renewal and the relevance as a therapeutic target in MDS initiating cells, NOD/SCID mice were injected sucutaneously with MDS-derived iPSCs or normal iPSCs then treated with PF-04449913 (100 mg/kg; p.o.) from day 10 for 28 days. We also used MDS-L, a myelodysplastic cell line establised from MDS patient with del(5q) and complex karyotypic abnormalities for *in vitro* studies.
Results: Both MDS-derived iPSCs transferred NOD/SCID mice and normal iPSCs transferred NOD/SCID mice demonstrated the engraftment of CD34+CD38- positive cells by flow cytometry. However, the treatment with PF-04449913 reduced the population of CD34+CD38- positive cells in MDS-derived iPSCs transferred NOD/SCID mice. We isolated human CD45+ cells from the spleen of mice from each treatment group and injected equivalent numbers of CD45+ cells into secondary recipients. Following 50 days, all mice treated with vehicle engrafted with CD34+CD38- positive cells. In contrast, CD34+CD38- positive cells engraftment was not detected in recipient mice (n=3) from PF-04449913-treated donors. These results demonstrate the persistent effects of PF-0449913 on long term self-renewing MDS-initiating cells. We further examined the effects of Nanog pathway modulation on *in vitro* clonogenic growth. CD34+CD38- cells from MDS-derived iPSCs transferred NOD/SCID mice and MDS-L cells were treated with 2 mM of PF-04449913 for 72 hrs, washed free of drugs, and plated in quadruplicate in methylcellulose. At 14 days, colonies were counted as initial plating. The representative plate was then washed and cells were re-suspended and re-plated. After an additional 14 days, colonies were counted as secondary re-plating. Clonogenic recovery of untreated cells was normalized to 100% and plating results from all treatment groups were expressed as % control. PF-04449913 had only minimum effects on colony formation after initial plating over control cells. However, upon serial re-plating, secondary colony formations were significantly inhibited by PF-04449913 ($p<0.001$). Also reduced expression of Nanog by shRNA also supressed the secondary colony formations.To identify the mechanisms that limit the self-renewal of MDS-initiating ells by PF-04449913, NOD/SCID mice engrafted with CD34+CD38- fractions from MDS-derived iPSCs were treated

with PF-04449913 (100 mg/kg; p.o.) for 14 days. PF-04449913 induced the expressions of p21Cip1, cleaved PARP and reduced the expression of BMI-1, c-Myc, Nanog, and Bcl-XL.
Summary/Conclusions: Our preclinical results indicate that PF-04449913 have potential as an important option for controlling the drug-resistant MDS-initiating cells. It is expected that the PF-04449913 may become extremely useful therapeutic interventions in a number of hematological neoplasms, including MDS, where the persistence of cancer stem cells.

S445

SF3B1 MUTATIONS IN MDS-RS ARISE IN MULTIPOTENT HEMOPOETIC STEM CELLS

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Background: Mutations in the RNA splicing gene *SF3B1* are found in more than 80% of patients with myelodysplastic syndrome with ring sideroblasts (MDS-RS), and predict for a stable clinical course and a favourable survival (Malcovati L. *et al., Blood* 2015 126(2):233-41). Little is known about the cellular origin of these mutations or their hierarchy in relation to other somatic mutations.
Aims: To establish the origin of *SF3B1* mutations within the bone marrow (BM) hematopoietic stem and progenitor cell compartment (HSPC) in MDS-RS.
Methods: We investigated BM mononuclear cells (MNC) from 13 MDS-RS patients (6 RARS and 7 RCMD-RS) and 4 healthy controls for mutational spectrum by targeted sequencing, and quantified HSPC subsets using flow cytometry. Functional analysis of the purified populations was performed *in vivo*, using xenotransplantation in NOD scid gamma (NSG) mice to establish repopulating and self-renewal ability, and *ex vivo* using clonogenic progenitor assays: colony forming-unit (CFU) and long-term culture initiating cell (LTC-IC). Hierarchy of molecularly and functionally distinct stem and progenitors cells was established using pyrosequencing.
Results: Screening for recurrently mutated genes in the MNC fractions revealed mutations in *SF3B1* in 12/13 cases, combined with *TET2* and *DNMT3A* in 4 and 2 patients, respectively. The frequencies of phenotypically defined MDS-RS HSPC in the BM did not differ from that of normal controls, whereas pro-B cells were significantly reduced ($p<0.005$) in MDS-RS. We tracked back all the mutations identified in the MNC fractions to each purified MDS-RS stem and progenitor population, and in all LTC-IC and CFU colonies generated *in vitro*. Importantly, we identified the *SF3B1* mutation at the BM pro-B level in 5 investigated patients, and also in a smaller fraction, in purified peripheral blood B-cells from these patients. Analysis of peripheral blood B cells from the same patients 2-3 years after the first sampling showed an increase in *SF3B1* allelic burden in all cases. Importantly, co-mutations including *DNMT3A* and *B-COR* were not detected in peripheral B cells. This demonstrates that the origin of *SF3B1* mutation arises in the very primitive lymphoid-myeloid multipotent stem cell compartment. We transplanted purified hematopoietic stem cells (HSC) and lineage restricted cell populations: CMPs, GMPs and MEPs from 4 *SF3B1* mutated MDS-RS patients into NSG mice; 2 of which also carried *TET2* mutations. Engraftment, as defined by ($hCD45^+CD19^+$, $hCD45^+CD33^+CD66b^+CD15^+$ or GPA^+CD71^+) of all four HSC samples was detected, while all more mature cell populations failed to engraft. Importantly, morphological development of ring sideroblasts was observed in mice transplanted with HSCs from all 4 patients, proving that the self-renewal potential as well as commitment to the RS erythroid phenotype is restricted to MDS-RS HSC.
Summary/Conclusions: Our findings provide evidence of a multipotent stem cell origin of the *SF3B1* mutation in MDS-RS patients and propose the existence of a mixed B-cell population formed by abnormal cells derived from the MDS-RS clone and a normal "pre-clonal" population. Stem cell potential and reproduction of the MDS-RS erythroid phenotype was restricted to MDS-RS stem cells only.

S446

CLONAL ORIGIN OF THERAPY-RELATED MYELOID NEOPLASMS

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Background: Therapy-related myeloid neoplasms (t-MNs) are secondary malignancies that develop in patients treated with chemotherapy and/or radiation therapy (C/RT). Previously, it was thought that genotoxic stress from C/RT induced driver mutations in hematopoietic stem cells leading to the development of t-MNs. However, a recent study suggested that pre-leukemic *TP53* mutations could pre-exist in t-MN patients years before t-MN development. Further, hematological driver mutations have been detected in peripheral blood (PB) from apparently healthy individuals or patients with solid tumors, a phenomenon referred to as clonal hematopoiesis of indeterminate potential (CHIP).
Aims: To test the hypothesis that pre-leukemic driver mutations are detectable in t-MN patients at the time of primary cancer diagnosis and prior to C/RT exposures.
Methods: We identified 14 patients with t-MNs who were found to have paired samples of diagnostic bone marrow (BM) at the time of t-MN diagnosis and PB obtained after the diagnosis of their primary cancers but prior to C/RT. Targeted gene sequencing of the 280 leukemia-related genes was performed on the 14 t-MNs diagnostic BM to detect driver mutations. We then assessed presence of the same driver mutations in the patients' matched PB samples taken at the time of primary cancer diagnosis. Because pre-leukemic driver mutations in the PB samples were expected to have very low variant allele frequency (VAF), we performed molecular barcoding deep sequencing of 32 genes that were reported as CHIP-associated mutation.
Results: Of the 14 t-MN patients, 5 (36%) had t-AML and 9 (64%) had t-MDS. The median age at primary cancer diagnosis and at t-MNs diagnosis was 62 years (range: 25-74) and 66 years (range: 28-77), respectively. The median latency from primary cancer to t-MNs was 3 years (range: 1-8). In the t-MN BM, 3 patients (21%) had normal karyotype and 7 (50%) had del 7q/-7, 4 (29%) had del 5q/-5 and 5 (36%) had complex karyotypes. Targeted gene sequencing of t-MN BM (median 289x): revealed 21 canonical hematological driver mutations in 14 t-MNs patients: mutations in *TP53* (29%), *DNMT3A* (21%), *TET2* (21%), *RUNX1* (21%), *IDH2* (14%), *SRSF2* (7%), *EZH2* (7%), *FLT3* (7%), *NRAS* (7%), *PTPN11* (7%) and *GATA2* (7%). Molecular barcoding deep sequencing of the PB samples (median 3,000x) taken at the time of primary cancer diagnosis revealed that pre-leukemic driver mutations were detected in 10 out of 14 (71%) patients. Figure 1 shows the model of clonal evolution from pre-leukemic driver mutations to t-MN in 3 representative cases. For example, Case UID6982 had limited stage small cell lung cancer and received concurrent chemo-radiation therapy with carboplatin and etoposide. He developed t-AML 3 years after C/RT and was found to have an *IDH2* p. R140Q (VAF 25%) and *SRSF2* p.P95fs (46%) mutations in the diagnostic BM. His PB samples obtained before C/RT showed the same *IDH2* p.R140Q and *SRSF2* p.P95fs mutations with VAF of 16.4% and 9.8%, respectively. By genes, 75% of *TP53*, 67% of *DNMT3A*, 100% of *TET2*, 67% of *RUNX1*, 50% of *IDH2,* 100% of *SRSF2*, 100% of *FLT3,* 100% of *NRAS*, and 100% of *PTPN11* mutations were detected as pre-leukemic driver mutations at time of primary cancer diagnosis. We did not detect *EZH2* and *GATA2* mutations as pre-leukemic mutations. The median VAF of the detected pre-leukemic driver mutations was 7.6% (range: 0.5-23%). There was no statistical difference between patients with and without detectable pre-leukemic mutations in terms of age at primary cancer diagnosis or latency time to t-MN development (P=0.73 and 0.54, respectively).

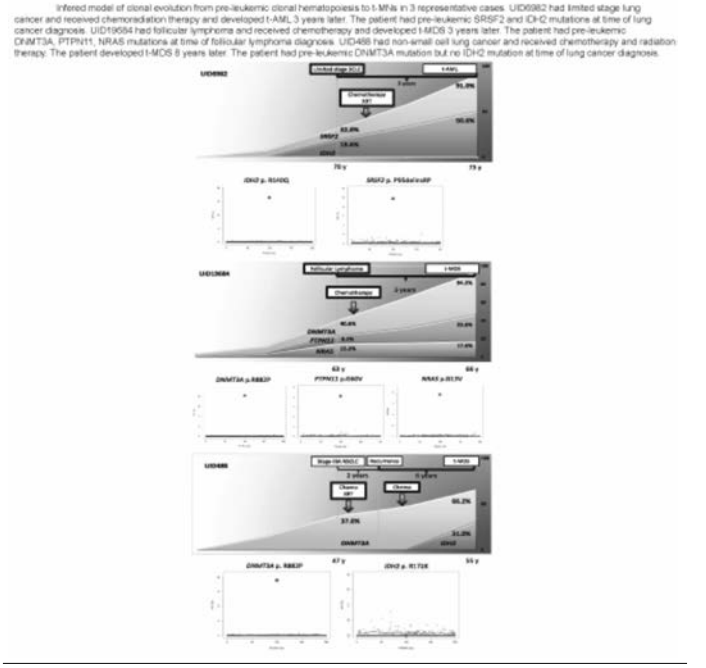

Figure 1.

Summary/Conclusions: In this study we have demonstrated evidence of detectable pre-leukemic driver mutations in multiple leukemia driver genes at time of diagnosis and before therapy of primary cancer in patients whose subsequent t-MNs also harbored identical driver mutations. These data suggest the potential to develop a risk stratification model based on presence of CHIP with canonical driver mutations at the time of primary cancer diagnosis. Determining predictive value of pre-leukemic driver mutations in t-MN development is currently undergoing.

New biological markers in MM

S447

A TARGETED SEQUENCING APPROACH IN MULTIPLE MYELOMA REVEALS A COMPLEX LANDSCAPE OF GENOMIC LESIONS THAT HAS IMPLICATIONS FOR PROGNOSIS

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Background: Next-generation sequencing (NGS) studies have shown that multiple myeloma is a heterogeneous disease with a complex subclonal architecture and few recurrently mutated genes. The analysis of smaller regions of interest in the genome ("targeted studies") allows interrogation of recurrent genomic events with reduces complexity of downstream analysis at a lower price.
Aims: Here, we performed the largest targeted study to date in multiple myeloma to analyze gene mutations, deletions and amplifications, chromosomal copy number changes and immunoglobulin heavy chain locus (IGH) translocations and correlate results with biological and clinical features.
Methods: We used Agilent SureSelect cRNA pull down baits to target: 246 genes implicated in myeloma or cancer in general in a mixed gene discovery/confirmation effort; 2538 single nucleotide polymorphisms to detect amplifications and deletions at the single-gene and chromosome level; the IGH locus to detect translocations. We sequenced unmatched DNA from CD138-purified plasma cells from 418 patients with multiple myeloma at diagnosis, with a median follow-up of 5.3 years. We sequenced at an average depth of 337x using Hiseq2000 machines (Illumina Inc.). We applied algorithms developed in-house to call genomic events, filtering out potential artifacts and germline variants. We then ranker each event on its likelihood of being "oncogenic" based on clustering, recurrence and cross-reference with the COSMIC database.
Results: We identified 2270 gene mutations in 412/418 patients, and of those 688 were oncogenic. 342 patients harbored at least one oncogenic mutation. 215/246 genes showed at lease one likely somatic mutation, but only 106 showed at least one oncogenic mutation. 63% of oncogenic mutations were accounted for by the top 9 driver genes previously identified (KRAS, NRAS, TP53, FAM46C, BRAF, DIS3, TRAF3, SP140, IRF4), implying our gene discovery effort did not identify novel mutated genes. We included deletion of tumor suppressors, amplification of oncogenes, chromosomal copy number changes and IGH translocations for a total of 76 variables, so that 413/418 patients showed at least one informative driver genomic event, (median 4/patient). We investigated pairwise associations between events and found significant correlations, such as TP53 mutations and del(17p), CYLD mutations and del(16), FAM46C mutations and del(1p), SF3B1 mutations and t(11;14). Hotspots mutations of IRF4 lysine p.123 showed an inverse correlation with a hyperdiploid karyotype and del(16) as opposed to other missense mutations scattered along the gene, which has pathogenic implications. Survival was negatively affected by the cumulative burden of lesions in an almost linear fashion, with median survival of 10.97 and 4.07 years in patients with <=2 or >=7 lesions respectively, and this was independent of the nature of the genomic events. Given the heterogeneity and complex interplay of the variables we fitted a cox-proportional hazard model to predict survival. We found that mutations in TP53, amplifications of MYC, deletions of CYLD, amp(1q), del12p13.31 and del17p13 where the only significant events, all promoting shorter survival. In particular, TP53 mutations and deletions, often co-occurring, had an additive effect so that carriers of both showed a dismal survival of 17 months (Figure 1).

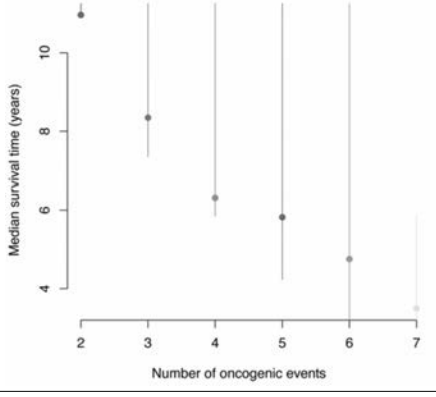

Figure 1.

Summary/Conclusions: Due to the complex genomic landscape in MM, a discovery effort still requires large studies to derive significant associations. We conclude that a targeted sequencing approach may provide prognostic models and give insights into myeloma biology.

S448

A NEW MULTIPLE MYELOMA CLASSIFICATION SYSTEM THAT CORRELATES TO DISEASE STAGE AND PROGNOSIS - INDICATION OF REVERSIBLE PHENOTYPIC PLASTICITY AS A HALLMARK

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Background: Today's diagnostic tests for multiple myeloma (MM) reflect the criteria of the updated WHO classification based on biomarkers and clinicopathologic heterogeneity.
Aims: To that end, we propose a new biological subtyping of myeloma plasma cells (mPC) by B-cell subset associated gene signatures (BAGS), from the normal B-cell hierarchy in the bone marrow (BM). Here we document the prognostic and biological value of subtyping, as shown for DLBCL (JCO 2015 Apr 20; 33:1379).
Methods: We combined FACS and GEP to generate BAGS classifiers for the normal BM subsets: PreB-I, PreB-II, immature (Im), naive (N), memory (M) and PC. Construction was based on median-centred probe sets from the BM data using regularized multinomial regression with six discrete outcomes representing BAGS, by a total of 55 genes varying from 15-24 per subtype. Each patient underwent BAGS assignment according to the highest predicted probability score above 0.45 or was otherwise unclassified. The impact of BAGS was analyzed using six clinical cohorts, gathered across geographical regions, time eras, and sampling methods. The analysis estimated subtype frequencies and included a prognostic meta-analysis of 926 patients treated with high dose melphalan as first line therapy in 3 prospective trials: UAMS, HOVON65/GMMG-HD4, MRC Myeloma IX data with the Affymetrix U133 plus 2.0 microarray data available from myeloma PC samples. To compensate for cohort-wise technical batch effects, each cohort was median centred and adjusted probe set-wise to have same variance as the BM data.
Results: *Validation of the normal B-cell subset phenotypes.* Normalized histograms of the fluorescence intensities (FI) of CD markers based on merged multiparametric flow cytometry reanalysis of pure sorted populations resulting from seven independent sorting procedures documented high purity. Principal component analysis (PCA) of the FI for each sorted cell in all samples documented specificity. Surface markers, transcription factors, and B-cell differentiation–specific genes were identified through a literature review, and their expression across subsets was evaluated. The most varying probe sets were included in an unsupervised hierarchical clustering analysis, supporting the biological differences. *Validation of MM patients subtyping by prognosis.* The resultant tumor assignments exhibited very similar BAGS subtype frequencies, across 1302 individual MM cases from 4 different cohorts. The 5 BAGS subtypes of 926 MM cases were significantly associated with overall ($P=5.2x10^{-8}$) and progression free ($P=1.5x10^{-6}$) survival in a meta-analysis of patients in the 3 clinical trials. The major impact was observed within the PreB-II and M subtypes conferred with significant increased ISS stage III and inferior prognosis compared to the Im, N and PC subtypes. Cox proportional hazard meta-analysis showed that the five BAGS subtypes added significant and independent prognostic information to the TC classification system and plasma Beta-2 microglobulin level. In parallel we found significant correlation between the PreBII subtypes and the proliferation index, risk profiling ($P<0.0001$) and Beta-2 microglobulin ($P<0.001$).
Summary/Conclusions: We have documented patient specific mPC differences with prognostic impact in support of reversible phenotypic plasticity in MM. This observation provides a new model for generating insight into the stages of clonal plasticity associated with oncogenesis and dedifferentiation.

S449

DEFINING NEW THERAPEUTIC AGENTS THAT TARGET THE ONCOGENIC TRANSLATION PROGRAM IN MULTIPLE MYELOMA

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Background: Despite significant therapeutic advances, Multiple Myeloma (MM) remains an incurable hematological malignancy. Therefore, there is a need for the development of new therapeutic options with novel mechanisms of action that have not been previously explored in MM.
Aims: To identify new potential therapies in MM, we screened a library of small molecules against lymphoid cell lines and defined the mode of action for the most active class of hits that we found. These compounds act as potent, highly selective inhibitors of translation initiation and therefore inhibit the oncogenic translation program of MM.
Methods: A chemically diverse small-molecule drug screen of over 3,000 compounds from the Boston University Center for Molecular Discovery was performed using H929 and NAMALWA cell lines. Follow-up validation and structure-activity relationship (SAR) studies were performed in several cell lines including H929, MM1S, MM1R, U266, RPMI and OPM2. RNA sequencing was performed using the NEBNext kit and Illumina HiSeq 2500. Quantitative proteomic analysis was performed by Tandem Mass Tag (TMT) with mass spectrometry (MS). For animal studies, SCID mice were injected with $5x10^6$ MM1S luc-GFP cells and followed by blood cell count, body weight, bioluminescence imaging (BLI) and survival.
Results: A primary screen of over 3,000 small-molecules identified 45 compounds with potent activity on H929 and NAMALWA. Further validation of these hits in 6 cell lines highlighted 3 compounds of the rocaglate class as by far the most effective. A follow-up SAR screen including 40 rocaglate derivatives identified a compound called CMLD10509 with an IC50 below 10nM against several MM cell lines, but no cytotoxicity on human PBMCs, suggesting a useful therapeutic window for this agent in MM. To define the mode of action for CMLD10509 against MM, we performed RNA-seq on drug- *vs* DMSO-treated cell lines. By GSEA analysis, the most enriched pathways were regulators of transcriptional activation and translation inhibition. To validate these findings we queried our compound's signature against the LINCS Cloud database - a large catalog of gene-expression profiles collected from human cells exposed to chemical and genetic perturbations - and identified a positive correlation between our drug signature and translation inhibitors as well as KD of ribosome subunits. To better define the consequences of CMLD10509-induced translation inhibition, we performed a quantitative proteomic MS experiment. We identified 7312 proteins of which 54 were significantly down-regulated by CMLD10509 exposure ($p<0.05$ and FC>2), among them key oncoproteins in MM such as MYC, MDM2, CCND1, MCL1 and MAF (Figure 1). Strikingly, down-regulated proteins were strongly associated with KEGG cancer pathways, representing the oncogenic translation program in MM. Correlation between TMT and RNA-seq (confirmed by immunoblot and qRT-PCR) validated a purely translational mechanism as responsible for their depletion in MM cells. Importantly, increased expression of these same rocaglate-sensitive genes was significantly enriched in MM patients in several datasets. Finally, CMLD10509 was both active and well tolerated in a xenograft model of MM (n=10 per group), with significantly lower BLI counts ($p<0.001$) and prolonged survival (median OS 35 *vs* 47 days, $p<0.001$).

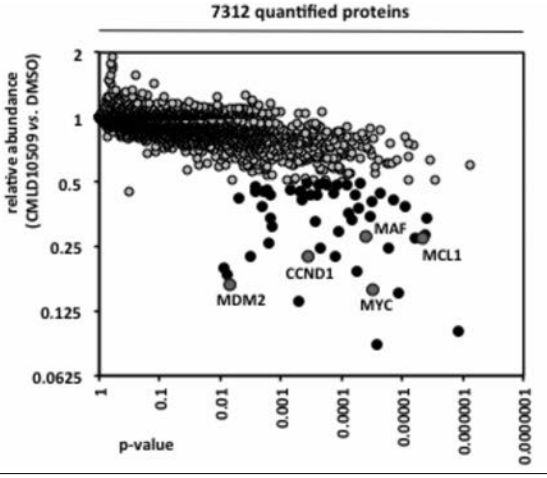

Figure 1. TMT mass spectometry of NCIH929 treated with CMLD10509 or vehicle (DMSO). Triplicate analysis, two tailed *t*-test.

Summary/Conclusions: From a large-scale small molecule screen, we identified CMLD10509 as a potent compound, which selectively inhibits the translation initiation and impairs an oncogenic translation program supporting MM cells that includes MYC, MDM2, CCND1 and MCL1. As proof of concept for the potential of CMLD10509 as a novel therapeutic strategy, we found it to be highly active and well tolerated in a mouse model of MM.

S450

PD-1 BLOCKADE ENHANCES THE EFFICACY OF ELOTUZUMAB IN MOUSE TUMOR MODELS

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Background: Elotuzumab is a humanized monoclonal antibody (mAb) that binds specifically to human SLAMF7 (hSLAMF7) and has been approved by the US Food and Drug Administration for use in combination with lenalidomide and dexamethasone for the treatment of patients with multiple myeloma (MM) who have received one to three prior therapies. Previous preclinical models demonstrated that elotuzumab inhibited the growth of SLAMF7-expressing human myeloma xenografts in immune-compromised mice. These models, however, lacked adaptive immune cells and therefore could not assess the mechanism of elotuzumab anti-tumor activity in the setting of a fully intact immune system.
Aims: To model elotuzumab mechanism of action, alone or in combination with PD-1 blockade, using human natural killer (NK) cells *in vitro* and mouse tumor models *in vivo*.
Methods: We evaluated elotuzumab-mediated anti-tumor activity alone or in combination with a mAb to PD-1 using mouse cell lines that stably express human SLAMF7 (A20-hSLAMF7 and EG7-hSLAMF7). The effect of treatment on the phenotype and functionality of tumor-infiltrating lymphocytes (TILs) using flow cytometry and immunohistochemistry was evaluated. In addition, the effects of elotuzumab on the phenotype of human NK cells isolated from either healthy donors or patients with MM treated with elotuzumab plus lenalidomide and dexamethasone was assessed.
Results: A murine IgG2a version of elotuzumab (elotuzumab-g2a) inhibited growth of hSLAMF7-expressing mouse tumor cells *in vivo*. Tumor-bearing mice treated with elotuzumab-g2a, anti-PD-1, or elotuzumab-g2a plus anti-PD-1 had significantly improved survival over those treated with control IgG2a (Figure 1). Anti-tumor activity mediated by elotuzumab-g2a required an Fc fragment that was able to interact with activating Fc receptors, was partially dependent on both NK cells and CD8+ cells, and synergized with anti-PD-1 treatment. TILs taken from mice treated with elotuzumab-g2a plus anti-PD-1 had increased numbers of activated tumor-specific and total CD8+ T cells. Elotuzumab-g2a and anti-PD-1 combination treatment also promoted NK cell activation and cytokine and chemokine release within the tumor. *In vitro* co-cultures of human NK cells, elotuzumab, and SLAMF7-expressing tumor cell lines led to NK cell degranulation, upregulation of inflammatory cytokines, and enhanced expression of activation markers. Similarly, NK cells isolated from bone marrow aspirates from patients treated with elotuzumab showed an enhanced activation state compared with NK cells isolated prior to treatment.

Kaplan–Meier curve showing survival in A20-hSLAMF7-bearing mice treated with control mIgG2a (n=9), elotuzumab-g2a 10 mg/kg (n=9), anti-PD-1 3 mg/kg (n=9), or elotuzumab 10 mg/kg plus anti-PD-1 3 mg/kg on Days 10, 14, and 17. Differences between survival curves were analyzed using Mantel-Cox test.

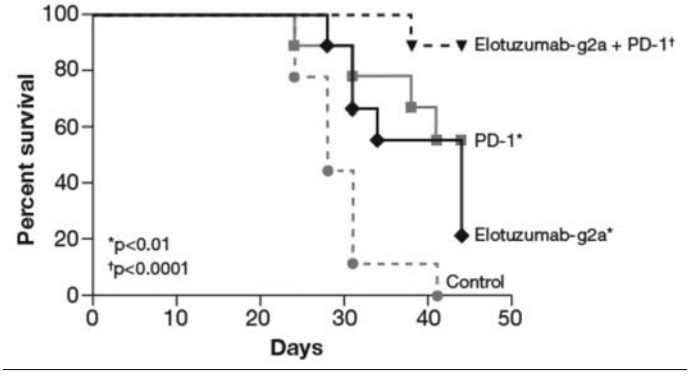

Figure 1.

Summary/Conclusions: The combination of the tumor-targeted antibodies elotuzumab-g2a and anti-PD-1 showed enhanced anti-tumor efficacy in mouse tumor models. The strong control of tumor growth observed with combination treatment was due to activation of both adaptive and innate immune cells local-

ized to the tumor microenvironment. These *in vivo* findings support the suggested mechanism of action of elotuzumab and PD-1 blockade and provides rationale for the clinical investigation of elotuzumab and anti-PD-1 combination therapy in patients with MM.
Study support: Bristol-Myers Squibb.

S451

THERAPEUTIC IMPACT OF TAK-1 INHIBITION ON TUMOR GROWTH AND BONE DESTRUCTION IN MYELOMA

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Background: Multiple myeloma (MM) is still incurable with progressive bone loss. The development of novel therapeutic options yielding better survival outcomes with bone restoration is urgently needed. We have reported that Pim-2 is overexpressed in MM cells and their surrounding cells, namely bone marrow stromal cells (BMSCs) and osteoclasts, in bone lesions, and that treatment with Pim inhibitors markedly suppressed MM tumor growth while preventing bone destruction in MM-bearing animal models, indicating Pim-2 as an important therapeutic target in MM (Leukemia 2011, 2015). We recently found TGF-β-activated kinase-1 (TAK-1) as an upstream mediator responsible for Pim-2 up-regulation.
Aims: We therefore aimed to clarify the role of TAK-1 in tumor growth and bone destruction in MM and therapeutic impact of TAK-1 inhibition.
Methods: TAK-1 and Pim-2 expression and TAK-1 phosphorylation was analyzed by Western blotting. Osteoclastogenic activity was analyzed by TRAP staining as well as resorptive pit formation. Osteoblastogenesis was estimated with mineralized nodule formation by MC3T3-E1 preosteoblastic cells. The TAK-1 inhibitor LLZ1640-2 and TAK-1 siRNA were used to block TAK-1 activation and reduce TAK-1 expression, respectively. To create MM animal models, murine 5TGM1 MM cells were inoculated into the tibia in SCID mice.
Results: TAK-1 was constitutively over-expressed and phosphorylated in MM cells while only marginally in normal peripheral blood mononuclear cells. The TAK-1 inhibitor LLZ1640-2 dose-dependently suppressed cell growth, and induced caspase-dependent apoptosis in MM cells. Although TNF-α and IL-6 upregulated Pim-2 expression in MM cells, LLZ1640-2 abolished TNF-α-induced NF-κB, p38MAPK and ERK activation and IL-6-induced STAT3 activation in MM cells, leading to Pim-2 suppression. The TAK-1 inhibition also reduced the transcription factor Sp1 expression upregulated in MM cells. Because Sp1 induces the expression of various factors responsible for MM cell growth and survival, Sp1 reduction by the TAK-1 inhibition appears to cause efficaciously suppression of MM cell survival. LLZ1640-2 as well as TAK-1 knock-down decreased VCAM-1 expression and IL-6 production in BMSCs, and MM cell adhesion to BMSCs to impair BMSC support of MM cell growth. Interestingly, phosphorylation of TAK-1 was induced in BMSCs and MC3T3-E1 preosteoblastic cells by addition of cytokines known as inhibitors of osteoblastogenesis in MM, including IL-3, IL-7, TNF-α, TGF-β and activinA, as well as MM cell conditioned media (MMCM), suggesting TAK-1 as a common mediator to suppress osteoblastogenesis in MM. Furthermore, LLZ1640-2 abolished up-regulation of Pim-2, an inhibitory mediator of osteoblastogenesis, in BMSCs and MC3T3-E1 cells by MMCM to restore mineralized nodule formation. Moreover, the TAK-1 inhibition up-regulated in MC3T3-E1 cells phosphorylation of Smad1/5 and p38MAPK by BMP-2 while suppressing Smad2/3 phosphorylation by TGF-β suggesting potentiation of BMP-2-mediated anabolic signaling. TAK-1 was also up-regulated in osteoclastic lineage cells along with osteoclastogenesis. Treatment with LLZ1640-2 suppressed the induction of c-fos and NFATc1 in RAW264.7 preosteoclastic cells as well as their osteoclastogenesis by RANK ligand. Finally, treatment with LLZ1640-2 potently suppressed MM growth in murine MM models.
Summary/Conclusions: TAK1 plays a pivotal role in MM tumor growth and bone destruction in MM. TAK1 may become an efficacious therapeutic target in MM to suppress tumor burden while restoring bone.

Myeloproliferative neoplasms - Clinical 2

S452

LONG-TERM OUTCOMES OF RUXOLITINIB (RUX) THERAPY IN PATIENTS (PTS) WITH MYELOFIBROSIS (MF): 5-YEAR FINAL EFFICACY AND SAFETY ANALYSIS FROM COMFORT-I

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Background: The JAK1/JAK2 inhibitor RUX has demonstrated rapid and durable improvements in splenomegaly and symptoms and improved survival in the two phase 3 COMFORT studies in pts with MF.
Aims: To report final long-term efficacy and safety results after 5 years (y) of RUX treatment in COMFORT-I.
Methods: In COMFORT-I, 309 pts were randomized (1:1) to RUX or placebo (PBO). RUX starting dose was based on baseline platelet count (100–200×10^9/L: 15 mg BID; >200×10^9/L: 20 mg BID). Pts receiving PBO could crossover to RUX after the primary analysis (when all pts completed week [wk] 24 and half completed wk 36) or at any time if they had prespecified worsening of splenomegaly. The primary endpoint was the proportion of pts achieving ≥35% reduction in spleen volume (SV) at wk 24. Overall survival (OS) was estimated by Kaplan-Meier analysis according to randomized treatment (intent-to-treat analysis).
Results: Median follow-up was 268 wk at the time of this analysis. Of 155 pts randomized to RUX, 43 were still on treatment at the time of study termination. Of 154 pts randomized to PBO, 111 crossed over to RUX; median time to crossover was 41.1 wk; 28 were still on treatment at the time of study termination. At wk 24, pts originally randomized to RUX had a mean SV reduction from baseline of 31.6%; this response was durable for pts who continued on RUX with a mean SV reduction of 37.6% at wk 264. At wk 264, 18.5% of pts randomized to RUX had a ≥35% reduction from baseline in spleen volume. Median duration of spleen response (≥35% SV reduction) was 168.3 wk for RUX (range, 107.7–NE; n=92 pts). OS favored RUX (HR=0.69; 95% CI: 0.50, 0.96; *P*=0.025), with 69 and 82 deaths among pts originally randomized to RUX and PBO, respectively. Median OS has not been reached for pts in the RUX group. Median OS was approximately 108 wk for pts randomized to PBO censored at crossover, and approximately 200 wk for PBO pts. Mean platelet count and hemoglobin (Hgb) initially decreased through 3 mo. Mean Hgb gradually increased toward baseline. After wk 24, mean Hgb and platelet count generally remain stable through 5 y. New onset grade 3 or 4 anemia was 25.2% for the RUX arm and 26.1% for PBO crossover; grade 3 or 4 thrombocytopenia occurred in 12.3% and 13.5% of pts, respectively. Notable AEs included herpes zoster (10.3% and 13.5% of RUX and PBO crossover pts, respectively); basal cell carcinoma (7.7% and 9.0%, respectively); acute myeloid leukemia (5 pts in each arm). The rate of leukemic transformation was 0.01 per pt-year for pts randomized to RUX and 0.02 per pt-year for the PBO crossover pts (Figure 1).

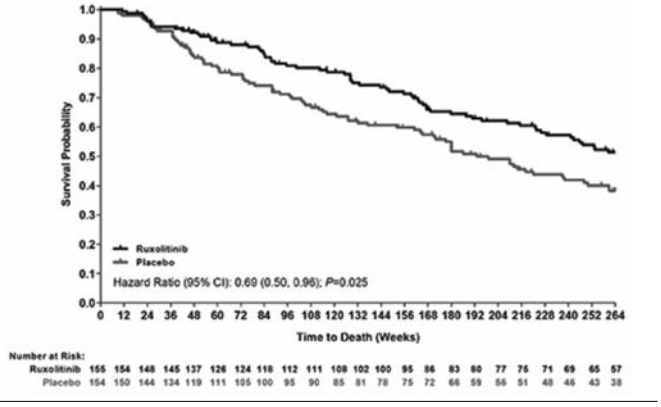

Figure 1.

Summary/Conclusions: After a median follow-up of 268 wk, the hazard ratio for OS favored pts randomized to RUX over those randomized to PBO, and SV reductions were sustained with long-term therapy. Collectively, these data support the durable efficacy and long-term safety of RUX in pts with MF.

S453

IMPACT OF THE MOLECULAR PROFILE ON THE RISK OF ACUTE MYELOID TRANSFORMATION IN PATIENTS WITH POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are myeloproliferative neoplasms (MPN) associated with driving mutations in *JAK2*, *CALR* or *MPL* genes that can evolve into acute myeloid leukemia (AML). Older age, abnormal cytogenetics and sequential exposure to cytoreductive agents have been associated with higher risk of AML.
Aims: To analyze the influence of the genotype on the risk of myeloid transformation in patients with PV and ET. In addition, the somatic mutational profile in paired samples from the chronic and the leukemic phases was studied.
Methods: A total of 1095 patients (PV n=371, ET n=724) were included. The risk of myeloid transformation according to age, sex, type of disease (PV or ET), leukocyte count at diagnosis, cytogenetics, cytoreductive treatment, and genotype (*JAK2*, *CALR*, *MPL,* and triple negative) was evaluated by Cox regression. Next generation sequencing (NGS) analysis of 66 AML-related genes was performed in paired samples from the chronic and leukemic phases, whenever possible. Supported by grants from the Instituto de Salud Carlos III, Spanish Health Ministry: PI13/00557, PI1300393 and PI1300636.
Results: Median age at MPN diagnosis was 63 years (females: 59%). An abnormal karyotype was documented in 30 (2.7%) out of 583 evaluable patients. Genotype was available in 806 patients: 92.5% of PV patients were *JAK2*-mutated, whereas 64%, 18%, 3%, and 15% of ET patients were *JAK2*+, *CALR*+, *MPL*+ or triple negative, respectively. Hydroxyurea plus P32, busulphan or melphalan was administered in 10% of patients. With a median follow-up of 8 years, 35 cases of AML and 5 cases of myelodysplastic syndrome (MDS) were diagnosed, resulting in a 10-year probability of myeloid transformation of 3.9%. There were no significant differences in transformation rate according to MPN type (10-year probability of 4.7% and 3.5% for PV and ET, respectively, p=0.8). In multivariate analysis, an increased risk of AML/MDS transformation was observed for patients older than 60 years (HR: 4.3, 95%CI: 1.6-11.6, p=0.004), with leukocytes >10x109/L (HR: 2.2, 95%CI: 1.01-4.7, p=0.048) and those treated with hydroxyurea plus leukemogenic agents (HR: 2.5, 95%CI: 1.2-5.4, p=0.02). An abnormal karyotype was associated with an increased risk of transformation in PV (HR: 5.1, 95%CI: 1.2-22.1, p=0.03) but not in ET. In ET patients, the 10-year probability of AML/MDS according to genotype was 25% for *MPL*+, 6.6% for triple negative, 4.6% for *JAK2*V617F+, and 0% for *CALR*+ (p=0.007). When the genotype was included in the multivariate model, the *MPL* mutation was an independent risk factor for higher risk of AML/MDS (HR: 6.1, 95%CI: 1.2-30.2, p=0.028), whereas *JAK2*V617F, *CALR* or triple negative genotypes lacked prognostic significance. NGS was performed in 24 post-MPN AML/MDS samples. Somatic mutations in genes other than *JAK2*, *CALR* or *MPL* were seen in 20 (83%) patients. Five (21%) patients have one, 7 (29%) two, 3 (12.5%) three and 5 (21%) four or more additional mutations. Mutational frequencies were: *TP53* 42%, *TET2* 21%, *RUNX1* 21%, *DNMT3A* 17%, *IDH1/2* 17%, *SRSF2* 12%, *ASXL1* 12%, *SETBP1* 8%, *SH2B3* 8%, and <5% for *SF3B1*, *NRAS*, *KRAS*, *NOTCH1*, *PTPN11, CBL, NPM* and *FLT3*. At time of submission, NGS molecular studies were performed in 10 paired samples at PV/ET diagnosis and AML/MDS transformation being 52% of the mutations present in both samples.
Summary/Conclusions: *MPL* genotype is associated with higher risk of AML/MDS transformation. Somatic mutations in genes other than *JAK2, CALR or MPL* are frequently observed at time of transformation, with 50% of them being already present at ET/PV diagnosis. TP53 is the gene most frequently mutated at time of transformation.

S454

RUXOLITINIB (RUX) REDUCES JAK2V617F ALLELE BURDEN (AB) IN PATIENTS (PTS) WITH POLYCYTHEMIA VERA (PV) ENROLLED IN THE RESPONSE STUDY

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Background: The *JAK2*V617F mutation leads to constitutive activation of downstream JAK/STAT signaling in pts with PV. In the phase 3 RESPONSE study evaluating Rux (an oral JAK1/JAK2 inhibitor) *vs* best available therapy (BAT) in pts with PV who had an inadequate response to or unacceptable side effects from hydroxyurea, Rux was superior to BAT in controlling hematocrit, reducing spleen volume, and improving symptoms. Rux also decreased *JAK2*V617F AB steadily over time up to Week 112.
Aims: This follow-up exploratory analysis of the RESPONSE study evaluated the effect of long-term Rux treatment on *JAK2*V617F AB in pts with PV.
Methods: 211 pts with the *JAK2*V617F mutation at Baseline were evaluable for this analysis. AB was defined as the percent of mutant allele relative to total (wild-type and mutant). Changes from Baseline in *JAK2*V617F AB among Rux-randomized pts and those who crossed over from BAT to Rux were reported up to Week 208. Baseline AB for the crossover cohort was defined as last observation before receiving Rux. Complete and partial molecular remission (CMR, PMR) were defined using the IWG-MRT/ELN consensus criteria. An exploratory analysis was performed to identify any potential relationship between reductions in AB and spleen volume.
Results: Pts randomized to Rux (n=104) experienced consistent AB reduction (mean percent change from Baseline, –12.2%, –18.3%, –22.0%, –30.0%, –38.1%, –34.9%, and –40.0% at Weeks 32, 56, 80, 112, 144, 176, and 208, respectively). On average, BAT-randomized pts did not experience AB improvements at Week 32 (mean percent change from Baseline, 1.2%); those who crossed over to Rux (n=80) had markedly reduced AB over time (–6.3%, –6.7%, –10.4%, –15.7%, –17.8%, and –13.0% at 32, 56, 80, 112, 144, and 176 weeks after crossover, respectively). Mean baseline AB for pts who did and did not have prior interferon (IFN) therapy was as follows: Rux: IFN=72.1%, no IFN=74.5%; BAT: IFN=88.2%, no IFN=72.0%. All BAT pts who received IFN (n=13) at randomization crossed over to Rux; mean maximal AB reduction in evaluable BAT pts receiving IFN was –6.6% before crossover and –25.6% after crossover. The average maximal percent reductions in AB (median time to maximal reduction) in Rux-randomized and Rux crossover arms were –35.9% (25.9 mo) and –21.2% (18.2 mo), respectively (Figure 1). Among evaluable pts, 2/102 (2.0%) Rux-randomized pts achieved CMR at Weeks 143 and 144 *vs* 1/94 (1.1%) Rux crossover pts at Week 123. Pts who achieved PMR included 33/101 (32.7%) evaluable Rux-randomized pts and 20/92 (21.7%) evaluable Rux crossover pts, after median times of 112 and 92 weeks, respectively. Seven pts had ≥90% AB reduction from Baseline (Rux-randomized, n=6; Rux crossover, n=1). Based on tertiles of maximal AB reduction for the Rux-randomized arm, there was no relationship between mean maximal change in AB (tertile 1, –72.2%; tertile 2, –29.1%; tertile 3, –5.7%) and mean PV duration (mo) from diagnosis (tertile 1, 81.6; tertile 2, 126.2; tertile 3, 105.2). No linear relationship was observed at last visit between percent change in AB from Baseline and corresponding clinical outcomes in terms of spleen volume reduction.

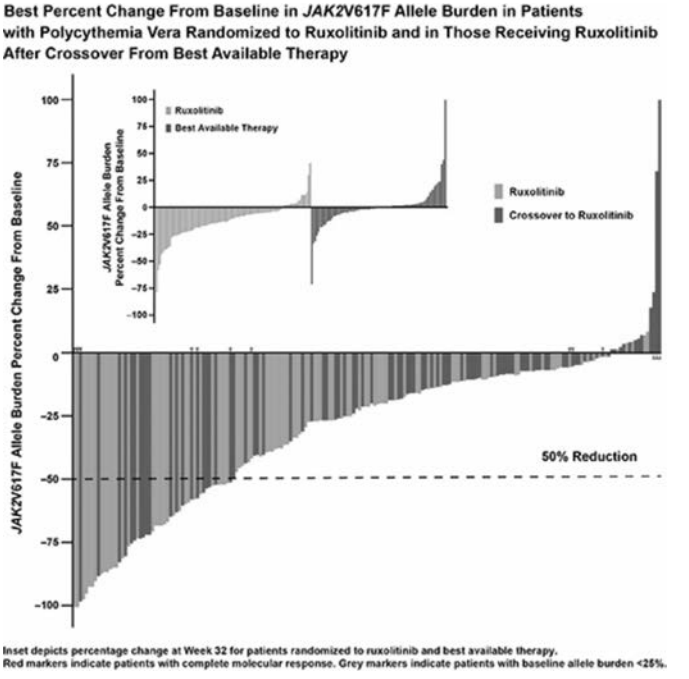

Figure 1.

Summary/Conclusions: Rux treatment (randomized and post-crossover) reduced *JAK2*V617F AB in RESPONSE study pts after up to 4 years of treat-

ment. Some pts may experience molecular responses with Rux treatment regardless of initiation timing during the disease course or previous IFN treatment. No clear relationship between AB reduction and spleen volume reduction was observed.

S455

HSC GENETIC HETEROGENEITY DETERMINES CLONAL DYNAMICS IN PMF

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Background: Primary Myelofibrosis (PMF) is a myeloproliferative neoplasm of stem cell origin, characterized by sequential distinctive waves of aberrant myeloid differentiation. Atypical erythroid-megakaryocytic progenitors expand at the initial stages of PMF and perish in the course of PMF development and/or the expansion of pre-leukemic clones. The continuum of clonal dynamics dominating the chronic phase disease, as exhibited in the variability of divergent myeloid progeny in PMF progression, reflects the molecular heterogeneity of malignant HSCs that sustain fluctuations in clone propagation.
Aims: In our previous work, we described a CD133+ stem cell population circulating in PMF patient peripheral blood, which exhibits multi-lineage differentiation capacity *in vivo* and *in vitro* and induces the PMF phenotype in the first xenotransplantation model of PMF. Single cell analysis of CD133+ patient-derived HSCs revealed multi-clonal lineage restricted differentiation potential of the stem cell pool in PMF. The correlation of the molecular burden of individual stem cells with their differentiation potential sheds light on the order of genetic lesions orchestrating the sequence of impaired stem cell function.
Methods: CD133+ HSCs from 100 PMF patients were molecularly characterized by whole exon sequencing. Sorted HSC cells were functionally analyzed at a single cell level for variable myeloid colony formation. 2230 colonies were phenotypically characterized and isolated. Analysis of the PMF HSC clonogenic potential indicates that the presence of mutations in the epigenetic regulator EZH2 correlates with granulo/monocytic differentiation but limited erythroid colony formation potential (0-0,05%), as determined in three different patient samples (2 JAK2-V617F+, 1 CALR-fs*+). Transplantation of these patient samples gave the highest engraftment in our mouse model and in one case, $EZH2^{mu}JAK2^{wt}$ leukemic transformation.
Results: CD133+ HSC-derived single colony analysis indicated 8 different genotypic clones of HSC, which exhibit variable granulo/monocytic differentiation capacity *in vitro*. From a total of 569 formed colonies, 538 were CFU-GM,-G,-M and 31 BFU-E. PCR analysis of colonies for JAK2-V617F and Sanger sequencing for EZH2-D265H and EZH2-Y733C mutations indicates that the presence of JAK2-V617F in hetero- or homozygosity can occur in the EZH2-D265H background without influencing the granulo/monocytic commitment of these mutated HSCs. Interestingly, the limited BFU-Es that arose contained only single JAK2-V617F mutations in the same patient. Moreover, the presence of single EZH2-D265H heterozygous clones, single JAK2-V617F hetero- or homozygous clones, as well as double mutated clones indicate two independent mutational events affecting the same locus and nucleotide have occurred in this patient. In view of the overall high frequency of JAK2-V617F mutations, we predict that the EZH2 mutation was the first mutation in double mutant clones in this patient. The presence of homozygous EZH2-Y733C mutations in other patients was detected irrespectively of JAK2-V617F mutation and is correlated with granulocytic-monocytic committed progenitors.
Summary/Conclusions: Our results indicate that mutations in epigenetic regulators precede the expanding mutations of the chronic phase PMF (JAK2, CALR). Thus, they confer the genomic instability connected with subsequent emergence of following mutations and they shape the genomic landscape supporting the expansion of pre-leukemic clones.

S456

ACTIVATION OF THE THROMBOPOIETIN RECEPTOR BY MUTANT CALRETICULIN IN CALR-MUTANT MYELOPROLIFERATIVE NEOPLASMS

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Background: Recurrent somatic mutations of *Calreticulin (CALR)* have been identified in patients harboring myeloproliferative neoplasms; however, their role in tumorigenesis remains to be elucidated.
Aims: We aim to elucidate the oncogenic property of mutant CALR and molecular mechanism of its action.
Methods: To examine whether mutant CALR exhibits oncogenic properties, its capacity to induce factor-independent growth was examined in a human megakaryocytic cell line UT-7/TPO that proliferates only in the presence of thrombopoietin (TPO). The genetic and biochemical interactions between mutant CALR and TPO receptor c-MPL were examined using a loss-of-function assay with shRNA and a co-immunoprecipitation assay, respectively. The functional and structural differences between mutant and wild-type CALR were examined using a domain analysis of CALRs for the c-MPL interaction. Mutant-CALR activation of c-MPL was determined by examining the phosphorylation status of c-MPL-downstream molecules using an immunoblot analysis. To demonstrate this activation process in more physiological circumstances, a loss-of-function assay for c-MPL was performed in iPS-derived hematopoietic stem cells that harbor the CALR mutation and exhibit TPO-independent megakaryopoiesis.
Results: We found that the expression of mutant, but not wild-type, CALR induced TPO-independent growth of UT-7/TPO cells. We demonstrated that c-MPL was required for this cytokine-independent growth. Mutant CALR preferentially associated with c-MPL that is bound to JAK2 over the wild-type protein. Furthermore, we demonstrated that the mutant-specific C-terminus portion of CALR interfered with the P-domain of CALR to allow the N-domain to interact with c-MPL; thus, providing an explanation for the gain-of-function property of mutant CALR. We showed that mutant CALR induced the phosphorylation of JAK2 and its downstream signaling molecules in UT-7/TPO cells and that this induction was blocked when the cells were treated with a JAK2 inhibitor. Finally, we demonstrated that c-MPL is required for TPO-independent megakaryopoiesis in iPS-derived hematopoietic stem cells harboring the CALR mutation.
Summary/Conclusions: These findings imply that mutant CALR activated the JAK2 downstream pathway via its association with c-MPL. Considering these results, we propose that mutant CALR promotes the development of myeloproliferative neoplasms by activating c-MPL and its downstream pathway.

AML Biology Mutant FLT

S457

NPM1 AND FLT3-ITD MUTATIONS SINERGISTICALLY INDUCE GATA1 EPIGENETIC SILENCING IN A AML MOUSE MODEL

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Background: Mutations in *NPM1* and *FLT3*-ITD are frequently associated in human AML and were also reported to induce leukemia in mice. However, the molecular consequences of this oncogenic cooperation in the development of AML still remain elusive.
Aims: We generated an *NPM1*/*Flt3*-ITD AML mouse model to address the following issues: i) to characterize the cellular origin of leukemia; ii) to identify the epigenetic/transcriptional signature in AML pathogenesis; iii) to analyze AML therapeutic response *in vivo*; and iv) to validate mouse data in human AML.
Methods: We crossed our *NPM1* mutant and *Flt3-ITD* mice and used: 1) FACS analysis to study the HSC/progenitor cell compartments in bone marrow (BM); 2) real-time PCR and Western blot analysis to measure GATA1 mRNA and protein expression; 3) bisulfite conversion followed by DNA sequencing to study methylation; 4) 5-Aza-dC and the FLT3 inhibitor AC220 for treatment experiments.
Results: *NPM1/Flt3-ITD* double mutated mice progressed to AML recapitulating *features of the human disease*. The AML phenotype was preceded by a preleukemic stage of variable length that inversely correlated with the burden of the mutant alleles. Pre-leukemic mice displayed leukocytosis (WBC 20.8±18x10^9/L) and macrocytosis (MCV 58.74±5.6 fl). In *NPM1*/*Flt3-ITD* mice, FACS analysis revealed expansion of multipotent and granulocyte-macrophage progenitor compartments. Conversely, long-term hematopoietic stem cells and immature megakaryocytic/erythroid compartments were significantly reduced as compared to other genotypes combinations. Strikingly, we found that the key regulator of normal myelo-erythroid differentiation GATA1 was transcriptionally silenced in *NPM1*/*Flt3-ITD* mice. Similarly, AML patients with *NPM1*/*FLT3*-ITD mutations displayed a significantly lower mean GATA1 expression compared to patients with a different genotype (0.42±0.09 *vs* 5.3±1.3 respectively, p<0.05). Genomic bisulfite sequencing of the GATA1 promoter revealed more than 80%±0.12 methylated CpGs in BM cells from double mutated mice as compared to only 53%±0.22 methylation in other genotypes, thus clearly implicating epigenetic changes as the cause of GATA1 transcriptional silencing. *In vivo* treatment of leukemic mice with the DNA methyltransferase inhibitor 5-aza-deoxycytidine (5-Aza-dC) reactivated GATA1 expression determining the normalization of leukocytosis and preventing a drop in platelets counts. Strikingly, real-time PCR analysis revealed a significant 3.45±1.01 upregulation of GATA1 mRNA levels in BM samples from *NPM1*/*FLT3*-ITD mutant AML patients that underwent 5-Aza-dC treatment (p<0.001). Finally, we assessed the sensitivity of *NPM1/Flt3*-ITD mice AML to targeted FLT3 inhibition. *In vitro* treatment with the FLT3 inhibitor AC220 significantly reduced the viability of BM cells isolated from *NPM1*/*Flt3-ITD* preleukemic or leukemic mice. *In vivo*, AC220 induced a reduction of WBC counts accompanied by a partial GATA1 re-expression in peripheral blood cells without improving mice survival. In a xenotransplant model of human *NPM1*/*FLT3*-ITD AML, the FLT3 inhibitor induced significant reduction in size of subcutaneous AML tumors with GATA1 upregulation.
Summary/Conclusions: We demonstrated that epigentic modification abrogated a GATA1-dependent transcriptional program and represented a prerequisite for *NPM1*/*Flt3*-ITD leukemogenesis. Mouse data are further supported by low GATA1 expression found in human AML samples and *in vivo* treatments rescues. Therefore, GATA1 represents a target for new tailored therapies in AML with *NPM1* and *FLT3*-ITD mutations.

S458

FLT3-D835Y AND NPM1C ONCOGENES COOPERATE TO INDUCE AN AGGRESSIVE MPN IN MICE

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Background: Activating mutations in FLT3 and NPM1 are most common alterations in AML and frequently coincidental. The exon 12 mutation of *Npm1* (NPM1c) leads to a mislocalization of the protein from the nucleus to the cytoplasm. Two types of mutations are present in FLT3: Tandem duplication of the juxtamembraneous domain (ITD) and point mutations of the tyrosine kinase domain (TKD). In murine models, both NPM1c and FLT3-ITD induce an MPN, while FLT3-TKD leads to a lymphoid disorder. Co-expression of NPM1c and FLT3-ITD rapidly induces an AML in mice.
Aims: In the present study, we investigated the impact of co-expression of NPM1c with the TKD mutation FLT3-D835Y in a mouse model.
Methods: For this purpose a heterozygous conditional *Npm1c* knockin mouse model was used where *Npm1c* can be induced via the Mx1Cre-system. Donor bone marrow was harvested from these mice and cells were retrovirally infected with the MiG-*Flt3-D835Y* vector. Infected bone marrow cells were injected into lethally irradiated C57Bl/6 wt recipient mice. The *Npm1c* expression was induced by p(I:C) injection post transplantation resulting in the activation of IFN-α and thereby Cre recombinase induction under the control of the Mx1 promotor. Control mice received no p(I:C) injection. Lymphatic/Leukemic organs of moribund mice were analyzed for lineage-specific cell surface markers by flow cytometry.
Results: Strikingly, *Npm1c Flt3-D835Y* mice rapidly developed a myeloproliferative neoplasia and succumbed after a median latency of 33 days. In contrast, *Flt3-D835Y Npm wt* control mice did not develop a leukemic disease. Detailed analysis *Npm1c Flt3-D835Y* mice revealed a high leukemic burden in the peripheral blood as well as in the bone marrow and a pronounced splenomegaly. Flow cytometry analysis revealed a myeloid phenotype in the peripheral blood, spleen and bone marrow of moribund mice, characterized by a high frequency of FLT3-D835Y$^+$/CD11b$^+$and Gr1$^+$ cells, whereas *Flt3-D835Y* control mice showed a normal immune phenotype. Interestingly, immunoblot analysis of spleen extracts from moribund mice indicated that Flt3-D835Y in combination with NPM1c induces STAT5 but not STAT3 signaling. These results suggest that the MPN induction in this model might be due to STAT5 activation. Previously, it has been shown that the myeloid leukemia of *Npm1c Flt3-ITD* mice is retransplantable. To discover whether the myeloid phenotype of *NPM1c Flt3-D835Y* mice is retransplantable as well, 1x10^5 and 1x10^6 spleen cells of an *Npm1 Flt3-D835Y* mouse were injected into sublethally irradiated C57Bl/6 wt recipient mice. Indeed, we were able to show retranplantability of *Npm1c Flt3-TKD* disease in the group transplanted with high cell numbers, whereas the retranplantability was not only partial inducible (15-30% of the mice) in the group transplanted with lower spleen cell numbers (1x10^5).
Summary/Conclusions: In summary, our data show that co-expression of NPM1c and FLT3-D835Y results in the development of a rapidly fatal MPN whereas FLT3-D835Y-expression alone is not sufficient to cause a myeloproliferative disease in C57Bl/6 mice. Moreover, the myeloid phenotype is retransplantable with 1x10^6 donor spleen cells in C57Bl/6 mice. Interestingly, myeloproliferative disease induction might be due to aberrant STAT5 activation by FLT3-D835Y in the background of NPM1c mutant.

S459

THE RET RECEPTOR TYROSINE KINASE PROMOTES ACUTE MYELOID LEUKEMIA THROUGH PROTECTION OF FLT3-ITD MUTANTS FROM AUTOPHAGIC DEGRADATION

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Background: Acute myeloid leukemia (AML) is a heterogeneous disease with diverse leukemogenic driver lesions. The genetic understanding of AML has resulted in major improvements in diagnosis, classification, prognostication, and outcome prediction. However, these insights have not yet translated into molecular mechanism-based therapies in the majority of cases, because AML cells are able to rapidly escape attempts at therapeutic targeting such as small-molecule inhibition of FLT3 internal tandem duplication (ITD) mutants, which occur in up to 30% of cases and confer a poor prognosis.
Aims: Given the propensity of AML to develop resistance to single-agent small-molecule therapy, we aimed to identify potential new targets for combinatorial treatment approaches.
Methods: We performed a series of large-scale RNA interference screens, using a subgenomic short hairpin RNA (shRNA) library targeting kinases, phosphatases, and known cancer-related genes, in a panel of AML cell lines.
Results: We identified that the RET receptor tyrosine kinase (RTK), which has not previously been implicated in AML pathogenesis, is critical for the viability and proliferation of cell lines representing various AML subtypes. Validation experiments demonstrated that depletion of RET by shRNA knockdown or CRISPR/Cas9-mediated knockout leads to cell cycle arrest in the G0/G1 phase, increased apoptosis, and reduced clonogenic activity. Moreover, transcriptome analysis identified elevated *RET* mRNA levels in 35 of 260 (13.5%) primary human AML samples. RTK profiling using ELISA-based antibody arrays demonstrated that RET is highly phosphorylated in RET-dependent AML cell lines. Analysis of known RET ligand/co-receptor pairs (GDNF/GFRA1, NRTN/GFRA2, ARTN/GFRA3, PSPN/GFRA4) by quantitative real-time PCR and shRNA knockdown suggested that RET signaling is facilitated mainly through NRTN/GFRA2 or ARTN/GFRA3. Interrogation of various signaling pathways known to promote myeloid leukemogenesis showed that RET knockdown resulted in decreased

phosphorylation of p70S6K (Ser371), S6RP (Ser240/244), and ULK1 (Ser758), pointing to mTORC1-mediated protein synthesis and/or suppression of autophagy as important effects of RET signaling in AML cells. Based on recent data showing that FLT3-ITD mutants can be autophagically degraded (Larrue *et al.* Blood 2016), we reasoned that the RET-mTORC1 signaling axis promotes AML through protection of FLT3-ITD mutants from autophagic degradation. Consistent with this hypothesis, RET knockdown or pharmacologic RET inhibition with vandetanib and danusertib predominantly affected FLT3-dependent AML cell lines and were accompanied by upregulation of autophagy and destabilization of FLT3, as evidenced by p62 degradation, increased numbers of autophagic vacuoles, and decreased FLT3 protein levels. Finally, we are currently investigating whether Ret is required for AML development and propagation *in vivo* using a murine bone marrow transplantation model.

Summary/Conclusions: Combined, our results indicate that in a proportion of AML, RET-mTORC1 signaling promotes cell viability and proliferation through suppression of autophagy, suggesting that targeting RET or, more broadly, depletion of critical leukemogenic drivers via induction of autophagy may provide a therapeutic opportunity in this subset of patients.

S460

A NOVEL EPIGENETIC RESISTANCE MECHANISM TO THE FLT3 INHIBITOR PKC412 INDUCES CROSS-RESISTANCE TO STANDARD CHEMOTHERAPY IN ACUTE MYELOID LEUKEMIA (AML)

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Background: In acute myeloid leukemia (AML) about 20-30% of patients possess a FLT3-internal tandem duplication (ITD) associated with a poor prognosis and a higher propensity for relapse after remission. Tyrosine kinase inhibitors (TKI) such as PKC412 have been developed for therapy of FLT3-ITD-positive AMLs. So far, resistance to tyrosine kinase inhibitors has been demonstrated to occur by point mutations in FLT3 whereas further mechanisms that render leukemic cells drug resistant remain incompletely understood.

Aims: We utilized a PKC412-resistant model of FLT3-ITD-positive MV4-11 cells to (1) identify novel mechanisms of drug resistance, (2) to analyze cross resistance to the standard chemotherapeutics cytarabine and daunorubicin and translate our findings to FLT3-ITD-negative AMLs and (3) to develop strategies to reverse chemoresistance in AML.

Methods: Drug resistance mechanisms were analyzed *in vitro* by immunohistochemistry, Western blot, shRNA-mediated mRNA knockdown, lentiviral overexpression, immunoprecipitation, label-free- and SILAC (Stable isotope labeling by amino acids in cell culture)- mass spectrometry, ChIP-seq and ex-vivo- culture of patient samples. For *in vivo* analysis xenograft mouse models were applied.

Results: We identified loss of the histone methyltransferase EZH2 with subsequent reduction of histone H3K27 trimethylation in PKC412- resistant MV4-11 cells which led to cross-resistance to cytarabine and daunorubicin. In AML patients, the absence of EZH2 protein expression was closely associated with poor overall survival (p=0.008), poor event-free (p=0.005) and poor relapse-free survival (p=0.047) as analyzed by tissue microarrays. The reduction of EZH2 protein levels via treatment with H3K27 methyltransferase inhibitors or lentiviral knockdown was sufficient to induce chemoresistance of Normal Karyotype (NK)- AML blasts and cell lines *in vitro* and in a xenograft mouse model. The loss of EZH2 in drug resistant MV4-11 cells was regulated by posttranslational mechanisms. Immunoprecipitation of EZH2 revealed a 2.6-fold increased phosphorylation of residue T487 in resistant cells (p=0.014) and the cyclin-dependent kinase 1 (CDK1) was associated with EZH2 specifically in resistant MV4-11. Several ubiquitin-E3-ligases such as TRIM21 were increasingly bound to EZH2 in resistant compared to sensitive cells resulting in a strong ubiquitination of EZH2 and subsequent degradation. Pharmacological inhibition of CDK1 and treatment with the proteasome inhibitor bortezomib, respectively, increased EZH2 protein and restored drug sensitivity in resistant cells. Functionally, reduction of EZH2 directly induced upregulation of *HOX* genes and the drug transporter MRP1, suggesting a stem-cell-like signature to be associated with the resistance phenotype, which could be reverted by proteasome inhibitors. In primary AML samples sensitivity to cytarabine was specifically increased in samples with elevated EZH2 protein levels upon bortezomib exposure whereas no change was observed in patient samples without EZH2 increase upon bortezomib treatment (p=0.0079, Fisher´s exact test).

Summary/Conclusions: In summary, our findings identify a novel epigenetic resistance mechanism that can be induced by prolonged exposure to PKC412 or cytotoxic drugs. EZH2 protein restoration by proteasome inhibitors in combination with established therapy approaches may be a promising therapy concept to restore drug sensitivity in AML.

S461

LOSS OF FUNCTION SETD2 MUTATIONS LEAD TO RESISTANCE TO DNA DAMAGING CHEMOTHERAPY IN LEUKEMIA

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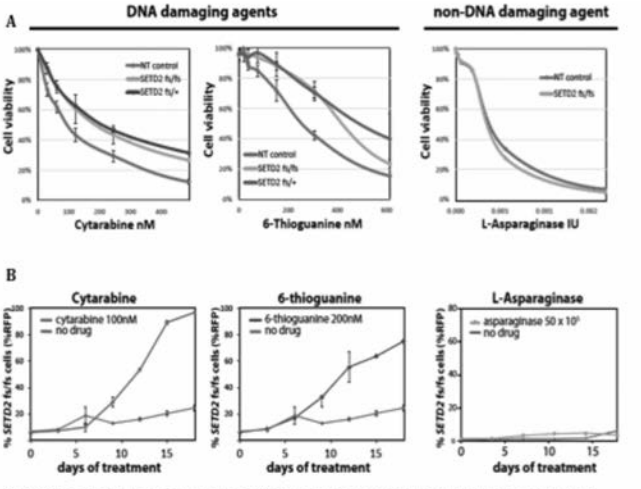
Background: Relapsed leukemia has a poor survival, which can be attributed to the relative chemotherapy resistance of this disease entity. While the consequences of chemotherapy resistance are clear, the genetic alterations underlying resistance remain poorly understood. We recently identified somatic mutations that are specifically enriched in relapsed leukemia, including a novel loss of function mutation in the epigenetic regulator *SETD2*. SETD2 is the only known mammalian Histone 3 Lysine (H3K36) trimethyltransferase and its role in DNA mismatch repair was recently established. In view of the presence of *SETD2* mutations in relapsed leukemia, and the role of SETD2 in DNA repair, we hypothesized that SETD2 plays a role in chemotherapy resistance in relapsed leukemia.

Aims: To investigate the role of *SETD2* loss of function mutations in chemotherapy resistance and identify therapeutic targets for *SETD2* mutant leukemia.

Methods: We employed two different models to study the role of SETD2 in chemotherapy resistance, which include an isogenic human leukemia model engineered with CRISPR-Cas9 and a murine leukemia with *Setd2* conditional knockout and retroviral expression of *MLL-AF9*. We studied the presence and mechanism of chemotherapy resistance in both models.

Results: We demonstrate that loss of function *SETD2* mutations cause resistance to chemotherapy. The resistance is specific to DNA damaging chemotherapies, including the commonly used agents 6-thioguanine and cytarabine. In contrast, no resistance to the non-DNA damaging agent L-asparaginase was observed. In addition to causing an increase in IC50 values, loss of SETD2 results in a strong competitive advantage in the presence of DNA damaging agents, mimicking the selective pressure of SETD2 mutant clones during treatment in patients (Figure 1). We further establish that the resistance is caused by an abrogated DNA damage response as demonstrated by a decreased phosphorylation of CHK1 and CHK2 and a reduced apoptotic response after treatment with DNA damaging therapies. In our conditional knockout model, we show that, in addition to causing a significant acceleration of leukemia, SETD2 loss causes resistance to cytarabine and doxorubicin treatment *in vivo*. Finally, we show that WEE1 inhibition specifically targets *SETD2* mutant cells and re-sensitizes to chemotherapy, hereby providing a treatment option for *SETD2* mutant, chemotherapy resistant leukemia.



SETD2 loss leads to resistance to DNA damaging chemotherapy. (A) Viability assays in Molm13 CRISPR Cas9 cells transduced with an sgRNA targeting *SETD2*, or a non-targeting control (NT control). *SETD2* heterozygous (fs/+), compound heterozygous (fs/fs) or *SETD2* WT control cells (NT control) were exposed to increasing concentration of Cytarabine, 6-Thioguanine or L-Aspargainase for 72 hours. **(B)** Molm13 *SETD2* mutant (fs/fs) cells were mixed in a 1:20 ratio with Molm13 control cells and exposed to Cytarabine, 6-Thioguanine or L-Asparaginase treatment for 18 days. Molm13 *SETD2* mutant cells were marked by RFP.

Figure 1.

Summary/Conclusions: While relapsed leukemia is known to be chemotherapy resistant, the mechanisms of resistance remain poorly understood. This study is the first to show that loss of function *SETD2* mutations confer chemotherapy resistance in leukemia. In addition, we show that treatment with a WEE1 inhibitor – which is currently in clinical trials - is able to specifically target *SETD2* mutant leukemia and reverse chemotherapy resistance. Hence, this study supports a prominent role for loss of function *SETD2* mutations in disease progression and chemotherapy resistance in leukemia, as well as providing evidence for a potential therapy to treat *SETD2* mutant, chemotherapy resistant leukemia.

Red blood cells and iron - Clinical

S462

RESULTS OF TRANSCRANIAL DOPPLER ULTRASOUND SCREENING FROM THE POST-STOP STUDY

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Background: The Stroke Prevention Trial in Sickle Cell Anemia (STOP) and Optimizing Primary Stroke Prevention in Sickle Cell Anemia (STOP 2) established that routine transcranial Doppler ultrasound (TCD) screening could identify children with sickle cell disease (SCD) at high risk of stroke. Furthermore, these studies demonstrated that patients with abnormal TCD require indefinite chronic red cell transfusions (CRCT) as standard of care. Thus, annual TCD screening is recommended from ages 2 to 16 years, with more frequent monitoring if the result is abnormal. Patients with abnormally high TCD who were started on CRCT had reductions in ischemic strokes compared to patients not receiving transfusions as shown in several clinical series and analyses utilizing large hospital databases when comparing rates before and after the publication of the STOP study in 1998.
Aims: This study sought to evaluate the outcomes of patients identified with abnormal TCD who had previously participated in the STOP and/or STOP 2 trials.
Methods: Between 1995 and 2005, STOP and STOP 2 (STOP/2) were conducted at 26 sites in the US and Canada. These studies included 3,835 children, ages 2 to 16 y with SCD type SS or S-beta-0-thalassemia. Participation in STOP/2 ranged from a single screening TCD to randomization. STOP 2 also had an observational arm for children on CRCT for abnormal TCD who's TCD had not reverted to normal. The Post-STOP study was designed to follow-up the outcomes of children who participated in one or both trials. 19 of the 26 original study sites participated in Post-STOP, contributing a total of 3,539 (92%) of the STOP/2 subjects. After exit from STOP/2, these children received TCD screening and CRCT (or other treatment) according to local practices. Data abstractors visited each clinical site and obtained retrospective data from STOP/2 study exit to 2012-2014 (depending on site) including follow-up TCD and brain imaging results, clinical information, and laboratory results. Two vascular neurologists, blinded to STOP/2 status and prior TCD and neuroimaging results, reviewed source records to confirm all ischemic and hemorrhagic strokes, discordant opinions were resolved through discussion.
Results: The two (STOP and STOP-2) study databases contained unique records for 3854 children at 26 sites. Nineteen of those original sites participated in post stop accounting for 3539 subjects (92%). Of these 3539, records of care at enrolling states were located for 2851 subjects (81% of possible). The mean age at the start of Post-STOP was 10.5 y and mean duration of follow-up after exiting STOP/2 was 9.1 y. During the STOP or STOP2 period, 333 (11.7%) patients had an abnormal TCD and 12 patients suffered acute stroke (included the post-STOP encounters). During the Post-STOP period, an additional 122 (4.34%) of children where identified with abnormal TCD. Of these patients identified with abnormal TCD in the Post-STOP period, 25 (20%) patients suffered an ischemic event only 15 (60%) of which were receiving appropriate CRCT prevention. An additional 7 patients (5.7%) identified with abnormal TCD suffered a hemorrhagic event, only 1 of whom was on CRCT therapy (4 patients were on HU and 2 patients had unknown treatment). The remaining 90 (74%) patients with abnormal TCD had no reported negative neurologic outcomes despite variations in compliance with CRCT during the post-stop period (or were lost to follow-up).
Summary/Conclusions: The Post-STOP study included 81% of the original STOP and STOP-2 patients. In total, 455 of those patients (16%) were identified with an abnormal TCD. While many of these patients were continued on CRCT, many individuals were treated with Hydroxyurea, had no preventative therapy or were lost to follow-up. Future prospective trials are needed to better evaluate which patients identified at high risk for stroke by TCD do not require lifetime CRCT.

S463

DOPPLER ULTRASOUND RELIABLY IDENTIFIES SICKLE CELL DISEASE ADULT PATIENTS WITH INTRACRANIAL VASCULOPATHY

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Background: Stroke is one of the leading causes of death in both children and adults with sickle-cell disease (SCD) most often related to a specific large-vessel vasculopathy. Prevention of stroke is based on blood exchange transfusion if stenosant vasculopathy is confirmed. However magnetic resonance angiography or angio scanner are not easily available in many AFrican countries instead of Doppler ultrasound which is the main diagnosis strategy in SCD children. Doppler ultrasound has never been demontrated to be effecient in SCD adults.
Aims: The objective of this study was to assess whether Doppler ultrasound is sensitive and specific to identify SCD adult patients with vasculopathy, compared with magnetic resonance angiography.
Methods: 80 adult SCD patients likely to have brain attempt were referred to our center for vasculopathy screening. 77 patients could benefit from our protocol including both a 3T brain MR imaging with a 3D time-of-flight MR angiography and a Doppler-Ultrasound examination performed on the same day. 3 patients presented contraindication to MRI.
On MR imaging, patients were categorized according to the grade of the highest stenosis and to the presence of Moya-Moya. On EchoDoppler-Utrasound examination, time-averaged mean of the maximum velocity (TAMMx) was measured and a TAMMx ratio was established using the highest TAMMx value recorded on one axis, divided by the TAMMx value of the ipsilateral extracranial internal carotid artery. Presence of Moya-Moya was also recorded.
Results: Among the 38 patients with an intracranial vasculopathy (stenosis >=50% on MR imaging, presence of an occlusion or Moya-Moya), 33 had a severe vasculopathy according to EchoDoppler criteria defined as a TAMMx ratio >=3 (n=5) or presenting an occlusion (n=11) or a Moya-Moya (n=17). Sensitivity and specificity of EchoDoppler to identify patients with >=50% vasculopathy on MRA is respectively of 87%, 90%. Positive and negative predictive values are respectively of 86% and 87%.
Summary/Conclusions: Doppler ultrasound may be used to identify SCD adult patients with vasculopathy.

S464

CHILDREN WITH SICKLE CELL ANEMIA ON CHRONIC TRANSFUSION FOR ABNORMAL TRANSCRANIAL DOPPLER VELOCITIES. PATIENT/SIBLING COMPARISON OF COGNITIVE PERFORMANCES AND ASSOCIATION WITH NEUROIMAGING DATA

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Background: Patients with sickle cell anemia (SCA) are known to have cognitive deficiency. However, only a comparison with non-SCA siblings allows true appreciation of the disease impact.
Aims: The aims of the present study were to compare the cognitive performances of SCA-children on long-term transfusion programs due to a history of abnormal transcranial Doppler (TCD) velocities with those of their siblings, and to assess the relation to neuroimaging data.
Methods: *Drepagreffe* is a French national multicenter prospective trial (APHP, NCT 01340404) involving SCA-children, younger than 15 years of age, placed on chronic transfusion for abnormal-TCD (TAMMX ≥200 cm/sec), and comparing the outcome of cerebral vasculopathy following transfusions or hematopoietic stem cell transplant. We present here, the results of cognitive testings done at enrollment in SCA-children and non-SCA siblings, and patient neuroimaging data. The MRI scoring was: 3=territorial, 2=borderzone (cortical and subcortical), 1=white matter or basal ganglia infarcts, 0 to 3=atrophia, and MRA scoring was: 1=mild stenosis (25-49%), 2=moderate stenosis (50-74%), 3=severe stenosis (75-99%), 4=occlusion for each artery and 0 to 2 for Moya presence. Cognitive testing, performed in patients and in siblings when possible, included WPPSI-3 (3-6 yr), WISC-4 (7-16 yr) or WAIS-3 (>16 yr) scales, depending on the subject age.
Results: Sixty-seven SCA-children (36F/31M) were included. Seven had a history of overt stroke. *At abnormal-TCD detection and chronic transfusion initiation*, the mean age (SD) was 5.5yr (2.5) and velocities≥200/cm/sec were found in right/left middle (n=29/22), anterior (n=7/5), internal carotid (n=10/7) and extracranial internal carotid (n=7/7) arteries, as abnormal velocities were observed in more than one artery in several patients. *At enrollment*, following a mean duration of chronic transfusion of 2.8 years, the mean age was 8.1yr (3.1), and mean(SD) maximum velocities had significantly decreased from 219 (26) to 169 (46) cm/s *(p<0.001)*. MRI/MRA data were available in 66/67 patients. Ischemic lesions were present in 25 patients and 18 of them had silent lesions. Stenoses were present in 35/66 patients in right/left middle (n=7/10), anterior (n=11/16), internal carotid (n=7/12) and extracranial internal carotid (n=11/12) arteries, as stenoses were observed in more than one artery in several patients. Cognitive testing was obtained in 64/67 patients (parental refusal for 3 patients), and in 56 siblings (8 were too young for testing). Paired analysis with siblings showed significant differences in Verbal Comprehension Index (VCI) with a mean difference of 7.6±14.5 (p<0.001), Processing Speed Index (PSI) 6.3±20.5 (p=0.04), and Full Scale IQ (FSIQ) 7.3±15.0 (p=0.001). Patient cognitive performance indexes were correlated negatively and significantly with the MRI and MRA scores. After exclusion of the 7 patients with stroke history, significant differences were still observed in VCI (p=0.013) and FSIQ (p=0.019), but were not correlated with the presence of silent cerebral infarcts.
Summary/Conclusions: SCA-children on transfusions for history of abnormal cerebral velocities had significantly lower cognitive performances than their siblings, even in the absence of stroke history. In the no-stroke patients, the differences observed with their siblings were not correlated with the presence of silent cerebral infarcts. These data suggest that stroke and silent infarcts are not the only underlying cause of cognitive deficiency in SCA children.

S465

A PHASE I/II STUDY OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS GENETICALLY MODIFIED WITH GLOBE LENTIVIRAL VECTOR FOR THE TREATMENT OF TRANSFUSION DEPENDENT BETA-THALASSEMIA

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Background: A clinical trial was initiated in April 2015 at Scientific Institute San Raffaele to evaluate safety and efficacy of autologous hematopoietic stem cells (HSCs) genetically modified with the GLOBE lentiviral vector for the treatment of transfusion dependent beta thalassemia (TIGET-BTHAL; EudraCT number 2014−004860−39).
Aims: The financial sponsor is the Italian charity organization Fondazione Telethon. The manufacture is MolMed S.p.A. The therapeutic potential of GLOBE was validated in thalassemic mice (Miccio *et al.*, PNAS 2008; Miccio *et al.* PlosONE, 2011) and human thalassemic cells (Roselli *et al.*, EMBO MolMed 2010) showing persistent transgene expression and disease correction. Preclinical GLP studies included a toxicology and tumorigenicity study in th3/+ mice and a biodistribution study of human of CD34+ cells in NSG mice demonstrating safety of the approach.
Methods: The clinical protocol will treat 3 adults followed by 7 minors, with a staggered enrolment strategy based on evaluation of safety and preliminary efficacy in adult patients by an independent data safety monitoring board before inclusion of pediatric subjects. Inclusion criteria: transfusion dependent beta thalassemia, age 3-65 years, adequate cardiac, renal, hepatic and pulmonary functions, absence of severe liver or cardiac iron overload, absence of severe liver fibrosis or cirrhosis. Exclusion criteria include active hepatitis, HIV infection or a previous allogeneic BMT and for pediatric patients availability of a well matched donor. The HSC source is mobilized peripheral blood stem cells (PBSCs) obtained after administration of lenograstim and plerixafor. PBSCs are transduced after apheresis and kept frozen in 10%DMSO until administration to the patient. Conditioning: treosulfan and thiotepa for adult and elderly children (≥8years), busulfan for younger children (age 3-7yrs old). The chosen route of administration of thawed and washed gene modified cells is intraosseous in the posterior-superior iliac crests, bilaterally. The aim of intraosseous infusion is to enhance engraftment and minimize first-pass intravenous filter. Autologous lymphocytes (previously cryopreserved from apheresis or phlebotomy) are re-infused at a target dose of 5x10^7 CD3+ cells/kg on day+3 to speed adaptive immune recovery.
Results: Between May and October 2015 three patients were included. As of March 2016, one patient is 6 months post gene therapy, one 1.5 months and one has completed mobilization. Age at enrollment: 31, 35, 35 years. Stem cell harvest was $30x10^6$ CD34+/kg, $21.3x10^6$ CD34+/kg and $23.5x10^6$ CD34+/kg, respectively. Cell dose of gene modified cells of the 2 treated patients: $19.4x10^6$ CD34+/kg and $18.4x10^6$ CD34+/kg. Adverse events recorded as of March 2016 were mild, reversible and related to the conditioning regimen and transplant procedure. The first patient had a neutrophil engraftment (>500 ANC/µl) on day+21 and platelet engraftment (>20.000/µl unsupported) on day+16. The second patient had a neutrophil engraftment on +25 and platelet engraftment on +21. The first patient treated showed multilineage engraftment of gene corrected cells with higher levels in erythroid lineage and received the last red blood cell transfusion 2 months after gene therapy. At 6 months follow up the patient was transfusion-free and conducting a normal working activity with a haemoglobin of 9.2 g/dL.
Summary/Conclusions: In conclusion, preliminary data on adult patients showed a good yield of PBSCs, a high number of cells infused, good tolerability of the procedure overall with reversible adverse events and preliminary positive efficacy data.

S466

EFFECTS OF AG-348, A PYRUVATE KINASE ACTIVATOR, ON ANEMIA AND HEMOLYSIS IN PATIENTS WITH PYRUVATE KINASE DEFICIENCY: EARLY DATA FROM THE DRIVE PK STUDY

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Background: Pyruvate kinase (PK) deficiency is a congenital hemolytic anemia caused by deficiency of the glycolytic enzyme red cell PK (PK-R), required for maintenance of adenosine triphosphate (ATP) in red blood cells. AG-348 is an orally-available, small molecule, allosteric activator of PK-R that activates wild-type and a range of mutated PK-R enzymes *in vitro* and increases PK-R activity and restores ATP levels in red blood cells from patients with PK deficiency *ex vivo*.
Aims: To describe early data from the ongoing DRIVE PK study (NCT02476916), an open-label, dose-ranging trial of AG-348 in transfusion-independent adults with PK deficiency.
Methods: After providing informed consent, patients are randomized to AG-348 50 mg or 300 mg orally twice daily (BID) for 6 months. Transfusion independence is defined as no more than 3 units of red blood cells transfused in the 12 months preceding first dose and no transfusions in the 4 months preceding first dose. Patients are followed weekly for Weeks 1–3, then every 3 weeks until Week 12, and then monthly until Week 24. Hormone and iron status are evaluated at Baseline, Week 12 and End of Study.
Results: As of 15 Jan 2016, 10 patients have received treatment for between 1 and 24 weeks. Adverse events (AEs) have been mild-to-moderate, with nausea (CTCAE Grade 1–2) the most commonly reported AE: 2 out of 5 (40%) patients in the 50 mg BID arm and 3 out of 5 (60%) patients in the 300 mg BID arm. No serious AEs have been reported. There have been no dose modifications for AEs. A single patient has had dose modifications for an increase in hemoglobin (Hb) above the protocol mandated maximum. Dosing was temporarily interrupted and reduced twice (100 mg BID, then 50 mg BID) and the patient has since been maintained on 50 mg BID for several weeks. Pharmacokinetic data showed exposure in line with expectations from earlier healthy volunteer studies with AG-348 for both doses. At data cutoff, pharmacodynamic analysis of 2,3-diphosphoglycerate (2,3-DPG) and ATP was available for 5 patients. Baseline 2,3-DPG levels were elevated in 4 of 5 patients, as expected. All patients with a Hb response of ≥1.5 g/dL also showed a substantial decrease in 2,3-DPG levels. Further data are being collected to evaluate relationships between hematological parameters and pharmacodynamic markers (2,3-DPG and ATP). Genotype–response relationships are also being assessed.
Summary/Conclusions: Early clinical data from DRIVE-PK indicate that AG-348 is well tolerated. Data on all patients (n~15–20), including safety, hematological responses, pharmacodynamic effects and genotype–response relationships will be presented.

Bleeding disorders

S467

FIRST RESULTS FROM A DOSE-ESCALATING STUDY WITH AAV5 VECTOR CONTAINING WILD TYPE HUMAN FACTOR IX GENE THERAPY IN PATIENTS WITH SEVERE OR MODERATELY-SEVERE HAEMOPHILIA B

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Background: Gene therapy for haemophilia B offers the potential to convert the disease severity from severe to a mild phenotype through continuous endogenous production of FIX. Adeno-associated viral (AAV) vectors of serotypes 2 and 8 (AAV2 and AAV8) containing the human factor IX (hFIX) gene have been used in previous clinical trials.[1-3] A single infusion with AAV8 vector resulted in dose dependent FIX expression for up to 4 years.[4] AMT-060 (AAV5-hFIX) consists of an AAV5 vector with an LP1 liver specific promotor, containing a codon-optimised wild type hFIX gene. A potential advantage of AAV5 is the lower prevalence of neutralising antibodies compared to other serotypes.
Aims: This study aims to investigate the safety and efficacy of AMT-060 in adult patients with severe or moderately severe haemophilia B.
Methods: This is an open-label, single dose escalating study in patients with FIX activity ≤1-2% of normal, and a severe bleeding phenotype. Five patients were enrolled in the first cohort of AMT-060 5×10^{12} gc/kg and a subsequent 5 patients will be enrolled in the second cohort of 2×10^{13} gc/kg. Patients receive AMT-060 via IV infusion over 30 minutes in an in-hospital setting. Safety assessments include treatment related adverse events (AEs) and serious AEs (SAEs). Efficacy assessments include FIX activity, rFIX usage and annualized bleeding rates. We report here preliminary results of the 5 patients treated in the first dose cohort. All patients gave informed consent.
Results: The age of the patients ranged from 35 to 72 years. None of the patients had pre-existing AAV5 antibodies. Four patients had documented FIX activity of <1% and one patient had 1.5%. Four patients had documented haemophiliac arthropathy. All patients were receiving rFIX prophylaxis weekly or twice-weekly prior to treatment with AMT-060. As expected, all 5 patients developed anti-AAV5 antibodies in response to study drug. None of the patients have developed inhibitory antibodies against FIX. Two SAEs have occurred. One patient had an asymptomatic, mild, transient elevation of ALT (peak level at week 10, 61 IU/L; upper limit of normal, 40 IU/L) that resolved after rapid institution of a tapering regimen of prednisolone. Another patient had a self-resolving febrile episode within the first 24 hours of AMT-060 administration. During the first 12 weeks post-gene therapy, 4 out of the 5 patients achieved FIX activity levels that allowed them to discontinue rFIX prophylaxis. FIX activity and other efficacy outcome measures for a minimum of 30 weeks follow-up of the 5 patients in the first dose cohort will be presented.
Summary/Conclusions: Treatment of haemophilia B with a single infusion of AMT-060 was well tolerated, and clinically relevant FIX activity has been achieved in the first dose cohort, relieving 4 out of 5 patients from rFIX prophylaxis. The study will continue with enrolment of the second dose cohort as planned.

References

1. Manno C, Chew A, Hutchison S, *et al.* AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe haemophilia B. *Blood* 2003;101:2963-2972.
2. Manno C, Pierce G, Arruda V, *et al.* Successful transduction of liver in haemophilia by AAV factor IX and limitations imposed by the host immune response. *Nat Med* 2006;12:342-347.
3. Nathwani A, Tuddenham E, Rangarajan S, *et al.* Adenovirus-associated virus vector-mediated gene transfer in haemophilia B. *N Engl J Med* 2011;365: 2357-2365.
4. Nathwani A, Reiss U, Tuddenham E, *et al.* Long-term safety and efficacy of factor IX gene therapy in haemophilia B. *N Engl J Med* 2014;371:1994-2004.

S468

RESPONSIVENESS OF HEMOPHILIA B-CAUSING NONSENSE MUTATIONS TO RIBOSOME READTHROUGH-INDUCING DRUGS STRICTLY DEPENDS ON THE NUCLEOTIDE AND PROTEIN CONTEXT

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Background: Nonsense mutations, caused by premature termination codons, are relatively frequent in Haemophilia (>10%) and are considered as "null mutations". However, they have been found also in moderate/mild Haemophiliacs

and, as compared with large gene deletions, are associated with lower risk for developing inhibitors. This observation point to the presence of residual expression levels arising from "ribosome readthrough" over nonsense triplets, whose sequence context has an impact on readthrough efficiency, as we have shown in a very small cohort of Haemophilia B (HB) patients. Drugs inducing readthrough such as aminoglycosides are proposed as potential therapy.
Aims: To investigate residual secreted and functional levels of factor IX (FIX) produced by an extended panel of HB nonsense mutations.
Methods: Recombinant FIX (rFIX) nonsense variants were expressed in HEK293 cells, and secreted and intracellular protein levels, as well as protein isoforms, were evaluated by ELISA and Western Blotting analysis. Residual activity was assessed by coagulant assays.
Results: We investigated a panel of *F9* mutations (R75X, L103X, R162X, W240X, R294X, R298X, Y330X, Q370X, R379X, R384X), associated to severe/moderate HB, representing the vast majority of patients with nonsense mutations in HB (324 patients out of 467, corresponding to ~70%). Appreciable levels of secreted FIX were detected for the R162X, W240X, R294X, R298X, Y330X mutants, with truncated isoforms being the large predominance. Truncated forms for Q370X, R379X and R384X were observed in cell lysates only, which suggests misfolding and intracellular retention. Noticeably, the full-length FIX form was appreciable for the R162X variant, indicating the occurrence of spontaneous readthrough, which was significantly increased by the use of aminoglycosides. Moreover, aminoglycosides promoted the synthesis of full-length FIX in the presence of the R75X, Y330X, Q370X, R379X, R384X mutations, not undergoing appreciable spontaneous readthrough. Intriguingly, we investigated the peculiar W240X nonsense mutation, caused by two different termination codons, which showed a differential impact of readthrough in restoring the full-length protein depending on the sequence context. Preliminary coagulant and functional assays revealed that only a few variants were prone to undergo efficient readthrough in terms of secretion and coagulant function.
Summary/Conclusions: These data demonstrate that nonsense mutations can be associated to residual FIX levels through a mechanism of "productive" readthrough. The identification of "leaky" nonsense mutations, and thus of patients with trace FIX levels and potentially "high responders" to readthrough-inducing drugs, might help diagnosis and treatment.

S469

A FUSION PROTEIN OF INTERLEUKIN-4 AND INTERLEUKIN-10 PROTECTS AGAINST BLOOD-INDUCED CARTILAGE DAMAGE *IN VITRO* AND *IN VIVO*

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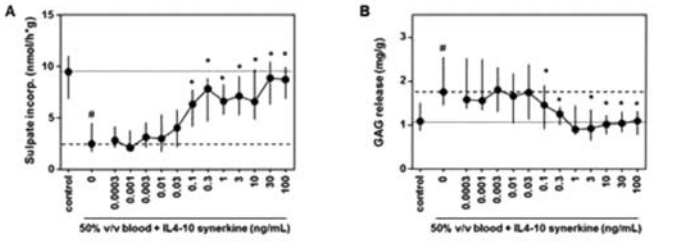
Background: Joint damage upon bleeding causes significant morbidity in patients with hemophilia, and adds to joint degeneration after trauma and major joint surgery. Interleukin (IL)-4 and IL-10 have been demonstrated to protect cartilage from blood-induced damage independently. Recently a novel fusion protein of both cytokines, IL4-10 synerkine, has been developed.
Aims: To evaluate whether IL4-10 synerkine protects against blood-induced joint damage similarly as the combination of the individual components, both *in vitro* as well as *in vivo*.



Healthy human cartilage (n=5) was cultured for 4 days in the absence (control: dotted line) or presence of 50% blood (dashed line). During blood-exposure, the IL4-10 synerkine was added in a broad concentration range. Proteoglycan synthesis rate (A), -release (B) and -content (C) are depicted as median ± IQR.
 # P<0.05 compared with control values, ** P<0.05* compared with blood-exposed cartilage without addition.

Figure 1. Concentration dependent effect of the IL4-10 synerkine.

Methods: *In vitro*, human cartilage explants were exposed to 50% v/v whole blood for 4 days and simultaneously to a broad concentration range (0-100ng/mL) of the IL4-10 synerkine. Effects of 10 ng/mL IL4-10 synerkine were compared to the same concentrations of the individual cytokines and the combination. Cartilage matrix proteoglycan turnover (proteoglycan synthesis, release, and content) was assessed after a recovery period of 12 days. Moreover, the influence of IL4-10 synerkine and its individual components on levels of IL-1β and IL-6 were investigated in a 4 days 50% v/v whole blood culture.
In hemophilia A mice, a joint bleed was introduced on day 0 and 14. Mice were randomized between intra-articular treatment with synerkine (7pmol), IL-4&IL-10 (both 7pmol) or PBS on day 0, 2,14 and 16. After 5 weeks, joint damage was evaluated by the Valentino score for synovitis (hematoxylin and eosin-stained sections) and the modified OARSI score for cartilage damage (Safranin O-stained sections).
Results: *In vitro,* the synerkine prevented blood-induced cartilage damage in a dose-dependent manner up to normalization already at a concentration of 1 ng/mL (see Figure 1). At 10ng/mL, the synerkine was equally effective as the combination of the separate cytokines. IL-1β and IL-6 release in whole blood cultures was suppressed. *In vivo*, treatment with the synerkine attenuated cartilage damage upon joint bleeding (difference between experimental and contralateral joint in synerkine group p=0.201; IL-4&IL-10 p=0.008; PBS p=0.001). In all groups, synovial inflammation was statistically significantly increased in the experimental paw, none of the treatments affected this (p<0.005 in all groups).
Summary/Conclusions: The IL4-10 synerkine fully prevented blood-induced cartilage damage in a human cartilage tissue *in vitro* model and ameliorated cartilage degeneration upon a repeated joint bleed in haemophilic mice when injected intra-articularly. These data support the need for further investigation of the potency of the IL4-10 synerkine in the treatment of blood-induced arthropathy.

S470

FIRST PROSPECTIVE RESULTS OF JOINT DISTRACTION IN SEVERE HEMOPHILIC ANKLE ARTHROPATHY

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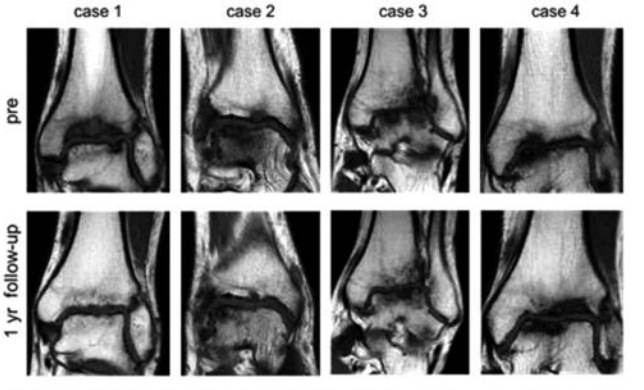
Background: The ankle joint is the most affected joint in youngsters with hemophilia. Joint distraction, an effective treatment in ankle osteoarthritis, has the advantage of preservation of the original joint without compromising subsequent conservative surgeries like arthrodesis, if still needed. In three cases evaluated in retrospect, good clinical and structural efficacy in hemophilic ankle arthropathy was demonstrated.
Aims: The aim of the current study is to prospectively investigate the clinical effectiveness of ankle joint distraction in hemophilia.
Methods: Hemophilia patients (n=10; ≥18 and <55 years) were eligible in case of severe complaints of arthropathy in the tibiotalar joint causing functional limitations, despite analgesics and conservative treatment. Ankle joint distraction using an Ilizarov external fixator was performed during 10 weeks. Clinical effectiveness was evaluated using standard questionnaires and physical examination. Functional tests, X-ray and MRI examination were performed at baseline and 1-year follow-up.
Results: At the moment, a 12 months follow-up is available in 4 patients, three severe hemophilia A patients and one severe hemophilia B patient. Age at time of surgery ranged from 21 to 33 years. During distraction, none of the patients experienced bleeding. Pin tract infection, commonly seen with external frame use, occurred in 3 patients, and was treated effectively with oral antibiotics.
Pain (visual analogue scale) decreased from 67 (47-79)mm at inclusion to 27 (7-84)mm at 6 months and 15 (1-43)mm at 12 months follow-up. Functional limitations, measured by the Haemophilia Activities List and the Ankle Osteoarthritis Scale, improved in three patients at 6 months, and in all four patients at 12 months. Functional tests improved considerably in all patients at 1-year follow up (*e.g.* 6-minutes walking test increased from 497 (434-560) to 621 (560-688) meters). Range of motion of the ankle was slightly decreased after 6 months due to stiffness of the ankle, but regained at 12 months in all patients. MRI revealed a decrease in volume of subchondral cysts and bone edema in all patients, and slight improvement of the joint space width in two patients (see Figure 1).



Note the decrease in volume of the subchondral cysts, and bone edema in all cases. Improvement of joint space width is visible in case 3 and 4.

Figure 1. Structural improvement after ankle distraction on MRI.

Summary/Conclusions: This first prospective study investigating the efficacy of joint distraction in haemophilic ankle arthropathy, showed clear clinical and structural improvement in all patients at 1 yr follow-up. Although preliminary, these data indicate that joint distraction may be a promising treatment postponing more rigorous surgery like ankle arthrodesis in those patients not benefitting from conservative therapy.

S471

THALIDOMIDE FOR HEREDITARY HEMORRHAGIC TELANGIECTASIA: EFFICACY AND SAFETY OF LONG-TERM TREATMENT

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Background: Hereditary hemorrhagic telangiectasia (HHT) is a genetic disease that leads to multiregional angiodysplasia. Severe recurrent epistaxis is the most common presentation often leading to severe anemia. In a previous open label, phase 2, non-randomized, single-centre study, we have shown that low-dose thalidomide (Thal) was safe and effective in reducing epistaxis in HHT patients, providing a rapid, long-lasting clinical improvement without serious adverse events (Lancet Haematol 2015; e465-73). However, the effect of Thal was not permanent. After the end of therapy, most patients relapsed at various time points.
Aims: To assess the effects of Thal on the severity of epistaxis in subjects with HHT who relapse after a first successful treatment with the drug; to evaluate safety and tolerability. The main purpose of our study is to obtain clinical data useful for the identification of a regimen for long-term prevention of HHT epistaxis by Thal.
Methods: Our study is the extension of a previous clinical trial (THALI-HHT, EudratCT 2011-004096-36, ClinicalTrials.gov Identifier: NCT01485224). HHT patients, successfully treated with Thal according to this protocol, may receive again Thal providing that eligibility criteria are still met and they relapsed at least 4 weeks after the end of previous therapy. Twenty-nine patients are potentially recruitable. Thal is given orally for 8 weeks at the same dosage that induced remission (50 or 100 mg/day, off label use). Thal courses may be repeated at most 3 times; in case of relapse occurring within 4 weeks from the end of a previous treatment course, Thal is permanently discontinued. Monthly follow-up evaluates the epistaxis severity according to well defined criteria (Am J Rhinol Allergy 2009;23:52-58) and the transfusion need, with adverse events being reported.
Results: Fourteen HHT patients for whom informed consent was obtained, 9 M and 5 F, aged 48-84 years (median 64), with mutations in either ACVRL1 (12 cases) or ENG gene (2 cases) who relapsed at 10-68 weeks (median 32) after the end of Thal induction therapy, have been retreated so far, 9 patients with 50 mg/day of Thal and 5 patients with 100 mg/day. Eight-week courses of Thal were effective in 13 (93%) patients with a significant reduction of nose bleeding and a concomitant increase of hemoglobin levels and decrease of the transfusion need. At a median follow-up of 81 weeks (range 39-117) after the end of the first course of retreatment, 3 (22%) patients maintained a response, whereas 10 (71%) relapsed again (median relapse-free survival 28 weeks). Eight patients were successfully retreated with a second 8-week course of Thal and 5 patients, who relapsed again, with an additional third course. Median relapse-free survival was 28 weeks after the end of the second course and 24 weeks after the end of the third course. Only nonserious, grade 1, drug-related adverse effects, including constipation, drowsiness, and peripheral edema were observed during Thal courses.
Summary/Conclusions: These preliminary results strongly support the hypothesis that repeated administrations of Thal maintain their efficacy and can be used for long-term treatment of HHT epistaxis. Recruiting a suitable group of patients and following them carefully is expected to confirm this suggestion and contribute to identifying maintenance treatment schedules that permanently reduce epistaxis.
Supported by Telethon Grant GGP13036.

Bone marrow failure syndromes incl. PNH - Biology

S472

DYSREGULATED MIR34A/DIACYLGLYCEROL KINASE Z INTERACTION ENHANCES T-CELL ACTIVATION IN ACQUIRED APLASTIC ANEMIA

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Background: As a paradigm of bone marrow failure syndrome, acquired aplastic anemia (AA) was thought to be a specific autoimmune disease for the aberrant T-cell immune homeostasis. MicroRNAs (miRNAs) down-regulate gene expression by binding to target messenger RNAs and play important roles in various immune processes. We screened for differentially expressed miRNAs in T-lymphocytes of sever AA (SAA) patients by miRNA genechip assays and found miR34a overexpressed in SAA. MiR34a has been demonstrated to directly and functionally target diacylglycerol kinase (DGK) ζ to promote T-cell response. There was no data referred to the role of miR34a or DGKζ in AA.
Aims: To investigate the role of miR34a and DGKζ in T-cell activation in AA.
Methods: Human bone marrow mononuclear cells (BMMCs) were isolated from 25 SAA patients, 16 moderate AA (MAA) patients and 20 healthy volunteers. Affymetrix miRNA genechip assays on $CD3^+$ T-cells purified from BMMCs of three SAA patients and three healthy controls were undertaken. Then the relative expression of miR34a and DGKζ in BMMCs from the 41 AA patients and 20 healthy controls was quantified by real time polymerase chain reaction. The BMMCs from AA patients were transfected with miR34a inhibitor sponge in lentivirus and the level of T-cell activation marker CD69 on the transfected cells was analyzed by flow cytometry. The lymphocytes isolated from lymph nodes (LN) of wild-type (WT) and miR34a-knockout (KO) mice were labeled with CFSE and left unstimulated or stimulated with McAbs to CD3 and CD28. The cell division and the CD69 and CD25 expression was analyzed by flow cytometry. A murine model of bone marrow failure was created in which LN cells from WT or miR34a-KO mice (C57/B6 background) were transferred into CB6F1 mouse recipients preirradiated with 5 Gy total body irradiation. After euthanasia on Day 12, $CD4^+$,$CD8^+$ and $Lin^-Sca1^+CD117^+CD34^-$(LSKCD34$^-$) BM cells were analyzed by flow cytometry.
Results: The microarray analysis demonstrated that miR34a expression increased 2.1-fold in T-cells from SAA patients compared with that in healthy controls. The significantly higher expression of miR34a was confirmed by PCR in BMMCs from the 41 AA patients compared with that from the 20 normal controls. The miR34a expression showed a negative correlation with peripheral blood neutrophil or reticulocyte counts in AA patients, and was higher in SAA than in MAA groups. The mRNA level of DGKζ in AA patients was much lower than that in controls, and was negatively correlated with the level of miR34a. The protein level of CD69 on T-cells transfected with miR34a inhibitor lentivirus was lower than that on cells transfected with control lentivirus. In murine models, upon stimulation with McAbs to CD3 and CD28, the $CD4^+$ and $CD8^+$ LN cells from miR34a-KO mice expressed much lower levels of CD69 and CD25 than those from WT mice, and proliferated less vigorously than the cells from WT mice. Consistently, the DGKζ expression in LN cells from miR34a-KO mice decreased to a less extent after stimulation than that in cells from WT mice. *In vivo*, the mouse recipients transferred with miR34a-KO lymphocytes demonstrated lower mortality on Day12 after irradiation, fewer $CD8^+$ T-cells in BM and increased BM LSKCD34$^-$ cells compaired to those transferred with WT cells.
Summary/Conclusions: The data demonstrated that miR34a/DGKζ was dysregulated in AA, which resulted in enhanced T-cell activation and bone marrow failure. Our study indicated that miR34a/DGKζ played a critical role in T-cell immunity in AA and might be a new therapeutic target for AA.

S473

BONE MARROW FAILURE IN PAROXYSMAL NOCTURNAL HEMOGLOBULINURIA IS ASSOCIATED WITH EXPANSION OF ANTIGEN-SELECTED T CELLS: HIGH-THROUGHPUT EVIDENCE

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Background: Paroxysmal nocturnal hemoglubinuria (PNH) is an acquired clonal disease of hematopoietic stem cells caused by somatic mutation of the *PIG-A* gene that is essential for the biosynthesis of the glycolipid molecule glycosylphosphatidylinositol (GPI). The clinical phenotype of PNH consists of hemolysis, thrombophilia and bone marrow failure (BMF). While hemolysis and thrombosis are largely due to deficiency of the GPI-linked complement inhibitors, the pathogenesis of BMF remains unknown. Previous studies have indicated that CD1d-restricted, GPI-specific T cells are present in PNH patients and might underlie BMF. Interestingly, these cells display a heavily biased T cell receptor

repertoire with enrichment for T cell receptor (TR) α chains encoded by the TRAV21/TRAJ31-1 genes and frequently carrying a distinctive complementarity-determining region 3 (CDR3: AVNNNARLM) combined by TR β chains encoded by the TRBV19 gene, often with restricted CDR3 as well, strongly alluding to selection by common (auto)antigen(s).
Aims: Prompted by these findings, here we sought to obtain more evidence about the nature of the implicated immune response through detailed analysis of the repertoire of GPI-specific T cell populations.
Methods: Our study included samples from 11 patients and 9 normal donors. TRAV21 gene rearrangements were RT-PCR amplified on RNA from T cell subsets sorted from peripheral blood mononuclear cells on the basis of: (i) expression of CD48, [positive in GPI+ cells] and vice versa; and, (ii) expression of TR β chains encoded by the TRBV19 gene *versus* TRBV19 negative. Both criteria applied to PNH patients, whereas the sole criterion used for normal donors was TRBV19 expression. PCR products were sequenced on 454 GS Junior (Roche) platform. Sequences were submitted to IMGT/HighV-QUEST, and metadata was processed by an in-house bioinformatics pipeline. Since all studied rearrangements utilized the same TRAV gene, we defined as clonotypes TRA rearrangements with identical TRA joining (TRAJ) gene usage and amino acid CDR3 sequence.
Results: Overall, significant differences ($p<0.05$) were identified in PNH patients *vs* normal controls for 13 of 50 functional TRAJ genes, the most pronounced concerning the TRAJ39, TRAJ57 and TRAJ58 genes. Similarly, significant differences ($p<0.05$) emerged when comparing the TRAJ gene repertoire between T cell subsets in PNH patients. In particular, (i) the TRAJ15 and TRAJ4 genes were over-represented amongst CD48+ cells; while the TRAJ24 and TRAJ33 were over-represented amongst CD48- cells, (ii) the "GPI-specific" CDR3 sequence (AVNNNARLM) was identified with higher frequency in CD48+ *versus* CD48- cells, however it was also present, with statistically significant lower frequency ($p<0.05$) amongst healthy donors ; (iii) the frequency of the "GPI-specific" CDR3 sequence was identified in GPI- positive up to 73.8% and in GPI-negative T – cell fractions up to 25.6%. When comparing the CDR3 length in i) patients *versus* healthy individuals, and ii) GPI-positive *versus* GPI-negative T cells, we noted a significant ($p<0.05$) bias to longer CDR3s in patients *vs* healthy donors; and, CD48- *vs* CD48+ cells. Cluster analysis of the CDR3 sequences of all PNH cases identified 21 different clonotypes that were shared by PNH patients but not by healthy individuals.
Summary/Conclusions: The present study documents noteworthy restrictions in the TR repertoire in PNH, indicative of antigen selection. The existence of clonotypes shared between different patients suggests that common antigen driven immune responses may be implicated in the pathogenesis of BMF.

S474

A SUBCUTANEOUSLY ADMINISTERED INVESTIGATIONAL RNAI THERAPEUTIC (ALN-CC5) TARGETING COMPLEMENT C5 FOR TREATMENT OF PNH AND COMPLEMENT-MEDIATED DISEASES: INTERIM PHASE 1 STUDY RESULTS

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Background: Uncontrolled complement activation plays a pivotal role in a variety of disorders such as paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic-uremic syndrome (aHUS). Despite the availability of eculizumab, treatment challenges, including heterogeneity in clinical response combined with inter-individual variation in clearance of eculizumab, remain. ALN-CC5 is a subcutaneous (SC) investigational RNA interference (RNAi) therapeutic targeting complement C5 (C5).
Aims: The aim of this abstract is to report safety, pharmacodynamics (PD) and clinical activity of ALN-CC5 in normal healthy volunteers as well as initial results in patients with PNH.
Methods: A phase 1/2 single-ascending dose (Part A) and multiple-ascending dose (Part B) study of ALN-CC5 in healthy adult volunteers and patients with PNH (Part C) is ongoing. Enrollment of several cohorts of healthy volunteers in both Part A and Part B has been completed. Part C is a 39-week multiple dose study in patients with PNH. Primary endpoints are safety and tolerability. Secondary endpoints include: pharmacokinetics (PK), reduction of circulating C5 and complement activity as measured by CAP/CCP Wieslab ELISA assays and sheep erythrocyte hemolysis assay as well as reduction in LDH (in PNH patients). ALN-CC5 is administered SC at a concentration of 200 mg/mL.
Results: Part A enrolled 32 healthy volunteers who were randomized (1:3) to placebo or a single SC dose of 50, 200, 400, 600 or 900 mg of ALN-CC5. Part B enrolled 24 healthy volunteers who were randomized (1:3) to placebo or up to 13 weekly doses of 100, 200, 400 or 600 mg of ALN-CC5. Among the initial healthy volunteers enrolled, (Part A: n=20; Part B: n=12) ALN-CC5 was considered generally well tolerated with no serious adverse events (SAEs), study discontinuations or clinically significant laboratory findings. Preliminary data showed C5 knockdown up to 99% with nadir residual C5 values as low as 0.6 mcg/mL; complement activity inhibition (CAP & CCP) up to 97% with mean max inhibition of 95±1% for CAP and 96±0.9% for CCP and reduction of serum hemolytic activity up to 98% with mean max inhibition of 84±7.6%, suggesting potentially clinically meaningful complement inhibition following ALN-CC5 administration. Notably, an up to 97.8% knockdown (KD) of serum C5 at day 98 after a single dose in the top dose cohort and up to 98.3% KD at day 112 after five weekly doses in the 200 mg cohort was maintained. Based on these initial findings, Part C was initiated. In Part C, patients with PNH receive 200 mg weekly doses of ALN-CC5. Updated safety, pharmacodynamics (PD) and clinical activity for all study participants, will be presented.
Summary/Conclusions: ALN-CC5 was shown to be generally well tolerated, with no clinically significant, drug-related adverse events to date in healthy volunteers. The PD effects of ALN-CC5 were found to be durable, with clamped C5 knockdown and complement activity inhibition supporting a less frequent as well as SC dose regimen. Collectively, these initial results suggest that the use of a novel RNAi therapeutic targeting C5 is a promising approach for inhibiting complement in PNH, aHUS and other complement-mediated diseases.

S475

NEXT GENERATION TARGETED SEQUENCING IMPROVED THE PRECISE DIAGNOSIS OF PEDIATRIC APLASTIC ANEMIA /INHERITED BONE MARROW FAILURE SYNDROMES

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Background: Inherited bone marrow failure syndromes (IBMF) is a group of diseases with heterogeneous clinical and genetic characteristics and overlapping symptoms. Diagnosis of IBMFS depend on classic clinical manifestation like early onset, physical anomalies associated, family history of cancer and/or bone marrow history, and abnormal laboratory test including chromosome breakage testing (MMC and/or DEB), mutation analyses and bone marrow chromosome analyses. At present, more than 70 pathogenic gene mutations had been identified. However, in some patients, physical anomalies is absent or delayed, and were diagnosed as acquired bone marrow failure. Therefore, precise genetic analysis is very important for establish a specific diagnosis, predict cancer risk, direct treatment and genetic counseling.
Aims: In this study, we focus on the application of next generation targeted sequencing in precise diagnosis of pediatric aplastic anemia (AA)/IBMF, and compared the results with clinical manifestation and chromosome breakage testing.
Methods: We designed a targeted capture next generation sequencing (NGS) assay to test a panel of 417 blood disease genes. Pediatic patients (≤14 year old) with suspected diagnosis of AA/IBMF were enrolled. Peripheral blood (PB) was used to genetic analysis and oral epithelia cells or PB from the parents were used to identify whether the gene mutations were somatic mutations or not. The results were validated by Sanger sequencing.
Results: We validated the assay using 30 samples with known mutations. The mean depth for targeted sequencing was 200×, 93.28% of targeted regions were covered with >10×.288 patients with pediatric IBMF were enrolled including 177 subjects were clinically diagnosed as acquired AA, 49 Fanconi anemia (FA), 26 DBA, 8 DC, 15 SCN, 4 congenital thrombocytopenia, 1 Shwachman-Diamond syndrome (SDS). Totally, 66 (23.6%) subjects have IBMF related genetic mutations. In subjects clinically diagnosed as acquired AA, 17 (9.6%) subjects have IBMF related gene mutations, including 10 subjects finally genetically diagnosed as FA, 3 DC, 2 Epstein syndromes, 1 Wiskott-Aldrich syndrome (WAS). In subjects clinically diagnosed as FA, 21 (42.1%) have FANC related gene mutations and 3 (6.9%) have other IBMF related gene mutations. In subjects clinically diagnosed as DC, 7 (85.7%) have telomere related gene mutations. In subjects clinically diagnosed as DBA, 11(37.9%) have ribosomal protein related gene mutations and 1 FA. In subjects clinically diagnosed as SCN, 5 (33.3%) have SCN-related gene mutations, 1 DC, 1 FA and 1 WHIM syndrome, 1 AKT, 1 GFI1 mutation. In subjects clinically diagnosed as congenital thrombocytopenia, 1 CAMT. In subjects finally genetically diagnosed as AA, FA or DC, 4.4%, 18.2% and 9.1% have family history of BMF or malignant blood diseases respectively, and 3.8%, 9.1%, 0% have family history of solid tumor respectively. In subjects finally genetically diagnosed as AA, FA or DC, 8.8%, 66.75%, 63.3% have physical anomalies respectively. Compared to subjects with AA, subjects with FA or DC are more likely to have physical anomalies of short stature and development retardation, Cafe au lait spots of the skin and finger(toe) malformation (24.2% *vs* 0%, P=0.000; 21.2% *vs* 1.2%, P=0.021; 36.4% *vs.*1.2%, P=0.000). Moreover, 8.1% subjects present physical anomalies before or at the same time with bone marrow failure. In subjects finally diagnosed as FA or DC, only 27% and 14% have positive MMC respectively.
Summary/Conclusions: Compared to traditional diagnostic methods, the targeted NGS analysis is economic, convenient, easy to update and very important for differential diagnosis, personal treatment and follow-up and genetic counselling, especially for make differential diagnosis between acquired and congenital BMF. Moreover, the targeted NGS can be used to make precise

diagnosis between IBMFs which have overlapping clinical symptoms. In subjects with IBMF, the positive rate of MMC is low. Therefore, further gene mutation analysis is necessary for highly suspicious subjects with IBMF who have negative result of MMC.

S476

THREE CASES OF CYCLIC NEUTROPENIA WITH ACQUIRED CSF3R MUTATIONS, ONE DEVELOPING AML

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Background: We recently reported a 17-year-old female with cyclic neutropenia (CyN) harboring *ELANE* mutations presenting with cycling hematopoiesis involving neutrophils, platelets and reticulocytes who developed AML (FAB M2) (presented at ASH 2015, Blood 126 (23) abstract 885). At the time of AML diagnosis, all *CSF3R*-expressing BM leukemic blasts were positive for the p.Gln741X mutation. We tested the patient's BM MNCs, obtained at the time of leukemia development, for *RUNX1* mutations, finding that the *RUNX1* mutation p.Asp171Asn was present at an allele frequency of 10%. So far, *CSF3R* mutations have been reported in congenital neutropenia patients (CN) only but never in patients with CyN.
Aims: Our aim was to determine whether other CyN patients harbor *CSF3R* mutations.
Methods: We performed deep sequencing of *CSF3R* in 19 additional CyN patients.
Results: Out of these 19 patients we identified two additional patients harboring acquired *CSF3R* mutations. One CyN patient aged 15.4 years and her sister inherited the *ELANE* mutation from their father. Time-course analysis of the acquisition of *CSF3R* mutations in this patient showed that 2.6% of the *CSF3R* alleles in BM MNCs were obtained at the age of 13 years possessing the p.Gln749X mutation. After additional 1.5 and 2.4 years, respectively, the mutant allele frequency increased to 9% and 8%. A third CyN patient, a 7 year old girl, harboring spontaneous *ELANE* mutations and revealing typical cycling of neutrophils, platelets, and monocytes was treated with G-CSF at a dose of 4.5 µg/kg/day and recently acquired *CSF3R* mutation (p.Gln739X) in approx. 30% of the *CSF3R* allele. The latter two CyN patients have not yet developed AML or myelodysplastic syndrome (MDS).
Summary/Conclusions: Taken together, these findings suggest that CyN patients with typical CyN-associated *ELANE* mutations may also acquire *CSF3R* mutations and are therefore at risk for leukemic development. Long-term data of the Severe Chronic Neutropenia International Registry (SCNIR) have shown that the risk of acquisition of *CSF3R* mutations and of myeloid transformation is low but not absent in patients with CyN compared to patients with CN. This new knowledge is important for prognostic counseling and long-term management of *ELANE*-CyN patients.

Diffuse large B-cell lymphoma

S477

RANDOMIZED PHASE III STUDY OF EARLY RITUXIMAB INTENSIFICATION IN COMBINATION WITH CHOP14 FOLLOWED BY RITUXIMAB OR NO MAINTENANCE IN DIFFUSE LARGE B-CELL LYMPHOMA: A HOVON-NORDIC LYMPHOMA GROUP STUDY

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Background: Rituximab (R)-CHOP is standard treatment for patients with diffuse large B-cell lymphoma (DLBCL). The optimal R dose and schedule is not known. In a small series of 20 patients serum levels of R, measured during the R-CHOP-14 regimen in elderly patients with DLBCL increased slowly up to cycle 5, reaching plateau thereafter (Reiser M, *et al.* J Clin Oncol 2006). We hypothesized that R serum levels might be suboptimal, especially early during treatment, and that the treatment outcome might improve through intensification of R during the first 4 cycles. In this study designed for both young and elderly DLBCL patients we compared standard treatment of R-CHOP-14 with the same regimen combined with 4 extra R administrations during the first 4 cycles. Patients in complete remission after induction treatment were randomized for a second time between observation and R maintenance. Here we report the efficacy and safety results of the first randomization.
Aims: This phase III, open-label randomized study was designed to detect superior metabolic complete remission rates of early intensification of R combined with CHOP-14+G-CSF *versus* no intensification of rituximab in patients with DLBCL of all age groups.
Methods: Patients with previously untreated DLBCL, CD20 positive, stage II-IV, ages between 18-80 years, were randomized 1:1 between standard R-CHOP-14 and an experimental arm with R-CHOP-14 combined with extra R 375 mg/m^2 IV on day 8 of the first 4 cycles. Prephase treatment of prednisone 100 mg orally for 5 days was mandatory in elderly patients. All patients received pegfilgrastim 6 mg sc on day 2. All patients underwent restaging after 4 cycles of therapy and at the end of induction therapy. Response was evaluated using PET-CT scans according to the Lugano criteria 2014. A Deauville score of ≤3 was considered as a complete response. The primary endpoint of the first randomization was metabolic complete remission rate after induction treatment. This trial was registered at www.trialregister.nl as NTR1014. All patients gave written informed consent.
Results: 575 patients were randomized (standard arm 286 patients, experimental arm 289 patients). Median age was 65 years (range, 18-80), 50% of patients were 66 years or older and 52% were male. The majority of patients (57%) had a high-intermediate or high aa-IPI score. Baseline patient and disease characteristics were well balanced between treatment arms. No significant difference in CR rate was observed between the two treatment arms, standard arm 84% CR and experimental arm 82% CR (odds ratio=0.83, 95% CI 0.54-1.28, p=0.40). The observed CR rates in patients ≤or >65 years were identical. With a median follow-up of 49 months (maximum 90 months), 3- and 5- year progression free survival (PFS) were 74% and 68% in the standard arm and 71% and 61% in the experimental arm (hazard ratio=1.23, 95% CI 0.92-1.63, p=0.16). No significant improvement in PFS was noted in subgroups by age (≤or >65 years) or gender. The frequencies of hematological and non-hematological adverse events were similar in both treatment arms.
Summary/Conclusions: In patients with DLBCL treated with R-CHOP-14 rituximab intensification early during treatment did not improve the CR rate or the 3-year PFS. No clinical subgroup benefited from rituximab intensification in this study.

S478

SURVIVAL OF DLBCL PATIENTS IN FIRST REMISSION RELATIVE TO A MATCHED BACKGROUND POPULATION

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Background: Communicating prognosis in a plain language to patients with diffuse large B-cell lymphoma (DLBCL) and their relatives can be challenging. Survival of DLBCL patients relative to a matched background population (MBP) represents a relevant measure of prognosis.
Aims: The purpose of this study was to investigate the post-treatment survival of DLBCL patients in CR/CRu relative to an MBP and to examine the duration of CR/CRu needed to obtain similar survival to the MBP.
Methods: This retrospective study utilized the nationwide Danish Lymphoma Registry (LYFO). We included patients fulfilling the following criteria: a) newly diagnosed DLBCL in the period 2003-2011, b) ≥15yrs of age at diagnosis, and c) achieving CR or CRu after 1st line R-CHOP(-like) therapy. Survival curves were generated using the Kaplan-Meier method and survival of the MBP was established from lifetime tables.
Results: In total, 1632 Danish DLBCL patients met the inclusion criteria and the baseline characteristics were similar to that seen in previous studies. The median follow-up from diagnosis was 85 months. The 5-year OS of the MBP was superior to that of the DLBCL patients (88% *vs* 78%, p<0.001). In analyses restricted to patients in continuous CR/CRu for >2 and 5 years, the OS of DLBCL patients remained inferior to that of an MBP (p<0.001 for 2 years; p<0.001 for 5 years). Age-stratified analyses (<50yrs *vs* ≥50yrs) showed, the OS of patients <50yrs became identical to that of an MBP after only two years in continuous CR/CRu (p=0.90). For DLBCL patients ≥50yrs, however, the OS remained inferior past 5 years of continuous CR/CRu. The OS of DLBCL patients ≥50 years were still inferior after censoring patients with relapses (p<0.001) (Figure 1).

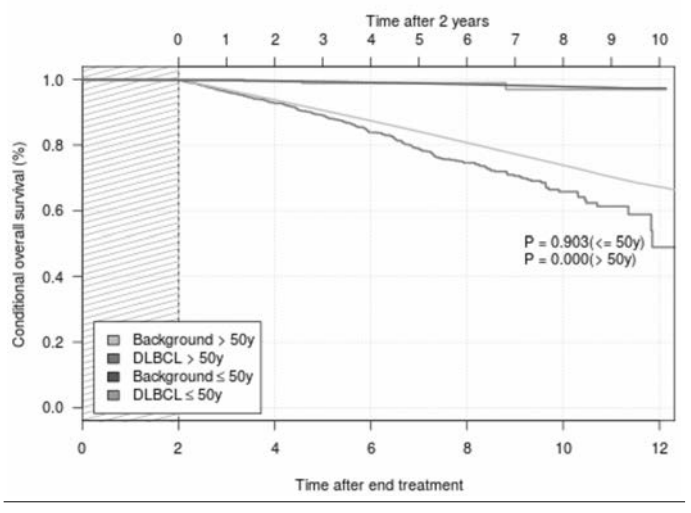

Figure 1.

Summary/Conclusions: The OS of DLBCL patients in CR/CRu after R-CHOP(-like) therapy was inferior to that of an MBP, but the differences were gradually reduced for patients with durable remission. Interestingly, the OS of young DLBCL patients (<50yrs) quickly became identical to that of an MBP. However this was not the case for patients ≥50yrs, even after excluding those with relapse. This suggests that the residual life expectancy of this subgroup of patients may be reduced due to late toxicities or other causes not directly related to DLBCL.

S479

A BIOCLINICAL PROGNOSTIC MODEL INCORPORATING MYC AND BCL2 PREDICTS OUTCOME TO SALVAGE THERAPY IN RELAPSED/ REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA: AN NCIC CTG LY12 CORRELATIVE SCIENCE STUDY

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Background: Less than 50% of patients (pts) with relapsed/refractory diffuse large B-cell lymphoma (rrDLBCL) achieve long-term event-free (EFS) and overall (OS) survival with salvage therapy. Better predictors of outcome are needed for these pts.
Aims: To determine clinical and molecular predictors of EFS and OS for rrDLBCL pts treated with rituximab (R) and either gemcitabine, dexamethasone, cisplatin (R-GDP) or dexamethasone, cytarabine, cisplatin (R-DHAP) followed by autologous stem-cell transplantation (ASCT) on the Canadian Cancer Trials Group LY12 study.
Methods: In total, 91 pts had DLBCL and sufficient histologic material to create tissue microarrays and undergo immunohistochemical (IHC) testing for CD10, BCL6, MUM1, FOXP1, LMO2, BCL2, CMYC, P53, pySTAT3 expression. In addition, 97 formalin-fixed, paraffin-embedded tissue samples underwent gene expression profiling (GEP) using NanoString to evaluate Cell of Origin (COO) by the Lymph2Cx assay, as well as BCL2, MYC, P53, STAT3, PDL1 and PD1 expression. Survival analysis was performed using the Kaplan-Meier method and Cox regression.
Results: COO was not associated with EFS or OS, according to the Hans IHC algorithm or the Lymph2Cx assay. Expression of py-STAT3 (≥50%), P53 (≥30%), MYC (≥40%), and BCL2 (≥70%) by IHC was found in 7.6%, 19.8%, 36.3%, 63.7%, of patient samples respectively. Expression of MYC was associated with lower 3-year (y) EFS rates (10% *versus* (*vs*) 42%, p=0.007) and OS rates (29% *vs* 56%, p=0.002) compared to MYC- patients. Similarly, 3y EFS rates were 25% *vs* 41% (p=0.029) and OS rates were 37% *vs* 63% (p=0.020) for BCL2+ *vs* BCL2- cases, respectively. The 22 pts with IHC-based dual expressing (DE) lymphomas (MYC+/BCL+) had significantly worse 3y EFS (0% *vs* 40%, p=0.0009) and OS rates (20% *vs* 54%, p=0.0004) relative to the 69 pts with without DE phenotype. In addition, p53+ *vs* p53- lymphomas had 3y EFS rates of 11% *vs* 36% (p=0.034). STAT3 was not associated with EFS or OS. Similar to IHC, both MYC and BCL2 expression using NanoString GEP (>1.5xmean) were significantly associated with inferior OS and EFS, and no patient who expressed both markers achieved 2y EFS or OS. For the 82 lymphomas with samples for both IHC and GEP, a concordance rate of 79% was seen for MYC and 57% for BCL2. Expression of PD1 (p=0.03) but not PDL1 (p=0.41) by GEP was associated with inferior EFS. In multivariate analyses, 4 factors were adversely associated with EFS and OS: primary refractory DLBCL, elevated serum LDH at relapse, MYC expression and BCL2 expression (assessed by either IHC or GEP). Using these 4 factors, a bio-clinical score predicted EFS and OS from initiation of salvage chemotherapy. Using IHC to assess MYC and BCL2, 3y EFS was 55% for 34 pts with 0-1 factor *vs* 16% for 53 patients with 2-4 factors (p<0.0001). Similarly, using GEP to assess MYC and BCL2, the 3y EFS was 46% for 58 pts with 0-1 factor *vs* 5% for 39 pts with 2-4 factors (p<0.0001, see Figure 1). The same 4 factor model predicted EFS for the 54 pts who received ASCT: 3y EFS 68% for 0-1 factor *vs* 34% for 2-4 factors (p=0.013) assessing MYC and BCL2 by IHC, or 53% for 0-1 factor *vs* 18% for 2-4 factors (p=0.008) when assessing MYC/BCL2 by GEP.

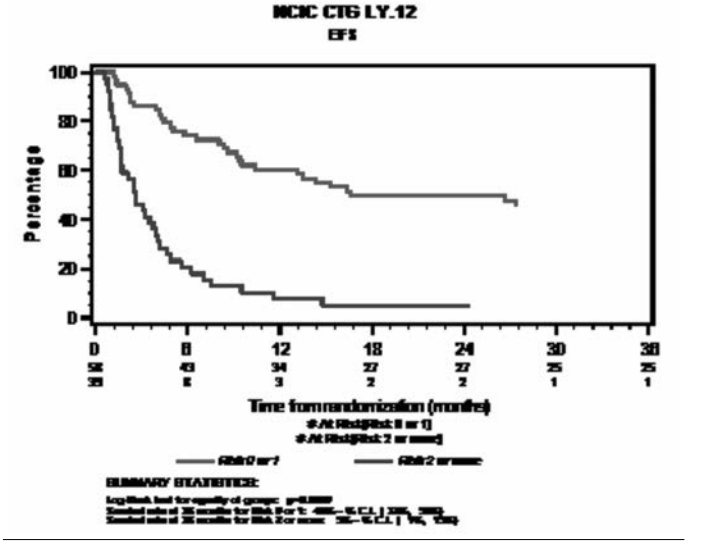

Figure 1.

Summary/Conclusions: In conclusion, MYC and BCL2 expression, determined by IHC or Nanostring GEP, are independent poor prognostic factors for rrDLBCL, and dual expression predicts dismal prognosis. Combining MYC and BCL2 expression, primary refractory disease and elevated LDH results in a robust bio-clinical model strongly associated with EFS and OS for rrDLBCL, including chemosensitive patients who proceed to ASCT.

S480

PHASE 1 TRIAL OF CUDC-907, A NOVEL, ORAL DUAL INHIBITOR OF HDAC AND PI3K: UPDATED ASSESSMENT OF PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA, INCLUDING DOUBLE EXPRESSOR LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL), the most common lymphoid malignancy in adults, is an aggressive form of non-Hodgkin lymphoma with significant heterogeneity in terms of gene expression, prognosis and response to treatment. Certain histone modifications and HDAC expression patterns have been implicated in the pathobiology of DLBCL and other cancers. Aberrant PTEN/PI3K/AKT signaling is also frequently observed in a variety of human cancers including DLBCL, where loss of PTEN and/or activating mutations in PI3K have been shown to inhibit apoptosis and promote tumor cell proliferation. Synergistic anti-tumor effects achieved with HDAC and PI3K inhibitor combinations in DLBCL xenograft models have provided a strong rationale for testing a highly potent dual HDAC and PI3K inhibitor, CUDC-907. Significant anti-tumor effects have been observed across a wide range of DLBCL models exposed to CUDC-907, including the U2932 ABC DLBCL model that harbors BCL2 amplification and over-expresses cMYC and BCL6, and the double hit WSU-DLCL-2 GCB DLBCL model that harbors *MYC* and *BCL2* translocations. These disease mechanisms and preclinical data have led to testing of CUDC-907 in an ongoing Phase 1 trial in patients with various hematologic cancers.
Aims: This study is evaluating the effect of CUDC-907 on treatment-related toxicity and efficacy in patients with relapsed or refractory (RR) lymphoma and multiple myeloma (MM).
Methods: Seventy-five heavily pre-treated patients with RR lymphoma or MM have received CUDC-907 in 21-day cycles according to once daily (QD), intermittent (BIW or TIW), or five days on/two days off (5/2) dosing schedules. CUDC-907 was escalated in 30 mg increments such that patients received 30-60 mg QD, 60-150 mg intermittently, or 60 mg 5/2. Dose expansion investigating the 60 mg 5/2 dose and schedule as monotherapy and in combination with rituximab is ongoing. To date, a total of 29 patients have been treated with the 5/2 dosing schedule, including 4 patients who were assigned to concomitant standard of care dosing of rituximab on day 1 of the first 6 cycles of treatment.
Results: The most common treatment-related adverse events (AEs) were Grade 1-2 diarrhea (56%; 4% Grade ≥3), fatigue (33%; 4% Grade ≥3), nausea (29%), thrombocytopenia (27%; 17% Grade ≥3), and neutropenia (12%; 9% Grade ≥3), with dose limiting toxicities of hyperglycemia (QD and BIW schedules) and diarrhea (QD and TIW schedules). Side effects were reversible and manageable. The AE profile for patients who received CUDC-907 on the 60 mg 5/2 dosing schedule, including those who received concomitant rituximab, was commensurate with that of the overall safety population. Among 20 response-evaluable patients with RR DLBCL, 9 (45%) achieved objective responses (3 CRs and 6 PRs); lasting a median of 2.6 months (range: <1-14+). Three response evaluable patients were found to overexpress MYC (≥40%) and BCL2 (≥70%) by IHC, meeting criteria applied to "double-expressor" (DE) DLBCL. Two of these patients attained objective responses: 1 CR (followed by autologous stem cell transplant) and 1 PR (lasting 4 months). The third patient has experienced lengthy disease stabilization (5.7+ months). Interestingly, all 3 CRs observed on this study occurred in patients with MYC gene copy number gain, including the patient who also had DE DLBCL.
Summary/Conclusions: Signals emerging from preclinical and clinical studies suggest that patients with DLBCL, including those with MYC-altered and therefore particularly aggressive disease, may derive benefit from treatment with CUDC-907. A Phase 2 trial examining CUDC-907 60 mg 5/2 as monotherapy and in combination with rituximab in RR MYC-altered DLBCL is currently ongoing. Patients are being enrolled based on locally reported MYC status, defined as gene translocation or copy number gain by FISH and/or protein expression ≥40% by IHC. Tumoral tissue will be assessed centrally to confirm MYC status of enrolled patients. Other genetic aberrations, such as BCL2 and BCL6, will also be evaluated for correlation with clinical outcomes (NCT02674750).

S481

RESULTS FROM SCHOLAR-1: OUTCOMES IN PATIENTS WITHREFRACTORY AGGRESSIVE DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Background: Non-Hodgkin lymphoma (NHL) is the most prevalent hematologic malignancy in the US and the fifth most deadly cancer with nearly 19,790 deaths/year in the US. DLBCL is the most common NHL subtype, representing 25-35% of new cases annually. Although five-year survival rates in the first-line setting range from 30-50%, approximately 1 in 3 of these patients will be refractory to or relapse post treatment. Diverse therapeutic outcomes are observed in these patients with relapsed/refractory aggressive DLBCL. The international, multi-cohort Retrospective Non-Hodgkin Lymphoma Research (SCHOLAR-1) was designed to study outcomes in patients with refractory DLBCL.
Aims: The aim of SCHOLAR-1 was to retrospectively evaluate response rate and overall survival in patients with refractory DLBCL to serve as a benchmark for future clinical trials in this underserved population.
Methods: Eligibility criteria included refractory DLBCL, which was defined as progressive disease (PD) or stable disease (SD) as best response to chemotherapy (duration of SD <12 months and at least 4 cycles of first-line or 2 cycles of later line therapy) or relapse ≤12 months after autologous stem cell transplantation. All patients must have received an anthracycline as part of a prior regimen. In addition, all patients with CD20-positive disease must have received prior treatment with an anti-CD20 monoclonal antibody. SCHOLAR-1 included data from 2 phase 3 studies (LYSARC-CORAL and Canadian Cancer Trials Group (CCTG)-LY.12) and 2 observational cohorts (MD Anderson Cancer Center [MDACC] and Mayo Clinic/University of Iowa [MC/IA] Specialized Program of Research Excellence). Response rates and overall survival were estimated from the time that salvage therapy was initiated.
Results: Among 861 patients with DLBCL, 597 patients with refractory disease were identified. Response rates ranged from 19% to 36% (complete responses from 2% to 18%). Median survival was poor, ranging from 4.6 months to 6.9 months across cohorts. Data for each study or cohort are shown in the Table 1.

Table 1.

	MDACC (n = 152)	MC/IA (n = 84)	CCTG LY.12 (n = 190)	CORAL (n = 171)
Median age[a],years (range)	55 (20 - 79)	61 (20 - 84)	54 (25 - 68)	54 (19 - 65)
RR, %	19	23	24	36
CR	7	8	2	18
PR	13	14	23	18
RR, %				
Primary refractory (n = 142)	NA	28	24	NA
Refractory to 2nd-line tx (n = 306)	19	26	NA	36
Relapsed ≤ 1 yr after ASCT (n = 149)	21	11	NA	35
Median OS, months (Q1, Q3)	6.9 (5.8, 8.2)	4.6 (3.9, 5.8)	6.8 (5.8, 8.2)	6.7 (5.6, 8.8)

[a]CORAL included subjects 18 - 65 years of age; CCTG LY.12 included subjects ≥ 16 years; subjects > 65 years were not recommended for inclusion.

ASCT, autologous stem cell transplantation; CR, complete response; NA, not applicable (based on study design); OS, overall survival; PR, partial response; RR, response rate; tx, treatment.

Summary/Conclusions: This is the first study to define patient outcomes for those with refractory DLBCL in a large group of patients. Outcomes were similar across cohorts. Our results suggest that patients with refractory, aggressive DLBCL represent a homogenous population with a response rate of 20% - 30% and median overall survival of approximately 6 mos. These consistently poor outcomes represent a significant unmet need in patients with refractory DLBCL.

Standard Treatment Results in AML

S482

IMPROVED SURVIVAL IN PEDIATRIC ACUTE MYELOID LEUKEMIA: A REPORT FROM AML-BFM TRIALS 1987-2011

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Background: Prognosis of children with acute myeloid leukemia (AML) improved within the recent decades. Study groups worldwide performed prospective randomized trials to select more effective and/or less toxic drugs. Several approaches such very high dosages of cytarabine *versus* high cumulative dosages of anthracyclins or performance of allogeneic stem cell transplantation (HSCT) *versus* chemotherapy only led to rather similar results in terms of event-free (EFS) and overall survival (OS).
Aims: Therefore we asked if the improvement of survival is dependent from particular therapy elements identified in randomized trials or rather a correlation with time. This would implicate that factors such as intensity, supportive care or risk group stratification have a much higher impact than the selection of a specific drug.
Methods: In total, 1873 children (0 to 18 yrs) enrolled to the AML-BFM trials from 1987 until 2011 were included (excluding Down's Syndrome and acute promyeloblastic leukemia). The AML-BFM Study group recruited on population-based trials all children with AML from Germany and since 1998 from Czech Republic, Austria and Switzerland. Outcome data were analyzed not only per trial but per 2- and 3-year-periods respectively (Kaplan-Meier).
Results: The 5–year EFS and OS (1987 to 2011) increased from 41±3% to 51±2% and 49±3% to 72±2%, respectively. The Table 1 shows EFS and OS of 3 year periods. The comparison to the AML-BFM trial episodes indicate that a stepwise increase in long-term survival was achieved –according to protocols- by more intensive chemotherapy from 1987-1997. Analysis of 2 and 3-yr-periods shows changes within these study protocols dependent on particular therapy elements and amendments (Figure 1). After 1998 the following years rather show a continuous improvement but only in OS. An increasing gap between EFS and OS (period 1987-1989: 9%; 2008-2010: 24%) was observed. A relevant and unexpected increase in OS was observed in 2001/02. At this time point there was no change in first line protocol, but in addition an international relapse protocol was implemented. The survival after relapse increased from 17±3 up to 44±3%.

Table 1.

3-yr-period	[n]	EFS±SE [%]	OS±SE [%]
1987-1989	134	43±4%	52±4%
1990-1992	161	39±4%	47±3%
1993-1995	249	52±3%	58±3%
1996-1998	284	48±3%	58±3%
1999-2001	250	50±3%	64±3%
2002-2004	246	51±3%	65±3%
2005-2007	245	52±3%	74±4%
2008-2010	232	46±4%	70±3%
AML-BFM 87 (1987-1992)	295	41±3%	49±3%
AML-BFM 93 (1993-1997)	488	50±2%	57±2%
AML-BFM 98 (1998-2003)	465	49±2%	63±2%
AML-BFM 2004 (2004-2011)	625	51±2%	72±2%

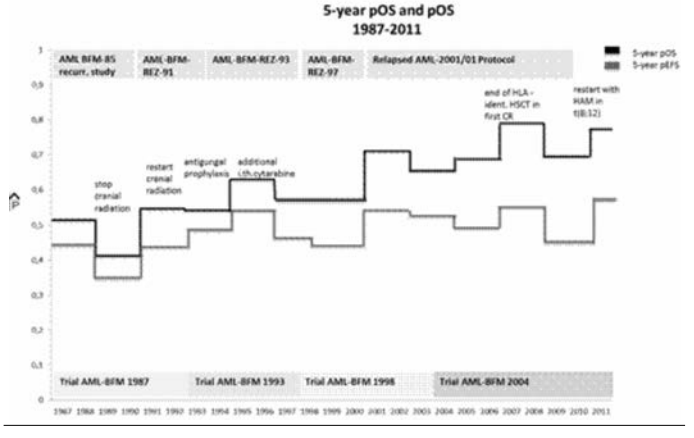

Figure 1.

Summary/Conclusions: Since 1987, several changes influenced AML therapy. The intensity of treatment increased until the AML-BFM 98 trial, in supportive care mainly the general introduction of antimycotics (recommendation 1993) might be the most relevant change. Since 1995 OS improved continuously until 2008, whereas EFS did not change significantly. Although, the slightly reduced relapse rate might suggest that 2nd line treatment becomes more difficult due to the selection of the more resistant AML, from the data it is clear that the efficacy of 2nd line therapy improved.

S483

MONOSOMAL KARYOTYPE AND TRISOMY 8 ARE POOR PROGNOSTIC FACTORS IN PEDIATRIC AML: AN AML-BFM 2004 TRIAL REPORT ON GENOTYPE-OUTCOME CORRELATIONS

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Background: Conflicting data on poor risk factors in pediatric acute myeloid leukemia (AML) hamper risk stratification systems and the improvement of risk-adapted therapeutic strategies.
Aims: We aimed to unravel the prognostic impact of specific chromosomal aberrations including complex and hypodiploid as well as monosomal karyotypes and trisomy 8 in pediatric patients with AML.
Methods: Karyotypes of a population-based cohort of 643 patients <18 years with *de novo* AML treated on the AML-BFM 2004 study protocol (excluding Down's Syndrome and patients with t(15;17)/PML-RARA fusion gene) were analyzed. For cytogenetic definitions see Table 1.
Results: Our analysis revealed overlapping groups of patients with monosomal (MK+), complex (CK+) or hypodiploid (HK+) karyotypes. Multivariate regression confirmed MK+ (n=25) as new independent poor risk factor for event-free survival (EFS) with a reduced EFS of 23±9% (p=0.008), even after exclusion of monosomy 7 (MK^{no-7}; n=19; EFS 25±10%, p=0.007). As expected, Monosomy 7 (n=8; pEFS 13%±12%; p=0.0026) was associated with poor outcome and resistance to induction chemotherapy. Importantly, after exclusion of MK, patients with CK did not experience a significantly worse outcome (n=47; 42±7%, p=0.12), while CK+/MK+ patients did poorly (n=11; EFS 24±14%, p =0.04). Similarly, hypodiploid karyotype (HK) did not predict worse outcome overall (n=38; EFS 43±8%, p=0.26). However, after exclusion of HK+ patients with concurrent t(8;21) (n=21; pEFS 70±10%), the remaining HK+ patients showed very poor prognosis (n=17, pEFS 9±8%, p=<0.0001). Finally Trisomy 8 without additional cytogenetic aberrations was associated with poor outcome (n=16; EFS 25±11%; p=0.009).

Table 1.

	Pts n (%)	5y pEFS (SE) %	p-value (Logrank)*	5ypOS (SE)%	p-value (Logrank)*
monosomal karyotype† (MK+)	**25(4)**	**23(9)**	**0.0008**	**43(10)**	**0.0008**
monosomal karyotype†excl. -7	19(3)	25(10)	0.0066	47(12)	0.0070
MK+/CK+	11(2)	24(14)	0.041	36(15)	0.0013
MK+/CK-	14(2)	21(11)	0.0047	50(13)	0.070
MK+/HK+	11(2)	9(9)	<0.0001	36(15)	0.0031
MK+/HK-	14(2)	34(13)	0.22	49(14)	0.057
hypodiploid karyotype¬ (HK+)	**38 (6)**	**43(8)**	**0.26**	**62(8)**	**0.27**
HK+/MK-	27(4)	57(10)	0.60	73(9)	0.59
complex karyotypes+(CK+)	**58(9)**	**42(7)**	**0.12**	**62(7)**	**0.30**
CK+/MK-	47(7)	47(7)	0.33	68(8)	0.94

**in comparison to other patients with cytogenetic data,+3 or more aberrations, at least one structural aberration; without favorable genetics; without MLL rearrangement; †Two or more autosomal monosomies or one autosomal monosomy with at least one structural abnormality. No favorable cytogenetics (t(15;17)(q22;q21); (t(8;21)(q22;q22); inv(16)(p13q22)/t(16;16)(p13;q22)); ¬ <46 chromosomes, SE=standard error, pOS=probability of OS, pEFS=probability of ES.*

Summary/Conclusions: Our study identified monosomal karyotype and isolated trisomy 8 for the first time as strong and independent prognostic factors in a pediatric AML cohort that are associated with poor outcome. In contrast, complex karyotype alone without MK or hypodiploidy per se does not seem to confer dismal prognosis. These results have important implications for risk stratification including the indication for stem cell transplantation and should be validated in ongoing pediatric trials.

S484

RISK ADAPTED THERAPY FOR ACUTE MYELOID LEUKEMIA (AML) BASED ON GENETIC DATA AND MINIMAL RESIDUAL DISEASE: RESULTS OF THE AML12 TRIAL OF THE CETLAM GROUP IN ADULTS UP TO THE AGE OF 70 YEARS

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Background: Refinement of the AML risk stratification based on genetic data (cytogenetics and molecular characteristics) and minimal residual disease (MRD) assessment may optimize the post-remission allocation to consolidation chemotherapy only or hematopoietic cell transplantation (HCT).
Aims: To evaluate the feasibility and results of an ongoing prospective phase II trial of intensive chemotherapy followed or not by HCT depending on genetics of AML and levels of MRD after consolidation.
Methods: Patients 18 to 70 years old with primary AML treated at 15 academic hospitals between February 2012 and November 2015 were enrolled in the trial. The number of patients included and the outcomes will be updated on May 1, 2016. Induction consisted of idarubicin 12 mg/m^2 days 1-2-3 as intravenous bolus and cytarabine 200 mg/m^{2}as continuous infusion days 1 to 7. Consolidation courses were the high-dose cytarabine schedule of the CALGB with reduction of cytarabine from 3 g/m^2 to 1.5 g/m^2 in patients older than 60 years. The number of consolidation courses was based on AML risk allocation: three in the low-risk category (CBF, NPM1mut/FLT3-ITDwild or ratio<0.5, CEBPA biallelic mutation, all of them with low MRD after consolidation (by flow cytometry and/or quantitative PCR of the specific transcripts); two in the intermediate risk category (intermediate cytogenetics without favorable or unfavorable mutations (these patients subsequently received an autologous or allogeneic transplantation depending on MRD levels and availability of an HLA matched donor); and one consolidation followed by mandatory allogeneic transplantation from any stem cell source available (identical sibling, unrelated, cord blood, haploidentical) in the high-risk group (adverse genetics and/or MRD persistence on high levels).
Results: In 372 patients enrolled so far, the median age (range) was 55 (18-70) years and 53% were male. MRC cytogenetic distribution was: CBF AML 12%, intermediate-risk 66% and poor risk 22%. FLT3-ITD was detected in 22% of 348 cases studied (29% in intermediate risk cytogenetics) group and NPM1 mutation was evident in 57% of patients with a NK.Complete remission (CR) rate in 365 evaluable patients was 74% (n=272), Induction death occurred in 9% and 19% in patients up to and above 60 years, respectively; 10% of patients had refractory leukemia in the two age categories. Two hundred forty patients completed the consolidation phase and were risk allocated; 104 (43%) to the genetics-MRD favorable category, 73 (30%) to the intermediate and 65 (27%) to the high-risk group; in the latter, 80% of patients received an allogeneic HCT. Median follow-up of the surviving patients is 16 months. Overall survival (OS) of the whole series was 47±3% at 30 months; disease-free survivals at that time point were 74±5% in the favorable risk group, 44±5% in the intermediate and 29±7% in the in the high-risk group (Figure 1), due to significantly difference in the relapse incidence (23%, 41% and 56%, respectively). Remarkably, we confirmed our previous finding that patients with intermediate-risk MRC cytogenetics with NPM1 mutation and FLT3-ITDwild had comparable outcomes to those with NPM1 mutation and an allelic ratio below 0.5.

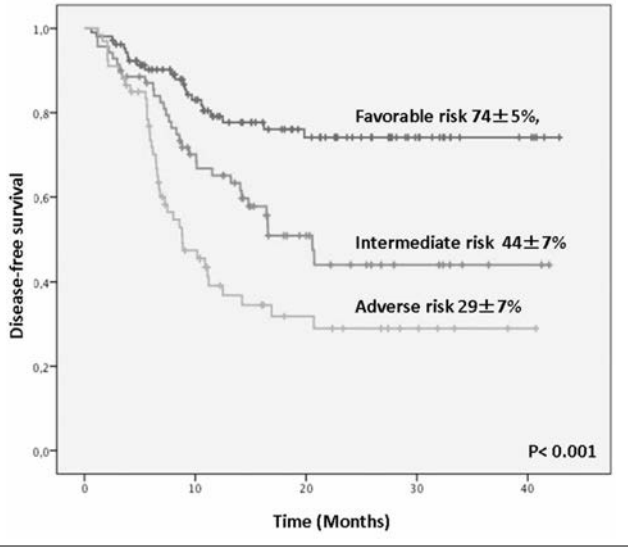

Figure 1.

Summary/Conclusions: Risk adapted therapy for primary AML based on genetics and MRD is feasible in a cooperative group setting. The proportion of patients in whom the risks of an allogeneic HCT in first CR may be avoided is higher than 40% when considering not only cytogenetics but also molecular and MRD information. Allogeneic HCT in high-risk patients was feasible in most instances but further approaches to decrease relapses such as novel agents and immune therapies are needed.

S485

SEQUENTIAL HIGH-DOSE CYTARABINE PLUS IDARUBICIN IMPROVES REMISSION RATE AND SURVIVAL DURATION IN ADULT ACUTE MYELOGENOUS LEUKEMIA (AML): RANDOMIZED TRIAL OF THE NORTHERN ITALY LEUKEMIA GROUP (NILG)

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Background: AML is curable in the fraction of patients who achieve complete remission (CR) and do not relapse following consolidation or allogeneic stem cell transplantation (ASCT). Therefore new strategies are continuously developed to overcome the risk of primary resistant or recurrent disease.
Aims: In NILG trial 02/06 [ClinicalTrials.gov Identifier: NCT00495287]a sequential high-dose (SHD) regimen was compared to standard induction, having CR rate as primary study objective. CR patients were eligible to risk-specific treatment.
Methods: Study patients were stratified by age 60 years and randomized to ICE (idarubicin 12 mg/m^2/d iv. dd1-3, cytarabine 100 mg/m^2/bd iv. dd 1-7, etoposide 100 mg/m^2/d iv. dd1-5) or SHD (cytarabine 2 g [1 g if age >65]/m^2/bd iv. dd 1-2 and 8-9, idarubicin 18 mg/m^2/d iv. dd 3 and 10), plus G-CSF from d11. Postremission consolidation consisted of IC (cycle 2), intermediate-dose cytarabine 1 g/m^2/bd iv. dd 1-4, with harvest of CD34+ blood stem cells (cycle 3), and allogeneic SCT if high-risk or second randomization to BUCY2-conditioned autograft or repetitive HD cycles (cytarabine 2 g/m^2/bd iv. dd 1-5 and idarubicin 8 mg/m^2/d dd 1-2, cycles 4-6) supported by 1-2 x10^6/kg CD34+ cells. The high-risk class was defined by cytogenetics, selected risk factors in intermediate/normal cytogenetic risk group (FLT3 ITD+, WBC >50, minimally differentiated/megakaryo-/erythro-blastic,secondary or MDS-related AML), and late CR. Results were analyzed by treatment intention and, to detect late survival differences, compared using the Kaplan-Meier estimate with weighted log-rank tests according to Fleming and Harrington.
Results: Between 2006-2012, 572 patients were enrolled (median age 52 years, range 16-73; 74% high-risk; 286 with comparable characteristics in each arm). After induction course, CR rate was 69.2% in ICE arm and 81.5% in SHD arm (*P* .001), due to lower resistance rate (25.2% *vs* 11.2%, *P* <.0001) without increased mortality (5.6% *vs* 7.3%, *P* .39). The benefit was confirmed in high-risk AML (n=201 *vs* 218: CR 64.2% *vs* 77.6%; *P* .002) and in patients aged ≤60 years with *de novo* AML (n=190 *vs* 189: CR 74.2% *vs* 86.2%; *P* .003). Overall CR rate after cycle 2/other salvage was 82.5% *vs* 86.0% (*P* .25). SHD increased the time to neutrophil/platelet recovery (*P* <.0001), the risk of infections (bacterial, *P* .0001; fungal, *P* .003) and hepatic, metabolic and cutaneous toxicity (*P* <.05), and was associated with longer intercycle intervals (P<.0001), lower feasibility of cycle 3 (*P* <.0001) and poorer stem cell mobilization (n=123 *vs* 93). One-hundred ninety-three patients (41%) had an allograft in CR1 (175 high-risk [96 in ICE arm and 79 in HDS arm] and 18 standard-risk [6 in ICE arm and 12 in HDS arm]). Median and 5-year overall survival (OS) were 2.14 years

and 38% in ICE arm compared to 4.51 years and 48% in SHD arm (*P* .0125), improving sensibly in the standard-risk subset (5-year OS 55% *vs* 72%, *P* .0068) and patients aged ≤60 years with *de novo* AML (5-year OS 43% *vs* 58%; *P* .0026). Median and 5-year DFS were 1.48 years and 36% compared to 3.41 years and 48% (*P* .0030), again improving in the standard-risk subset (5-year DFS 40% *vs* 64%; *P* .0064) and patients aged ≤60 years with *de novo* AML (5-year DFS 38% *vs* 54%; *P* .0023) (Figure 1).

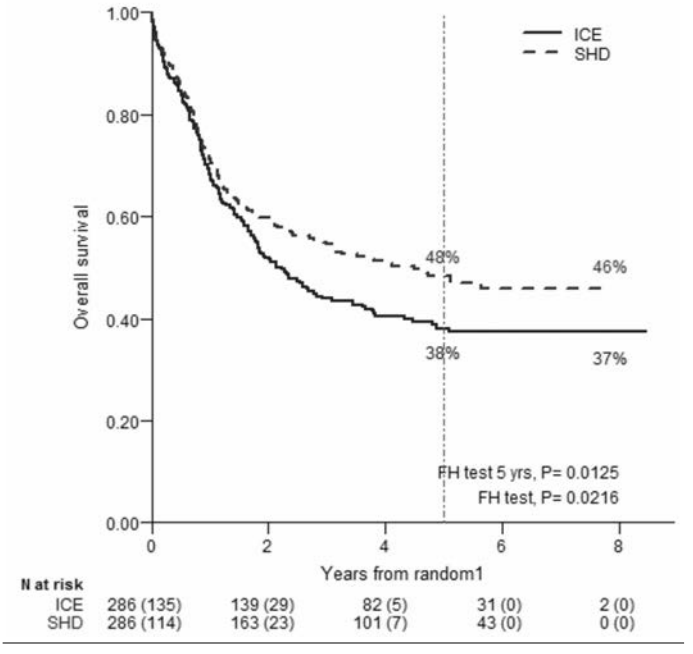

Figure 1.

Summary/Conclusions: The current SHD schedule, albeit proving more toxic than standard ICE did not increase the risk of induction death and improved the CR rate and 5-year OS and DFS results, particularly in patients with standard-risk AML and aged ≤60 years with *de novo* AML.

S486

LONG-TERM OUTCOME OF ELDERLY PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH ANTHRACYCLINE MONOTHERAPY AND ATRA-BASED PETHEMA PROTOCOLS

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Background: Outcomes of elderly patients with acute promyelocytic leukemia (APL) have been reported as less effective than in younger patients because of lower compliance and higher mortality rate related to toxicity of the treatment. This fact has led to design the risk-adapted and age-adapted PETHEMA LPA2005 protocol, in which the anthracycline dose in second consolidation was reduced with respect to previous protocols.
Aims: This study aims at comparing elderly patients (age ≥60 years old) who were treated according to PETHEMA LPA2005 schedule and those who were included in the more intensive previous protocols (LP96&LP99 trials).
Methods: Elderly patients (age equal or greater than 60) who were reported to the multicenter PETHEMA APL registry, diagnosed with APL and demonstration of the t(15;17) or *PML/RARA* rearrangement were included in this study. They were excluded if they met any of the following criteria: 1) Eastern Cooperative Oncology Group (ECOG) performance status at presentation of more than three, 2) severe medical comorbidities limiting the administration of chemotherapy in opinion of the treating physician, 3) antecedents of primary malignancy or previous therapy with leukemogenic agents, and 4) protocol violation. Elderly patients who received treatment according to the risk and age-adapted protocol LPA2005 were compared to those included in LPA96&LPA99 protocols.
Results: From November 1996 to November 2014, 389 elderly patients were reported to PETHEMA APL registry and 268 (69%) were considered as eligible. Causes of ineligibility were secondary APL (19%), ECOG 4/unfit for intensive chemotherapy (11%), and protocol violation (1%). Median age of eligible patients was 67 years (range, 60-84), and the distribution of relapse-risk categories was low (29%), intermediate (50%), and high (21%). Two-hundred sixteen out of 268 eligible patients (81%) achieved complete remission and 52 (19%) died during induction treatment due to hemorrhage and infection, mainly. Leukemic resistance was not observed. Comparing patients according to protocol, no differences in biological and clinical characteristics at diagnosis were identified between the LPA96&99 and LPA2005 cohorts. The median follow-up of patients alive was 62 months (range 2-191). Patients treated with the less intense schedule (LPA2005 trial) had lower toxicity, with a lower duration of neutropenia and thrombocytopenia during the second consolidation and fewer days of hospitalization, resulting in reduced 5-year non-relapse mortality compared with the LPA96&99 trials (5% *vs* 18%, *P*=0.02). At 5 years, patients treated according to LPA2005 protocol had higher overall survival (74% *vs* 60%, *P*=0.02), and higher disease-free survival (87% *vs* 69%, *P*=0.009) than patients who received LPA96&99 protocols. There were no differences in the cumulative incidence of relapse between the two groups (*P*=0.25) (Figure 1).

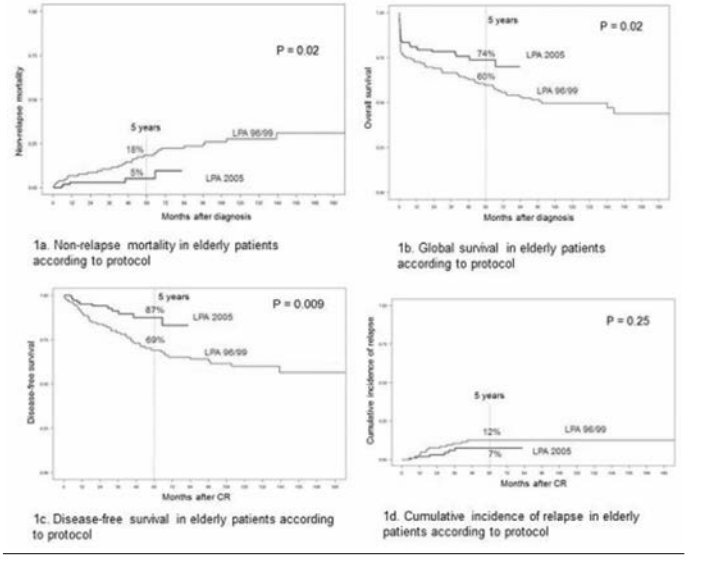

Figure 1.

Summary/Conclusions: Anthracycline dose reduction during consolidation for elderly patients with APL treated with the PETHEMA LPA2005 trial resulted in lower toxicity and non-relapse mortality, while maintaining a high antileukemic activity.

Non-Hodgkin & Hodgkin lymphoma - Biology

S487

GENETIC LANDSCAPE OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: Primary central nervous system lymphoma (PCNSL) is a rare subtype of non-Hodgkin lymphoma, of which approximately 95% are diffuse large B-cell lymphomas (DLBCLs). However, PCNSL shows very different biological and clinical characteristics from systemic DLBCL, and our knowledge about the molecular pathogenesis of PCNSL is still incomplete.
Aims: The purpose of this study is to delineate the entire spectrum of gene mutations, copy number alterations (CNAs) and structural variants (SVs) in PCNSL to better understand its pathogenesis.
Methods: We first analyzed paired tumor/normal DNA from 37 PCNSL cases by whole-exome sequencing (WES). Significantly mutated genes identified by WES and previously known mutational targets in PCNSL and systemic DLBCL were further screened for mutations using targeted sequencing in an extended cohort of PCNSL cases (N=92). CNAs have been also investigated using SNP array-karyotyping (N =56). Whole-genome sequencing (WGS, N=9) was also performed to identify the recurrent mutations in non-coding regions and S*Vs.*
Results: The mean number of nonsynonymous mutations identified by WES was 183 per sample, which was comparable to the figure in systemic DLBCL. A higher representation of C>T transition involving CpG dinucleotides and hotspot mutations within the WRCY motif targeted by somatic hypermutations (SHMs) suggested the involvement of activation-induced cytidine deaminase (AID) in the pathogenesis of PCNSL. We found 12 genes significantly mutated in PCNSL (q <0.1), including *MYD88*, *PIM1*, *HLA-A*, *TMEM30A*, *B2M*, *PRDM1*, *UBE2A*, *HIST1H1C*, *GRB2* as well as several previously unreported mutational targets in systemic DLBCL or PCNSL, such as *SETD1B*, *ITPKB*, *EIF4A2*. Copy number analysis identified recurrent genomic segments affected by focal deletions (N=27) and amplifications (N=10), most of which included driver genes targeted by recurrent somatic mutations or known targets of focal CNAs such as *CDKN2A* and *FHIT*. Subsequent targeted sequencing finally identified a total of 107 significantly mutated genes, of which 43 were thought to be targeted by SHM according to their mutational signature. Most cases with PCNSL (98%) had mutations and CNAs involving genes that are relevant to constitutive NF-κB/Toll-like receptor (TLR)/B-cell receptor (BCR) activity, including those in *MYD88*, *CD79B/A*, *CARD11*, *TNFAIP3*, *GRB2* and *ITPKB*. Genetic alterations implicated in escape from immunosurveillance were also frequently identified in as many as 76% of cases, including mutations of *HLA-B*, *HLA-A*, *HLA-C*, *B2M* and *CD58* as well as CNAs in 6p21.32 (HLA class II), 1p13.1 (*CD58*) and 15q15.2 (*B2M*), suggesting the importance of immune escape in the pathogenesis of PCNSL. SHMs were observed in most cases (98%), which affected not only known non-immunoglobulin targets of AID including *PIM1*, *IGLL5* and *BTG2* but also previously unreported genes involved in cell proliferation, apoptosis, or B cell development. WGS identified 52 loci in which somatic mutations were enriched, including 5'-region of *BCL6*, *RHOH*, *BACH2*, genes known to undergo SHM. Furthermore, breakpoints clusters of S*Vs* included *IG* loci (*IGK*, *IGH* and *IGL*), *CDKN2A/B* as well as known targets of AID, such as *BCL6, BTG2* and *PIM1*.
Summary/Conclusions: Comprehensive genetic analyses of a large cohort of PCNSL cases revealed the genetic landscape of PCNSL, which was characterized by constitutive NF-κB/TLR/BCR signaling, escape from immunosurveillance, as well as SHMs/S*Vs* caused by AID.

S488

DECODING THE ENTIRE DNA METHYLOME OF MANTLE CELL LYMPHOMA: NEW BIOLOGICAL AND CLINICAL INSIGHTS

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Background: Mantle cell lymphoma (MCL) ranges from cases with rather indolent clinical behavior to very aggressive tumors with poor survival rates. Aggressive cases seem to be associated with low levels of somatic hypermutation (SHM) in the Immunoglobulin Heavy Chain (*IGHV)* gene, and *de novo* expression of SOX11. In contrast, indolent MCLs have high levels of IGHV somatic mutation and lack SOX11. Although many studies have focused on biological and clinical features of MCL, its methylome remains largely unknown. As recent studies have suggested a relationship between dynamic methylation during normal differentiation and neoplastic transformation, we hypothesized that a similar link exists in MCL.
Aims: We aimed to explore the DNA methylation landscape in MCL in light of the methylome in the entire B-cell lineage in order (i) to generate new insights into the biological and clinical aspects of MCL and (ii) to further understand the link between the dynamic methylation during normal differentiation and neoplastic transformation.
Methods: We generated genome-wide DNA methylation profiles of 86 MCL samples using the HumanMethylation450 BeadChip and we sequenced the DNA methylome of two representative MCLs by whole genome bisulfite sequencing (WGBS). As normal controls, we used samples (n=67 for BeadChip and n=12 for WGBS) from different B-cell subpopulations (n=10 for BeadChip and n=6 for WGBS). We applied a principal component analysis and consensus clustering to define epigenetic MCL subgroups and to determine their normal counterparts. Furthermore, to tackle the high individual epigenetic variation in MCL, we compared the DNA methylation profile of each individual MCL to a fixed reference point, the hematopoietic progenitor cells, and further analyzed the observed changes in the context of DNA methylation changes observed during B-cell differentiation. To understand the functional and clinical impact of DNA methylation changes we have linked our data with histone modification profiles (chIP-seq), the three-dimensional chromatin structure (4C-seq), the mutational landscape (whole genome/whole exome sequencing) and clinical data in MCL.
Results: First of all, we identified two epigenetic MCL subgroups that carry epigenetic imprints of pre- *versus* post-germinal center B cells.
Secondly, we observed that the majority of individual DNA methylation changes in MCL also occur during normal B-cell differentiation and that pure tumor-specific changes are rare; most (89-99%) DNA methylation alterations in MCL are within or in close proximity to those regions showing dynamic methylation in normal B cells. Thirdly, several thousand differentially methylated regions in MCL show differential enhancer-associated histone modifications, including a region 650 Kb away from *SOX11*. In SOX11 expressing MCL cells, this distant region is hypomethylated and shows high contact frequencies with the *SOX11* promoter in three-dimensional space, suggesting that we have identified a new regulatory element of SOX11 in MCL. Lastly, at the clinical level we observed that epigenetic and genetic changes co-evolve during MCL progression and that the magnitude of epigenetic changes is associated with overall survival of MCL patients.
Summary/Conclusions: Our results (i) provide new insights into the cellular origin, pathogenetic mechanisms and clinical behavior of MCL, (ii) show that pure tumor-specific DNA methylation changes in MCL are rare and (iii) highlight that differential methylation in cancer can target potential epigenetic drivers at distant regulatory elements of key oncogenes.

S489

B CELL-SPECIFIC CONDITIONAL EXPRESSION OF MYD88P.L252P LEADS TO THE DEVELOPMENT OF DIFFUSE LARGE B CELL LYMPHOMA IN MICE

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Background: The adaptor protein MYD88 is critical to relay activation of Toll-like receptor signaling to NF-κB activation. *MYD88* mutations, particularly the *p.L265P* mutation, have been described in numerous distinct B cell malignancies, including diffuse large B cell lymphoma (DLBCL). 29% of activated B cell (ABC)-type DLBCL, which is characterized by constitutive activation of the NF-κB pathway, carry the *p.L265P* mutation. In addition, ABC-DLBCL frequently displays focal copy number gains affecting *BCL2*.
Aims: Here, we aimed to investigate the potential role of the *Myd88$^{p.L265P}$* point mutation in lymphomagenesis.
Methods: We generated a novel mouse model (termed *Myd88$^{c\text{-}p.L252P}$*), in which Cre-mediated recombination, specifically in B cells, leads to the conditional expression of *Myd88$^{p.L252P}$* (the orthologous position of the human *MYD88$^{p.L265P}$* mutation) from the endogenous locus. More precisely, the endogenous exons 2 to 6 of *Myd88* (which consists of 6 exons) were flanked by *loxP*-sites. Downstream of the last exon, a second set of exons 2 to 6 was inserted, harboring the point mutation *p.L252P*. Cre-mediated recombination leads to the excision of the endogenous exons 2 to 6 and expression of the inserted, mutated set of exons. This system very closely mimicks the situation observed in the clinic, as it allows for the heterozygous expression of *Myd88$^{p.L252P}$* from the endogenous locus.
Results: To assess the functionally of our allele, we generated mouse embryonic fibroblasts (MEFs) homozygous for the *Myd88$^{c\text{-}p.L252P}$* allele, recombined them *in vitro* by lentiviral transduction. After puromycin selection, cDNA was sequenced and only the mutant transcript was detected. Western blotting verified the expression of this mutant transcript and increased phospho-p65 levels as a marker for NF-kB activation. We activated our allele B cell-specifically by crossing it to the *Cd19-Cre* mouse. The resulting *Myd88$^{c\text{-}p.L252P}$;Cd19$^{Cre/wt}$* animals had a lifespan of around 500 days, significantly shorter than that of the *Cd19$^{Cre/wt}$* control. Magnetic resonance imaging and autopsy showed the development of splenomegaly and lymphadenopathy starting at around 60 weeks of age. Further histological and immunohistochemical investigation revealed the existence of lymphoproliferative disease and the sporadic emergence of large B cell lymphoma with an Bcl6$^-$/Mum1$^+$/B220$^+$/Cd138$^-$ immunotype. Analysis of V(D)J recombination by southern blotting showed clonal populations in samples from animals diagnosed with lymphoma by histology. BCL-2 is highly expressed in most ABC DLBCL cases. To potentially enhance lymphomagenesis in our mice, we aimed to combine the *Myd88$^{p.L252P}$* mutation with *Bcl-2* overexpression by making use of a newly genrated *LSL.BCL2* allele, where human *BCL2* expression is driven by the *CAGGs* promoter. Indeed, all *Myd88$^{c\text{-}p.L252P}$;LSL.BCL2;Cd19$^{Cre/wt}$* mice die of aggressive large B cell lymphoma at a median of 36 weeks. All animals showed splenomegaly and/or lymphadenopathy, accompanied by infiltration of the liver. Bone marrow involvement was only detected once and was locally restricted, indicating a secondary lesion. Clonality analysis by southern blot showed oligoclonality, suggesting a strong oncogenic potential of *Myd88$^{p.L252P}$* in combination with *BCL2* overexpression. Interestingly, lymphoma cells were of a Bcl6$^-$/Mum1$^+$/B220$^-$/Cd138$^+$ immunotype, in accordance with the plasmoblastic morphology that was histologically observed.
Summary/Conclusions: In summary, we generated a mouse model that enables Cre-mediated expression of *Myd88$^{p.L252P}$* from the endogenous locus. *Myd88$^{c\text{-}p.L252P}$;Cd19$^{Cre/wt}$* mice develop and eventually die of lymphoproliferative disease. The emergence of diffuse large B cell lymphoma (DLBCL) was observed. Combination of B cell-specific *Myd88$^{p.L252P}$* with *BCL-2* overexpression results in the development of an aggressive lymphoma with plasmoblastic features, most reminiscent of ABC-type DLBCL.

S490

BET INHIBITORS SUPPRESS PD-L1 TRANSCRIPTION TO ENHANCE ANTI-TUMOUR IMMUNITY AND IMMUNOTHERAPEUTIC APPROACHES

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Background: Bromodomain and Extra-Terminal (BET) proteins are a highly conserved family of epigenetic 'readers' that recognise and bind acetylated lysine residues on histones and other proteins to modulate gene expression. BET proteins are enriched at enhancer regions regulating oncogenic transcription and inhibitors (BETi) displace BET proteins from chromatin leading to suppression of oncogenes (*e.g. cMYC*). BETi elicit a heterogeneous range of tumour cell responses including apoptosis, growth arrest, differentiation, and senescence. However, responses to single agent BETi are self-limited *in vivo* and drug resistance uniformly emerges in preclinical modelling. As BET-inhibitors (BETi) are now being clinically evaluated in both haematological and solid malignancies, the focus is now shifting to the design of effective combination strategies for phase II studies.
Aims: BETi have potent anti-inflammatory properties, including chromatin-independent downregulation of NFkB signalling. However, broader mechanisms of immunomodulation by BETi in the context of anti-tumour responses remain poorly defined. Having previously discovered NK- and T-cell immunostimulatory activity of an acetyllysine mimetic fragment (n-methylpyrrolidone), we sought to evaluate the immunomodulatory activity of the prototypical theinodiazapine BETi, JQ1.
Methods: Utilising the syngeneic model of transplanted Eμ-*Myc* aggressive 'Burkitt-like' lymphoma we first compared the efficacy of JQ1 in wild-type (immunocompetent) and immunodeficient RAG1$^{-/-}$ or RAG2$^{-/-}$cγ$^{-/-}$mice. To interrogate the functional interaction between JQ1 and the immune system, we profiled changes in tumour cell immunogenicity following drug exposure by flow cytometry.
Results: We observed a 50% reduction in the survival advantage conveyed by JQ1 in mice deficient in T- and/or B-lymphocytes compared to immunocompetent controls. Notably, tumour-infiltrating lymphocytes (TILs) and peripheral blood T-cells from wild-type mice failing JQ1 therapy expressed high levels of PD-1, suggesting suppression of an endogenous anti-lymphoma immune response during disease progression. Having previously identified recurrent copy number amplification of PD-L1 (universally juxtaposed to the Eμ-*Myc* transgene), we hypothesised this endogenous host response may be dampened by the PD-L1/PD1 axis and further modulated by JQ1. Strikingly, JQ1 rapidly and potently suppressed PDL1 transcription in Eμ-*Myc* lymphoma cells *in vitro* and *in vivo*. ChIP assays revealed significantly reduced BRD4 occupancy of the PDL1 promoter/enhancer within 2 hours of JQ1 exposure (with 90% suppression of PD-L1 mRNA levels). shRNA knockdown of BRD4 likewise downregulated PD-L1 transcription. Moreover, JQ1 treatment suppressed both constitutive and IFNγ-inducible PD-L1 expression in human myeloma, Burkitt and Hodgkin cell lines. Finally, treatment of mice bearing Eμ-*Myc* lymphoma with JQ1 in combination with a checkpoint inhibitor (anti-PD1) or immune stimulating antibody (anti-4-1BB) was highly synergistic responses despite minimal single-agent activity of these therapeutic antibodies.
Summary/Conclusions: We suggest that oncogenic PD-L1 transcription (including IFNγ-induced expression) is directly regulated by BRD4 and can be suppressed for therapeutic gain by BETi leading to augmented anti-tumour immunity, particularly in the context of immune checkpoint inhibitors. BETi/anti-PD1 combination studies should be evaluated in genetically leveraged (*e.g.* cMYC positive) aggressive lymphoid malignancy.

Chronic myeloid leukemia - Biology

S491

ROLE OF THE MSC-DERIVED EXOSOMAL AND ENDOGENOUS JAK2-SET/PP2A-B-CATENIN-MODULATOR MIR-300 IN LEUKEMIC STEM/PROGENITOR PROLIFERATION AND SURVIVAL IN CML

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Background: Nanostring arrays and RNAseq analysis of total, $CD34^+$, $CD34^+CD38^-$ bone marrow (BM) cells from healthy individuals (n=6) and CML patients (n=16) showed gradual inhibition of miR-300 expression (CML-$CP^{miR-300}$>CML-$BC^{miR-300}$). MiR-300 is a human intergenic microRNA predicted to target multiple components of the BCR-ABL1/JAK2/hnRNPA1/SET/PP2A/β-catenin pathway that, as reported is essential for survival/self-renewal of leukemic progenitors and quiescent TKI-resistant Ph^+ hematopoietic stem cells (HSCs).
Aims: This work is aimed at understanding the mechanisms regulating miR-300 in CML and assessing the importance of its downregulation for proliferation, survival and self-renewal of CML (CP and BC) cells.
Methods: Lentiviral-mediated modulation of miR-300 expression was used in CML cells and cell lines to assess effect of miR-300 on its mRNA targets, and on the clonogenic potential, LTC-IC, and number of quiescent leukemic stem and/or progenitor cells, and on the leukemogenic potential in animal models of CML. MSC-derived exosomes and conditioned medium, hypoxic/normoxic conditions and BCR-ABL1 inhibition were used to determine the mechanisms of regulation of miR-300 expression.
Results: MiR-300 transduction in CML^{CD34+} cells and BCR-$ABL1^+$ cell lines decreased JAK2, β-catenin, hnRNPA1 and SET expression and increased PP2A activity. Targets were confirmed by miR-300 expression in BCR-$ABL1^+$ cells expressing Flag-tagged miR-300-targets lacking or carrying a wild-type or mutated 3'UTR. Restored miR-300 expression in CML^{CD34+}cells and/or BCR-$ABL1^+$ cell lines impaired proliferation and clonogenic potential, markedly reduced LTC-ICs, and increased TKI sensitivity. Notably, miR-300 expression was inhibited by BCR-ABL1 in proliferating cells. Accordingly, imatinib restored miR-300 expression in $CD34^+$ dividing progenitors and BCR-$ABL1^+$ cell lines without altering miR-300 levels in quiescent ($CFSE_{MAX}$) CML^{CD34+}cells (n=3), consistent with the BCR-ABL1 kinase-dependent activation of the Jak2/SET/PP2A/β-catenin pathway in CML progenitors but not quiescent Ph^+ HSCs. Surprisingly, miR-300 levels were increased in $CD34^+CD38^-$ compared to $CD34^+CD38^+$ CML cells, and >20-fold higher in $CFSE_{MAX}$ compared to dividing CML^{CD34+}cells (n=4). Furthermore, it appears that forced miR-300 expression decreases engraftment of BCR-ABL^+ cells (32D-BCR-ABL) in immunocompromised mice. Bone marrow transplant experiments in NSG mice with miR-300-transduced human CD34+ CML-BC cells, and with miR-300-transduced Lin^- and LSK cells from SCL-tTA-BCR-ABL mice are ongoing and will allow us to determine the role of miR-300 on leukemic HSC engraftment and ability to propagate disease. To determine whether enhanced miR-300 expression in quiescent cells depends on cell autonomous events or is induced by the BM microenvironment, we exposed BCR-ABL^+ cells to conditioned medium (CM) of HS-5 or hTERT mesenchymal stem cells (MSC). CM strongly decreased proliferation, induced imatinib but not FTY720 (PP2A activator) resistance, increased miR-300 levels, decreased BCR-ABL1 activity and Jak2 expression but not its activity, and did not alter b-catenin levels or PP2A activity. Interestingly, miR-300 was found in MSC-derived exosomes, and its expression increased in BCR-$ABL1^+$ cells exposed to exosomes. Accordingly, proliferation of CML-$BC^{CD34+}$and LAMA-84 cells was strongly reduced upon exposure to MSC-derived exosomes. These effects were abolished when we used CM from MSCs transduced with a miR-300 antagomir. Interestingly, culturing of leukemic cells in hypoxic conditions dramatically induced miR-300 expression that, in turn, impaired proliferation of primary $CD34^+$ CML cells and BCR-ABL cell lines.
Summary/Conclusions: Altogether our results indicate that downregulation of miR-300 appears necessary for the activation of JAK2/SET/PP2A/b-catenin survival signals in CML progenitors. Conversely, increased miR-300 levels (endogenous and MSC-derived) seem to be required for HSC quiescence.

S492

IDENTIFYING, TRACKING AND VISUALIZATION CML BLAST CRISIS STEM/PROGENITORS CELLS BASED ON SINGLE-CELL MASS CYTOMETRY ANALYSIS

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Background: Tyrosine kinase inhibitor (TKI) has dramatically improved the outcome of CML, but it could not eliminate leukemia stem cell (LSC). Self-renewal is a key characteristic for LSC. Emerging evidence indicates that β-catenin, the Wnt pathway's central effector molecule, is required for development and maintenance of LSC in CML.
Aims: We hypothesize that the combination of β-catenin and tyrosine kinase inhibition could represent a promising strategy for preventing the development of TKI resistance as well as a novel approach in the therapy of BC CML by targeting CML-BC LSC.
Methods: Aided with single-cell mass cytometry analysis and data visualization with SPADE and viSNE, we investigated the activity of the combination of TKI with a Wnt/b-catenin signaling modulator C82 *in vitro* and with the C82 prodrug PRI-724 *in vivo* in a xenograft murine model.
Results: Primary CML-BC cells were stained with multiple cell surface markers and intracellular survival signaling molecules and subjected to CyTOF analysis. It revealed that β-catenin expression was high in various CML stem/progenitor cells and particularly highest in $CD34^+CD38^+CD123^+Tim3^+$ subset. viSNE visualization demonstrated that β-catenin overexpression was associated with high levels of pCRKL, c-Myc, pAKT, pTyr, pSTAT3, and pSTAT5. Primary CML-BC cells from were then treated with C82, nilotinib, and both. viSNE visualization and SPADE tree analysis revealed that C82 and the combined regimen more significantly decreased various stem/progenitor cells, especially $CD34^+CD38^+CD123^+Tim\text{-}3^+$ cells, which represents for the candidate for CML-BC LSC. Next, NSG mice engrafted with CML-BC^{T315I}cells were treated with PRI-724, nilotinib, or the combination. At the end of treatment, cells were collected from the mice and subjected to CyTOF analysis. viSNE visualization showed that PRI-724 and combination strikingly reduced $CD34^+$b-$catenin^{high}$, $CD34^+CD38^-$, and $CD34^+CD38^+$ different CML-BC stem/progenitor subsets. Furthermore, SPADE tree analysis revealed the combination more profoundly than PRI-724 alone reduced $CD34^+CD38^-$, $CD34^+CD38^+$ and Tim-3^+GMP progenitor cells in NSG mice, which was confirmed by flow cytometry.Furthermore, PRI-724 and the combination induced CD11b expression, suggesting PRI-724 and the combination promoted CML-BC stem/progenitor cell differentiation. Notably, PRI-724 and nilotinib combination significantly inhibited CD44 in $CD34^+CD38^+CD123^{high}$ and $CD34^+CD38^+CD123^{high}Tim\text{-}3^{high}$ subsets, and Tim-3 in $CD34^+CD38^+CD123^{high}Tim\text{-}3^{high}$ subset, respectively. Finally, PRI-724 and the combination significantly improved the overall survival of CML-BC-loaded NSG mice, as compared to control and the nilotinib treatment.
Summary/Conclusions: Collectively, by identifying, tracking and visualization CML-BC LSC based on CyTOF analysis, our results show that inhibition of β-catenin effectively targets CML-BC stem/progenitor cells and prolongs survival of CML-BC-loaded NSG mice, which is further enhanced by combination with inhibition of Bcr-Abl.

S493

DYNAMICS OF SOMATIC MUTATIONS COMMONLY DETECTED IN OTHER MYELOID NEOPLASMS BEYOND THE BCR-ABL GENE REARRANGEMENT IN RESPONSE TO TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is mainly characterized by a translocation event t(9;22)(q34;q11.2) which fuses the *ABL1* gene and the *BCR* gene, yielding a *bcr-abl* onco-protein. Despite remarkable improvement in the treatment of CML via tyrosine kinase inhibitors (TKIs), a portion of patients show resistance to these treatments and have a much higher risk of progression to accelerated phase (AP) or blastic phase (BP) as a result. The survival of patients who progress to BP is comparable to the pre-TKI era, even with other salvage treatment modalities such as allogeneic stem cell transplantation. Using high-throughput sequencing technologies, extensive investigations in other myeloid malignancies have discovered commonly mutated genes and the biological pathways they effect. Other than *ABL1* kinase domain (KD) mutations,

very little is known about the origins and dynamics of relevant somatic variants, as well as their associations with *BCR-ABL* transcript changes and their clinical implications on TKI response in CML.
Aims: We aimed to determine whether patterns of mutation acquisition, persistence, and clearance can provide insight into treatment outcomes in CML.
Methods: 100 CML patients were included for deep sequencing targeting 92 genes that are recurrently mutated in other myeloid neoplasms. Treatment failure was estimated to be 10.2% at 3 years while PFS at 5 years was 91.0%. The OS at 5 years was 92.6%. Based on ELN criteria, 74 patients were determined to have optimal response, 18 failed but remained in CP, and 8 had progressed to accelerated or blast phases. For each patient, samples taken at the initial diagnosis (prior to TKI therapy) and after TKI treatment, as well as T-cell samples, were sequenced in this study.
Results: In total, 64 variants from 32 genes in 37 patients were detected in 300 serial samples from 100 CML patients. *ASXL1*, *ABL1*, and *TET2* were the 3 genes most frequently mutated (n=9, n=6, and n=6, respectively). Unsupervised hierarchical clustering of the 51 non-silent mutations across all serial samples revealed 5 distinct pattern of mutation dynamics throughout the course of CML (Figure 1). A majority of cases with mutations only had mutations from one pattern. Pattern 1 mutations arise at diagnosis and persist at follow-up. Despite this, all patients with Pattern 1 mutations were TKI-responsive. Since these mutations were not cleared in spite of significant reduction of *BCR-ABL* transcript level at the time of follow-up, they are likely to be indicative of abnormal and clonal *Ph*-negative hematopoiesis that existed prior to *Ph*-positive clones. Pattern 2 mutations are acquired during TKI treatment. This pattern included a high portion of *ABL1* KD mutations (7/13 mutations in 6 patients). Perhaps unsurprisingly, all patients with Pattern 2 mutations failed TKI therapy. Pattern 3 mutations rise at the time of diagnosis and vanish or substantially decrease following TKI therapy. Patients with these mutations showed mixed patterns of clinical outcomes. Interestingly, Pattern 3 mutations were frequently within genes associated with chromatin modification and DNA methylation, which are epigenetic regulation pathways (11/17, 64.7%). Patients with Pattern 4 and 5 mutations showed evidence of preleukemic mutations in CML. Too few cases had these mutations to assess their association with treatment outcomes.

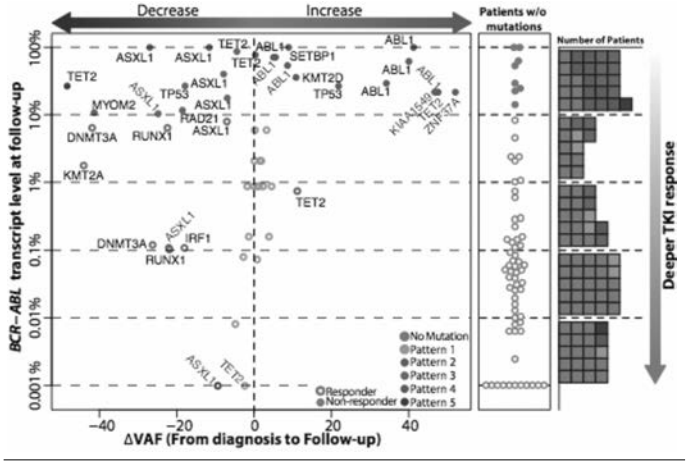

Figure 1. BCR-ABL level indicated on a log10 percentage scale for both subplots. Each circle in the left-hand plot represents a variant. Filled circles indicate TKI treatment failure. The right-hand plot shows patients without extra mutations. Each dot indicates an individual patient. The boxes on the right indicate the number of patients within each decade on TKI response coloured by pattern.

Summary/Conclusions: Overall, this study demonstrates that patterns of mutation acquisition, persistence, and clearance vary but have a number of interesting correlations with clinical outcomes. Our data show that mutation burden often persists despite successful TKI response in CML, particularly in mutations that are likely in persistent *Ph*-negative clones, while mutation clearance is not associated with particular outcomes. Patients that acquired new mutations during treatment all failed TKI therapy. We found evidence of preleukemic mutations in some CML patients. These patterns show that CML mutation dynamics following TKI therapy are markedly distinct from other hematologic malignancies.

S494

THE ADVERSE EFFECTS OF CANCEROUS INHIBITOR OF PROTEIN PHOSPHATASE 2A, ARE LINKED WITH REDUCTION OF FUNCTIONAL B56GAMMA, A REGULATORY SUBUNIT OF PROTEIN PHOSPHATASE 2A, IN CHRONIC MYELOID LEUKAEMIA

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Background: Protein phosphatase 2A (PP2A) is an important phosphatase which opposes the deregulated kinase activity that typifies many malignancies. It comprises a scaffold (A) subunit linked to a catalytic (C) and regulatory (B) subunit; the latter dictates activity, substrate specificity and intracellular localisation and has 26 isoforms. Of these, B56γ confers a PP2A nuclear localisation signal, and mutations interfering with its PP2A binding are reported in solid tumours. We have shown that a high diagnostic level of CIP2A, a key PP2A inhibitor, is a predictive biomarker of poor outcome in both acute and chronic myeloid leukaemia (CML), and ~30 studies in non-haematological tumours all also show a similar association with poor outcome. PP2A is known to dephosphorylate MYC at serine 62, thus destabilising it and shortening its half-life. PP2A also regulates mTORC1, MAPK/ERK, AKT, P53 and NfKB pathways which are associated with malignancy, but whether CIP2A involvement is direct or indirect is not understood; furthermore the detailed molecular and biochemical consequences of high CIP2A levels in malignant cells remain elusive.
Aims: We aim to test whether CIP2A binding to PP2A is dependent on which regulatory subunits are attached in the PP2A heterotrimeric complex. This will help to explain how CIP2A can mediate some but not all of PP2A's specific functions on pathways involved in cancer progression.
Methods: The CML cell line K562 expresses high levels of CIP2A. Its CIP2A levels and those of MYC, S62 MYC, and PP2A components were assessed by flow cytometry. Primary CML cells, both mononuclear and $CD34^+$, with either low or high levels of CIP2A were also studied, after obtaining informed consent. CML cells and cell-lines were used in fractionation (to seperate into distinct cellular compartments), western blotting, co-immunoprecipitation and qPCR.
Results: CIP2A co-immunoprecipitates not only with PR65, the heat repeat scaffold (A) subunit of PP2A, but also with B56γ, one of the B regulatory subunits of PP2A, though not with the alternative B subunits PPP2R4, B56α, B56β, PPP2R5E or B72/130. In primary CML cells with high CIP2A levels, B56γ mRNA expression is lower than in either control or CML samples with low CIP2A levels, to suggest it has an inhibitory effect on B56γ protein levels. In high CIP2A K562 cells, CIP2A is predominantly cytoplasmic, as is B56γ. In addition, S62 MYC is almost entirely cytoplasmic. CIP2A knockdown by transient transfection of siRNA will increase the level of B56γ and vice versa, suggesting that expression is negatively correlated.
Summary/Conclusions: These data suggest that CIP2A reduces the active PP2A-B56γ associated complex and is compatible with the following model: In normal cells (which have very little CIP2A present) and malignant cells with low CIP2A, the PP2A scaffold plus C unit complex moves freely around the cell with full activity, getting access to the nucleus by the nuclear localisation signal obtained on binding B56γ. CIP2A is at too low a level to interfere with this. Cytoplasmic PP2A is also fully active and can thus regulate important cytoplasmic pathways such as MAPK/Jun which can promote proliferation. If CIP2A is high (exclusively a feature of some malignant cells), it binds B56γ as part of the PP2A complex. This results in the specific loss of function performed by the PP2A-B56γ attached complex. This could be achieved by CIP2A in a number of ways, including interfering with PP2A- B56γ nuclear transport, by restricting access of the nuclear localisation signal (NLS) or a direct inhibitory effect on function (*i.e.* blocking of the active site). Concurrently, a reduction in B56γ mRNA is observed. Overall, these effects reduce the amount of PP2A- B56γ phosphatase function, leading to deregulation of known pathways that require functional B56γ; these include the P53 tumour suppressor function and MAPK/ERK in uncontrolled cell proliferation. The effect on S62 MYC may also be affected by reducing total nuclear levels of PP2A or causing a misbalance of other active B regulatory subunits that compete to bind PP2A. Thus, high CIP2A results in a specific deregulation of PP2A function likely to promote clonal progression, with potential overlap in other haematological malignancies.

S495

MODELLING PONATINIB RESISTANCE IN BCR-ABL1+ CELL LINES: IMPLICATIONS FOR PONATINIB THERAPY

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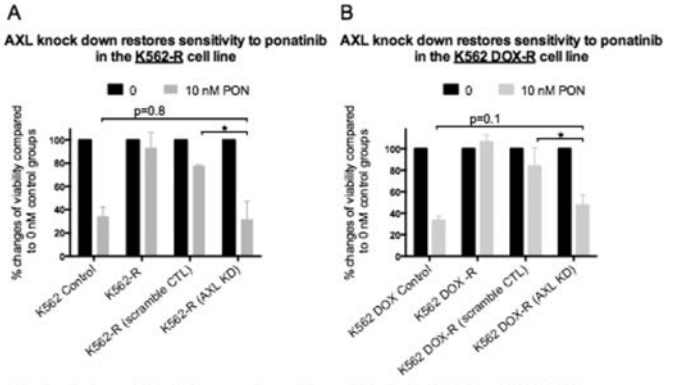
Background: Ponatinib overcomes TKI resistance (imatinib, nilotinib and dasatinib) that develops due to *BCR-ABL1* kinase domain (KD) mutations including T315I. Besides KD mutations, there are several identified TKI resistance mechanisms, such as *BCR-ABL1* overexpression, regulation of the expression of TKI transporters, and Bcr-Abl independent Axl kinase overexpression. However, little is known about the mechanisms that may cause ponatinib resistance. Here, modes of ponatinib resistance have been investigated in TKI naïve and pre-treated *BCR-ABL1*+ cell lines.
Aims: To characterise ponatinib resistance mechanisms in *BCR-ABL1*+ cell line models.
Methods: Ponatinib resistance was generated by using four *BCR-ABL1*+ cell-lines: 1) K562, 2) K562-DOX (ABCB1 overexpressing variant) 3) K562 DOX-55D (resistant to 55 nM dasatinib) and 4) K562 T315I (resistant to 200 nM dasatinib, harbouring the T315I mutation). The average steady state of ponatinib plasma concentration in patients is 101 nM for dosage at 45 mg/day.

Hence, the resistant cells were exposed to increasing concentration of ponatinib to 100 nM (if higher concentration could not be achieved) or 200 nM, as these concentrations are close to clinically relevant. Parental ponatinib naïve controls were maintained in parallel. R indicates ponatinib resistant variants.

Results: Four resistant cell lines were established. All of the four cell lines were cross-resistant to other TKIs (2000 nM imatinib, 1000 nM nilotinib and 200 nM dasatinib). The two dasatinib pre-treated resistant cell lines developed *BCR-ABL1* KD mutation(s). The level of T315I in the K562 T315I-R line (survived in 100 nM ponatinib) increased from 44% to 66%, and the expression level of *BCR-ABL1* increased 6-fold compared to the naïve line ($p<0.05$). The K562 DOX 55D-R (survived in 200 nM ponatinib) developed compound mutations G250E/E255K (54%). However, KD mutations were not observed in the cell lines with no prior exposure to TKI. The ponatinib resistance in K562-R and K562 DOX-R cell lines (both survived in 200 nM ponatinib) were Bcr-Abl independent: active Bcr-Abl and CrkL (surrogate marker of Bcr-Abl activity) levels were reduced, while Axl protein and *AXL* mRNA expression levels were increased significantly (all $p<0.05$). After incubation with Axl inhibitors, 1 μM R428 or 12.5 μM BMS-777607, the K562-R and K562 DOX-R cell lines were re-sensitised (approximately 50% reduction in viability) to 200 nM ponatinib while the controls remained resistant to the drug (both $p<0.05$). Next, retroviral vector was used to reduce *AXL* gene expression in the K562-R and K562 DOX-R cell lines. This *AXL* gene reduction in the two cell lines resulted in re-sensitisation to 10 nM ponatinib (Figure 1A-B), while controls remained resistant to 200 nM ponatinib. These results suggested that Axl overexpression is critical for Bcr-Abl independent ponatinib resistance.



The knock-down of AXL (A-B) restored ponatinib sensitivity in the K562-R and K562 DOX-R resistant cell lines to the same degree as the ponatinib naïve control cell lines. Error bars represent mean+SD. *$p<0.05$.

Figure 1.

Summary/Conclusions: We show that in the setting of prior-TKI exposure, *BCR-ABL1* KD mutations are likely to cause ponatinib resistance. In the TKI naïve setting, Bcr-Abl independent modes of resistance may be more likely, suggesting combination therapeutic approaches may be required in this setting. In addition, ponatinib resistance are unlikely to be overcome by the use of the first or second generation TKIs.

Acute lymphoblastic leukemia - Clinical

S496

HIGH THROUGHPUT SEQUENCING AS A MEASURE OF EARLY RESPONSE TO THERAPY IN CHILDHOOD ALL

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Background: Early response to induction chemotherapy has been demonstrated to be a highly significant prognostic factor in the outcome of children with acute lymphoblastic leukemia. Multiparametric flow cytometry (mpFC) has been the routinely used methodology in the US for determination of this response. New high throughput sequencing (HTS) technologies of rearranged immune receptor (TCR and Ig) genes have raised the possibility of a more accurate, sensitive, and standardizable approach to determination of early response to therapy in ALL patients.

Aims: In this study, we investigated whether the Adaptive Biotechnologies assay of IgH and TCRG would be able to quantify residual disease at the end of induction therapy for children with ALL and be of prognostic value with regard to outcome (event free survival) in these patients.

Methods: The first study involved a total of 480 patients enrolled on Children's Oncology Group (COG) clinical trials AALL0331 and AALL0232 for whom mpFC measurement of residual disease and outcome data are available. A second study involved samples from 73 patients enrolled in a former Pediatric Oncology Group trial, POG 9905, who were mpFC negative at d29. For increased statistical power the patients in POG 9905 were selected for analysis so that ~50% had relapsed disease determined in follow-up monitoring. MpFC was performed at COG reference laboratories the University of Washington or Johns Hopkins Hospital as part of the evaluation for MRD. Genomic DNA was extracted from frozen bone marrow specimens collected at diagnosis and at day 29 post the start of induction therapy. High throughput sequencing of CDR3 regions of IGH and TCRG was performed on all samples, with the testing laboratory blinded to patient outcome. Diagnostic and d29 matched samples from a given patient were sequenced and dominant clonal CDR3 sequences from diagnosis were searched for in the corresponding d29 sample. Both the presence and the frequency of the MRD clone relative to the total IGH repertoire and total nucleated cell population were determined.

Results: The assays defined the dominant clonal sequences in 93% of the patients. 70% of this subgroup was found to have residual disease present at d29. Clones from some of the patients demonstrated a single "trackable" sequence while clones from other patients demonstrated multiple trackable sequences either within or between the two immune receptor loci being assessed. 60% of the residual disease detected by HTS was previously reported as MRD negative by mpFC. For "standard risk" patients, 53% were positive for MRD by HTS and negative by mpFC. With the combined COG data, using a MRD cutoff of 10^{-4}, HTS was able to define a correlation with event-free survival ($p=0.0003$). Furthermore, for the "standard risk" patients, being MRD positive or negative as determined by the more sensitive HTS assay was also correlated with outcome ($p=0.02$). In addition, a correlation was noted between poorer outcome and a "germline" or TCRG only (*i.e.* IgH loci retained in the germline configuration) genotype ($p=0.04$). In the second study of patients enrolled in the POG 9905 trial, the more sensitive HTS cut-off of $10^{-5}$was correlated with outcome ($p=0.0228$).

Summary/Conclusions: This is the largest patient cohort studied to date for which mpFC, HTS, and outcome data are available. This work suggests that HTS is an accurate and standardized assay whose increased sensitivity compared to mpFC is relevant to determination of patient outcome.

S497

DURABLE REMISSIONS AFTER MONOTHERAPY WITH CD19-SPECIFIC CHIMERIC ANTIGEN RECEPTOR (CAR)-MODIFIED T CELLS IN CHILDREN AND YOUNG ADULTS WITH RELAPSED/REFRACTORY ALL

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Background: Targeted immunotherapy with chimeric antigen receptor (CAR)-modified T cells can produce potent anti-tumor responses. We previously reported complete remissions (CR) and prolonged persistence in children and adults

with relapsed/refractory acute lymphoblastic leukemia (ALL) treated with CD19-specific CAR-modified T cells (CTL019). We now report on outcomes and longer follow-up of 59 children and young adults with relapsed/refractory ALL enrolled on the pediatric phase 1/2a trial of CTL019.
Aims: Determine the safety and efficacy of CTL019 in pediatric patients with relapsed/refractory CD19+ ALL. Determine the safety and efficacy of CTL019 in pediatric patients with a history of CNS involvement of ALL. Assess relapse-free survival (RFS) and overall survival (OS) after CTL019.
Methods: After informed consent, patient-derived T cells were transduced with a lentiviral vector encoding a CAR composed of anti-CD19 scFv, CD3z, and 4-1BB domains, activated/expanded *ex vivo* with anti-CD3/CD28 beads, and then infused at a dose of 10^7 to 10^8 cells/kg with a transduction efficiency of 2.3-45%. 54/59 patients received lymphodepleting chemotherapy the week prior to cell infusion.
Results: Of 59 patients aged 20mo-24y with CD19+ ALL, 44 had detectable disease prior to CTL019 cell infusion, while 15 were minimal residual disease (MRD)-negative. 39 were treated for relapse after prior stem cell transplant (SCT). 15 patients had CNS disease within a year of infusion. At assessment 1 month after infusion, 55/59 (93%) were in CR. MRD <0.01% by flow cytometry was achieved in 52 patients. CTL019 cells were detected in the CSF, all patients achieved CR in the CNS (including 4 patients with CSF blasts detected the day prior to infusion), and no CNS relapses have been seen. With median follow-up 12 mo (1-43 mo), 34 patients had ongoing CR, with only 6 receiving subsequent therapy (5 SCT, 1 donor lymphocyte infusion), RFS was 76% (95% CI, 65-89%) at 6 mo and 55% at 12 mo (95% CI, 42-73%), and OS was 79% (95% CI, 69-91%) at 12 mo. 20 patients subsequently relapsed, 13 with CD19-negative disease. CTL019 persistence was accompanied by B cell aplasia, which continued up to last assessment (1-39 mo) in 24/34 patients with ongoing CR. Cytokine release syndrome (CRS) was seen in 88% of patients. Severe CRS requiring hemodynamic or respiratory support occurred in 27%, was associated with high disease burden, and was reversed with the anti-IL6R agent tocilizumab. We could predict development of severe CRS through regression modeling using IFNγ, sgp130, and IL1RA measured in the first 72h (sensitivity 86%, specificity 89%, AUC 0.93).
Summary/Conclusions: Single-agent CTL019 immunotherapy can induce potent responses in patients with relapsed/refractory ALL and can control CNS leukemia. Durable remissions were observed without subsequent SCT. Phase 2 multisite and global registration trials of CTL019 are in progress.

S498

IMPACT OF DISEASE BURDEN ON LONG-TERM OUTCOME OF CD19-TARGETED CAR MODIFIED T CELLS IN ADULT PATIENTS WITH RELAPSED B-ALL

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Background: We have previously reported high response rates in adult patients with relapsed or refractory (R/R) B-cell ALL following CD19-targeted 19-28z chimeric antigen receptor (CAR) modified T cells regardless of the disease burden at the time of T cell infusion. However, the impact of disease burden on toxicity and long-term clinical outcome in patients who received 19-28z CAR modified T cells has not been examined in detail.
Aims: In order to better understand the association between pre-treatment disease burden and tolerability and long-term efficacy of 19-28z CAR T cells, herein we report the result of of a focused analysis from the phase I clinical trial of 19-28 CAR T cells in adults patients with R/R B-ALL (NCT01044069).
Methods: Adult patients with R/R B-ALL were enrolled to the phase I clinical trial. All patients underwent bone marrow (BM) biopsy immediately prior to 19-28z CAR T cell infusion, and were divided into two cohorts based on the blast % in BM: minimal disease (<5% blasts) *vs* morphologic disease (≥5% blasts). Subsequently, patients received lymphodepleting chemotherapy followed by 19-28z CAR T cell infusion.
Results: Of 46 patients treated, 21 patients had minimal disease and 25 patients had morphologic disease at the time of CAR T cell infusion. Baseline disease and treatment characteristics were similar between the two cohorts, except less HSCT in the minimal disease cohort (29 *vs* 48%). Complete response (CR) and minimal residual disease-negative CR (MRD-CR) rates were 91% and 71% in the minimal disease cohort, and 75% and 65% in the morphologic disease cohort, respectively. Severe cytokine release syndrome exclusively occurred in patients with morphologic disease (44% *vs* 0%) but grade 3/4 neurotoxicity was observed in 14% of patients with minimal disease *vs* 40% with morphologic disease. Although overall relapse rates did not differ between the two cohorts, no relapse or death occurred in the minimal disease cohort beyond 12 months. At a median follow-up of 12.0 months (range, 1-45), the estimated 6-month overall survival (OS) rates for all patients in the minimal disease and morphologic disease cohorts were 73% and 57%, respectively. Among the patients who achieved MRD-CR, the estimated 6-month OS rates were 92% for the minimal disease cohort with remissions extending beyond 3 years, and 65% for the morphologic disease cohort.
Summary/Conclusions: These data confirm the potent anti-tumor efficacy of 19-28z CAR T cells (JCAR015) in adult patients with R/R ALL regardless of pre-treatment disease burden. Patients with minimal disease appear to have more favorable toxicity profile and long-term survival rates. These findings support the use of 19-28z CAR T cells in earlier lines of ALL treatment, such as in a frontline setting.

S499

INOTUZUMAB OZOGAMICIN FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN THE GLOBAL PHASE 3 RANDOMIZED CONTROLLED INO-VATE TRIAL: EFFICACY AND SAFETY BY PRIOR THERAPY

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, showed superior response *vs* standard care for relapsed/refractory acute lymphoblastic leukemia (ALL) in the ongoing phase 3 INO-VATE trial.
Aims: To assess the effects of prior therapy on response and toxicities in patients with relapsed/refractory ALL receiving InO in the phase 3 trial
Methods: Per protocol, intent-to-treat analyses of complete remission [CR]/CR with incomplete hematologic recovery [CRi] included the first 218 of 326 patients randomized (ITT218). The safety population included 139 patients who received ≥1 InO dose (max 1.8 mg/m^2/cycle [0.8 mg/m^2 on day 1; 0.5 mg/m^2 on days 8 and 15 of a 21–28 day cycle for ≤6 cycles]). Minimal residual disease (MRD) negativity was assessed by central flow cytometry (<0.01%). Data as of October 2, 2014 are presented (trial ongoing). Informed consent was obtained from all patients.
Results: 109 patients in the ITT218 received InO (CR/CRi rate, 81% [95% CI, 72–88]; MRD negativity rate in responders, 78% [95% CI, 68–87]; median remission duration [DoR], 4.6 [95% CI, 3.9–5.4] months). 67% and 32% of patients received InO as salvage (S) 1 and S2 (missing, n=1). For S1 *vs* S2, response was numerically higher, MRD-negativity was similar, and DoR was numerically longer (Table 1). CR/CRi rate was numerically lower for patients with (n=17) *vs* without (n=92) prior SCT (77% [95% CI, 50–93] *vs* 82% [72–89]). In the safety population, grade ≥3 febrile neutropenia rates were similar for S1 (n=95) *vs* S2 (n=43) (both 23%) and for patients with (n=24) *vs* without (n=115) prior SCT (29% *vs* 23%). Any grade hepatobiliary AE rates were significantly higher in S2 *vs* S1 (47% *vs* 17%; P<0.001) and numerically higher for patients with *vs* without prior SCT (42% *vs* 23%). For S1 *vs* S2, 36 *vs* 11 patients had poststudy SCT. Veno-occlusive liver disease (VOD) including post-SCT VOD occurred in 8% of S1 *vs* 16% of S2 patients (2 fatal in S1); in 21% with *vs* 9% without prior SCT (1 fatal in each cohort).

Table 1.

% (95% CI)[a]	S1 (n=73)	S2 (n=35)
CR/CRi[b]	88 (78–94)	69 (51–83)
CR	43 (31–55)	23 (10–40)
CRi	45 (34–57)	46 (29–63)
MRD-negativity in responders	78 (66–88)	79 (58–93)
Median DoR, months	5.2 (3.0–5.8)[c]	4.2 (3.2–4.6)[d]

[a]ITT218; [b]Best response (1–6 cycles); [c]n=62; [d]n=23

Summary/Conclusions: InO may provide clinical benefit in patients with relapsed/refractory ALL for both S1 and S2 therapy; hepatotoxicity risk increases with number of prior therapies and prior SCT.

S500

EARLY IMMATURE T-ALL AS DETERMINED BY ABSENCE OF BI-ALLELIC DELETION AT THE TCR GAMMA LOCUS IS NOT AN ADVERSE PROGNOSTIC FACTOR ON THE MRC UKALL2003 TRIAL

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Background: Despite a marked improvement in the survival of children with T-cell acute lymphoblastic leukaemia (T-ALL), a significant proportion of patients will experience refractory disease or relapse. Previous studies using immunophenotyping and gene expression profiling identified the early T-cell precursor (ETP) phenotype as a subgroup of T-ALL with an inferior outcome. However, the diagnosis of ETP by immunophenotyping remains difficult to standardise, and gene expression profiling on T-ALL cases is not widely utilised. An alternative marker of immature T-ALL is Absence of Bi-allelic Deletion (ABD) at the TCRγ locus which has been shown to be associated with a very poor outcome. It is a marker that can potentially be standardised and assessed in real time for clinical use.
Aims: To investigate whether ABD status at the TCRγ locus adds prognostic information to paediatric T-ALL patients treated on the MRC UKALL2003 trial.
Methods: After informed consent, diagnostic DNA from 152 paediatric patients with T-ALL on the UKALL2003 trial was analysed by qPCR to amplify the region between the most 3' V region (*TRGV11)* and the most 5' J region (*TRGJP1)* of the TCRγ locus. Presence of an undeleted sequence in this region would indicate developmental arrest of the thymocyte at an early immature stage prior to V-J recombination. Patients were assigned to the ABD and non-ABD groups if fold change was ≥0.5 and <0.25 respectively. The undetermined group (fold change 0.25-0.5) were excluded from further analysis. Results were validated using the Illumina CytoSNP-850K array. Overall Survival (OS) and Relapse-free Survival (RFS) were plotted using Kaplan-Meier survival analysis. Cox regression and the log rank test were used to compare groups.
Results: Of 152 patients analysed by qPCR, 23 (15%) had ABD, 110 (72%) were non-ABD, and 19 (13%) were undetermined. Of the 133 informative patients, 118 had SNP array data that was consistent with the qPCR findings. There was no statistical difference in OS by ABD group (HR for ABD 1.34 (0.37–4.79); p=0.65). RFS was also very similar between the two groups (HR for ABD 1.68 (0.54-5.22); p=0.36) (Fig 1). MRD results were available for 102 patients. Median MRD levels were higher in the ABD group than the non-ABD group (2.5% positive cells at day 29 *vs* 0.02% respectively, p=0.031). The proportion of patients with indeterminate MRD was also higher in the ABD group (n=13, 57%) than in the non-ABD group (n=18, 19%) (p<0.0001). All eight ABD patients with high risk MRD received regimen C compared to 29 of 53 (55%) MRD high risk non-ABD patients (p=0.01). The good OS in ABD patients could not be attributed to coincidence of a favourable–risk NOTCH/FBXW7 genotype, as only 9% (2/23) of ABD patients were double mutant as compared to 31% (34/110) of non-ABD patients (p=0.03) (Figure 1).

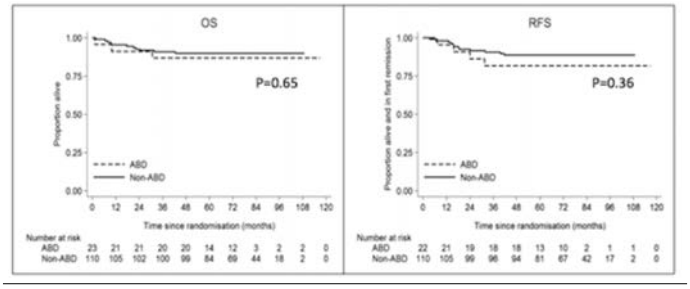

Figure 1. Kaplan-Meier curves for OS and RFS for T-ALL patients according to ABD status treated on the UKALL2003 trial.

Summary/Conclusions: We found no evidence that the molecular marker ABD added further prognostic value to the outcome of paediatric patients with T-ALL treated on the UKALL2003 trial. Therefore escalation of treatment to more intensive regimens based on ABD status is not justified on this protocol. Our failure to confirm previous reports of a poor outcome for the ABD patients could be due to the fact that this trial employed MRD risk directed therapy.

New Compounds in AML Treatment

S501

PHASE III RANDOMIZED TRIAL OF VOLASERTIB PLUS LOW-DOSE CYTARABINE (LDAC) *VERSUS* PLACEBO PLUS LDAC IN PATIENTS AGED ≥65 YEARS WITH PREVIOUSLY UNTREATED AML, INELIGIBLE FOR INTENSIVE THERAPY

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Background: Volasertib (V) is a potent and selective cell cycle kinase inhibitor that induces mitotic arrest and apoptosis by targeting polo-like kinase. In a randomized, open label, Phase II trial in patients (pts) with previously untreated AML, ineligible for intensive therapy, V+LDAC *vs* LDAC improved remission rate (31% *vs* 13%; p=0.052), event-free survival (EFS; HR 0.57 [95% CI 0.35–0.92]) and overall survival (OS; HR 0.63 [95% CI 0.40–1.00]; Döhner *et al.,* Blood 2014).
Aims: A Phase III trial (NCT01721876) was conducted to confirm the Phase II results.
Methods: Pts were randomized 2:1 (stratified by ECOG [0/1 *vs* 2] and type of AML [*de novo vs* secondary]) to receive LDAC (20 mg s.c. BID Days 1–10 Q4W) and either V (350 mg; 1-hr iv infusion Days 1 and 15 Q4W) or placebo (P). The primary analysis was performed after completion of recruitment (Nov 2014) and focused on efficacy in pts randomized ≥5 months before clinical cut-off. Objective response (OR; complete response [CR]+CR with incomplete hematological recovery [CRi]; blinded central review) was the primary endpoint, and OS was the key secondary endpoint. An additional OS analysis was performed in all randomized pts (Nov 2015).
Results: 666 pts were treated (444 V+LDAC; 222 P+LDAC). The primary analysis included 371 pts (246 V+LDAC; 125 P+LDAC); pt characteristics were balanced: median age, 75/75 yrs; secondary AML, 47%/49%; adverse genetics, 32%/32%, respectively. The percentage of pts with OR was not statistically significantly higher with V+LDAC *vs* P+LDAC (25.2% *vs* 16.8%; Odds ratio 1.66 [95% CI 0.95–2.89; p=0.071]), and thus the primary endpoint was not met. A negative OS trend was seen for V+LDAC *vs* P+LDAC (median OS: 4.8 *vs* 6.5 mos; HR 1.26 [95% CI 0.95–1.67; p=0.113]). Adverse event severity increased with V+LDAC *vs* P+LDAC, with fatal infection frequency of 16.6% *vs* 5.1%, considered to be the main reason for the negative OS trend. Consequently, the study was unblinded, and investigators/pts could stop/continue treatment based on individual benefit-risk evaluations. Additional exploratory analyses were conducted. The protocol allowed doses to be missed or delayed for medical reasons; this dosing flexibility resulted in differences of overall treatment intensity, and a better outcome for pts treated in the V+LDAC arm at lower-dose density *vs* higher-dose density was shown. Competing risk modeling of survival endpoints (events resulting from lack of efficacy or non-tolerability) confirmed the antileukemic effect of V+LDAC. An additional OS analysis was performed on all randomized pts from a Nov 2015 snapshot. The HR for OS was 1.06 (95% CI 0.88–1.28; p=0.552); these OS results may be affected by the communication of primary analysis results, unblinding and subsequent treatment decisions.
Summary/Conclusions: This Phase III trial did not meet the primary endpoint and did not confirm the promising efficacy results from the randomized Phase II study. Pts treated with V+LDAC had higher risk for fatal infections compared with P+LDAC, resulting in a negative OS trend at the primary analysis. Pt management/medical decision making (dose density) influenced the outcome and might have been affected by the blinded trial design. Updated results one year after unblinding show largely overlapping OS for both treatment arms. Com-

peting risk modeling of survival endpoints confirmed the antileukemic effect of V+LDAC and supports investigation of modified V doses and schedules to improve tolerability. The trial is still ongoing; updated/final results will be presented at the meeting as available.

S502

CPX-351 TREATMENT OF PREVIOUSLY UNTREATED OLDER AML PATIENTS WITH HIGH RISK AML MARKEDLY INCREASES THE RESPONSE RATE OVER 7+3 IN PATIENTS WITH FLT3 MUTATIONS

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Background: CPX-351 is a nano-scale liposomal formulation of 5:1 molar ratio cytarabine and daunorubicin that exhibits marked efficacy improvements among poor risk AML patients. A phase III study of CPX-351 *vs* 7+3 treatment in older high-risk (*e.g.* secondary) AML patients is nearing completion (NCT01696084).
Aims: This report correlates induction response (CR+CRi) with baseline FLT3 ITD/TKD+, NPM1+, and CEBPA+ mutations from the Phase III study.
Methods: Patients 60-75 years of age with t-AML, $_{MDS}$AML, $_{CMML}$AML and *de novo* AML with MDS cytogenetic abnormalities were eligible for this open label randomized study. 153 patients were randomized to CPX-351 (100units/m^2, days 1, 3, and 5) and 156 patients to 7+3 (cytarabine 100mg/m^2/dayx7 days, daunorubicin 60mg/m^2 days 1, 2, and 3) induction. Data for response was reviewed independently and in a blinded manner by the study's hematopathologist. Patients were assessed at baseline for FLT3, NPM1, and CEBPA mutations.
Results: Mutation status was determined in 278/309 (90%) patients for FLT3 ITD, 275/309 (89%) for FLT3 TKD, 282/309 (91%) for NPM1, and 270/309 (87%) for CEBPA. FLT3 mutations (ITD+ and/or TKD+) were found in 44 (16%) patients. Mutations occurred in 26 pt (9%) for NPM1, and 15 pt (6%) for CEBPA. The complete remission data stratified by FLT3 ITD+, FLT3 TKD+, NPM1+, and CEBPA+ status are presented in the Table 1. Compared to control, CPX-351 treatment produced higher response rates among all patients (47.7% *vs* 33.3%), all FLT3 mutated patients (68.2% *vs* 27.3%, p=0.148), FLT3 ITD+ patients (12/19 (63.2%) *vs* 3/14 (21.4%)), FLT3 TKD+ patients (5/6 (83.3%) *vs* 4/11 (36.4%)), and NPM1+ patients (92.3% *vs* 53.9%). Most FLT3 ITD+ patients were NPM1- (26/33, 78.8%) with responses in 8/15 (53.3%) CPX-351 *vs* 2/11 (18.2%) in 7+3 patients.
Summary/Conclusions: Treatment with CPX-351 appears to be associated with improved response rates in patients with common AML mutations, including those associated with poor outcomes, such as FLT3 ITD mutations. Preliminary findings from cytotoxicity assays using fresh AML patient blasts suggest that AML blasts exhibiting FLT3 ITD mutations were more sensitive to CPX-351 than FLT3 WT blasts. Moreover, increased cytotoxicity was associated with increased uptake of CPX-351 liposomes by AML blasts. While the clinical findings are based on small patient numbers, the remission data presented here, combined with the early preclinical data provide a provocative hypothesis for how CPX-351 might be advantageous for specific types of AML mutations and provide a rationale for expanded trials of CPX-351, particularly in the FLT3 ITD mutated population, which is expected to respond poorly to conventional treatment regimens.

Table 1.

	CR+CRi Rate n (%)		
Group:	**CPX-351 Arm**	**7+3 Arm**	**p-value**
All patients	**73/153 (47.7)**	**52/156 (33.3)**	
FLT3 mutated (all)	**15/22 (68.2)**	**6/22 (27.3)**	**0.0148**
FLT3 ITD+/TKD-	10/16 (62.5)	2/11 (18.2)	
FLT3 ITD+/TKD+	2/3 (66.7)	1/3 (33.3)	
FLT3 ITD-/TKD+	3/3 (100)	3/8 (37.5)	
FLT3 ITD+/NPM1-	8/15 (53.3)	2/11 (18.2)	
NPM-1mutated (all)	**12/13 (92.3)**	**7/13 (53.9)**	
NPM1+/FLT3 wt	5/6 (83.3)	6/7 (85.7)	
NPM1+/FLT3 mutated	6/6 (100)	1/5 (20.0)	
NPM1+/FLT3 unknown	1/1 (100)	0/1 (0.0)	
CEBPA mutated (all)	**3/11 (27.3)**	**1/4 (25.0)**	

S503

SGN-CD33A IN COMBINATION WITH HYPOMETHYLATING AGENTS: A NOVEL, WELL-TOLERATED REGIMEN WITH HIGH REMISSION RATE IN OLDER PATIENTS WITH AML

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Background: Many older patients with AML are treated with hypomethylating agents (HMAs) or other low-intensity therapy (Dombret 2015, Kantarjian 2012). SGN-CD33A (vadastuximab talirine; 33A) is a CD33-directed antibody conjugated to 2 molecules of a pyrrolobenzodiazepine (PBD) dimer. Upon binding, 33A is internalized and transported to the lysosomes where PBD dimer is released via proteolytic cleavage of the linker, crosslinking DNA, and leading to cell death. Enhanced cytotoxicity was observed in preclinical studies combining 33A+HMA (azacitidine or decitabine), with HMA priming upregulating CD33 and increasing incorporation of PBD dimer (Sutherland 2015).
Aims: A combination cohort in a phase 1 study (NCT01902329) was designed to evaluate the safety, tolerability, pharmacokinetics, and antileukemic activity of 33A in combination with an HMA.
Methods: Eligible patients (ECOG 0– 1) must have previously untreated CD33-positive AML, and have declined intensive therapy. 33A (10 mcg/kg) was administered via outpatient IV every 4 weeks on the last day of HMA (azacitidine or decitabine [5-day regimen], standard dosing). Patients with clinical benefit could continue treatment until relapse or unacceptable toxicity. Investigator assessment of response was per IWG criteria; CRi required either platelet count of ≥100,000/μL or neutrophils of ≥1,000/μL (Cheson 2003).
Results: Fifty-three patients (64% male) with a median age of 75 years (range 60 – 87) were treated with the combination therapy. Nineteen patients (36%) had adverse cytogenetic risk (MRC) and 30 patients (57%) had intermediate cytogenetic risk. Forty-eight patients (91%) were treatment naïve and 5 patients (9%) had received prior low-intensity therapy for MDS. At baseline, patients had a median of 45.9% BM blasts. Patients were on treatment for a median of 15.6 weeks (range 2 – 68) and 27 patients (51%) remain on study treatment; no DLTs were reported. Grade 3 or higher adverse events (AE) reported in ≥20% of patients were febrile neutropenia (47%), thrombocytopenia (42%), anemia (34%), and neutropenia (28%). Other common treatment-emergent AEs regardless of relationship to study treatment were fatigue (55%), nausea (43%), constipation (38%), decreased appetite (36%), and peripheral edema (36%). Thirty- and 60-day mortality rates were 2% and 8% respectively with no treatment-related deaths reported. Thirty-seven of the 49 efficacy evaluable patients (76%) achieved CR (17), CRi (19), or PR (1), with a median time to remission of 2 cycles (range 1 – 4); the median relapse-free survival in CR/CRi patients is currently 6.9 months (range 0+ – 11+). Thirteen of 17 patients (76%) with adverse cytogenetic risk achieved remission. Of the responding patients, 14 of 33 (42%) achieved MRD negativity by local or central flow cytometry.

Thirty-seven patients (70%) were alive at the time of this data cut with a median follow-up of 4.7 months (range 0.5-15.6). Survival trends appear similar in patients ≥75 years as in patients <75 years.

Summary/Conclusions: The combination of 33A with HMA was well tolerated and capable of inducing deep and durable remissions. In ~50 treated patients, activity with the combination appeared greater and occurred more rapidly than what is historically expected from HMA alone in this patient population. The observed low early mortality rate and the 76% overall remission rate in AML patients with poor risk factors are particularly encouraging. A global phase 3 randomized trial of 33A+HMA *vs* HMA alone is planned for 2016.

S504

PHASE 1B RESULTS OF IDASANUTLIN+CYTARABINE (ARA-C) IN ACUTE MYELOID LEUKEMIA (AML) PATIENTS (PTS)

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Background: Idasanutlin is a potent, oral MDM2 antagonist. A Phase 1b study was conducted to assess the safety and efficacy of idasanutlin+ara-C in relapsed/refractory (R/R) AML pts.

Aims: The primary endpoint was to identify the maximum tolerated dose and/or recommended dose and safety profile of idasanutlin+ara-C. Secondary endpoints included complete remission (CR) rate, complete remission with incomplete platelet (CRp) or hematologic recovery (CRi), morphologic leukemia free state (MLFS), composite complete remission (CRc, CR+CRp+CRi) rate, pharmacokinetics (PK), and exploratory analyses of predictive/prognostic biomarkers.

Methods: In the dose escalation phase, R/R AML pts or those not considered candidates for standard induction therapies were treated with escalating doses of the initial idasanutlin formulation dailyx5 days (d)+ara-C 1 g/m^{2}x6d. After identification of the recommended dose, an expansion cohort in R/R AML pts treated with ≤2 prior regimens was enrolled. A bridging arm was added to characterize the safety and PK of a spray dried powder (SDP) formulation of idasanutlin. CRs were confirmed ≥28d after initial assessment. TP53 mutation and flow cytometry of MDM2 expression in blasts were assessed as potentially predictive/prognostic by association with CRc.

Results: A total of 76 pts (1 pt was subsequently identified to have CMML and not AML) were treated with the combination therapy. Dose escalation patients (n=23) were treated with 400 mg qd (n=10), 400 mg bid (n=7), or 600 mg bid (n=6) of idasanutlin with ara-C. While not meeting protocol specified DLT criteria, diarrhea incidence and grade tracked with increasing dose and thus the recommended dose was 600 mg bid. Twenty-one pts were subsequently treated in an expansion cohort with 600 mg bid idasanutlin+ara-C. Thirty-two pts were enrolled in the bridging arm with either 300 mg bid (n=19) or 400 mg bid (n=13) of SDP idasanutlin+ara-C. Demographics and best responses are noted in the Table 1. The CR proportion was 25% (19/75 pts); the CRc proportion was 29% (22/75 pts), and the CR+CRp+CRi+MLFS proportion was 33% (25/75). Patients with CRc were followed until relapse or until 1 year (yr) from start of therapy; median duration of response is ~6.4 months (range 1.1 to 11.9 mos). Five pts remain in CR and continue in the 1 yr follow up period; 4 pts were in CR at the final visit ~1 yr from start of treatment. PK exhibited C_{max} at ~6h, $t_{1/2}$ of 1d, and dose proportionality. The SDP formulation doubled relative bioavailability. MDM2% positivity in CD45dim AML blasts by flow cytometry suggests that higher levels of MDM2 expression are associated with response (p=0.00049). TP53 mutation status was not associated but did trend with response (p=0.08) as predictive/prognostic association derives from negative predictive value of the small proportion of mutant patients. By contrast, MDM2 protein expression by flow cytometry displayed pronounced association with CRc when analyses were restricted to TP53 WT-only patients (p=0.0021).

Summary/Conclusions: Treatment with idasanutlin+ara-C resulted in durable CRs. Five of the 22 pts who achieved a CRc (23%) proceeded to transplant following therapy, demonstrating that this combination is a promising therapeutic option in R/R AML. Biomarker data suggests that identifying pts in which TP53 activity may be therapeutically enhanced may provide for improved outcomes. A Phase 3 trial is open and accruing.

Table 1.

	Dose Escalation (n=23)	Expansion (n=21)	Bridging Arm (SDP, n=32)
Median Age (range)	64 y (32-76)	64 (45-74)	61 (32-79)
Median prior regimens for AML (range)	1 (0-5)	1 (1-4)	2 (0-3)
# prior ara-C	19	19	30
# prior hypomethylator	7	3	8
Prior SCT[1] (allograft or autograft)	2	0	7
30 day mortality	4 (17%)	5 (24%)	2 (6%)
Best Response for AML to study therapy[2]	n=22	n=21	n=32
CR	6 (27%)	5 (24%)	8 (25%)
CRp	0	0	1 (3%)
CRi	0	0	2 (6%)[3]
MLFS	1 (5%)	0	2 (6%)
CRc (CR + CRp + CRi)	22/75 (29%)		
CR + CRp + CRi + MLFS[4]	25/75 (33%)		

[1] includes adoptive transfer of NK cells

[2] prior to transplant or non-study therapy, if applicable

[3] 1 pt went to transplant prior to count recovery and achieved a CR post-transplant

[4] Assessment performed ≥ day 18

S505

PHASE I/II STUDY OF VOSAROXIN AND DECITABINE IN NEWLY DIAGNOSED OLDER PATIENTS (PTS) WITH ACUTE MYELOID LEUKEMIA (AML) AND HIGH-RISK MYELODYSPLASTIC SYNDROME (MDS)

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Background: Vosaroxin, is a first-in-class anti-cancer quinolone derived (AQD) DNA topoisomerase II inhibitor currently under evaluation for the treatment of pts with AML and high-risk MDS.

Aims: To determine the overall response rate including complete response (CR)+CR without platelet recovery (CRp)+CR with insufficient hematological recovery (CRi), safety and early mortality of vosaroxin and decitabine in newly diagnosed older pts with AML.

Methods: Pts were eligible if they had untreated AML or high-risk MDS (≥10% blasts), were 60 years of age or older, had adequate performance status (ECOG ≤2) and organ function. In the phase I the first six pts received vosaroxin 90 mg/m^2 daily on Days 1 and 4 with decitabine 20 mg/m^2 daily for 5 days repeated in approximately 4 to 5 week intervals for up to 7 cycles. This dose was well tolerated in the 6 pts. However, due to occurrence of 8 episodes of grade 3/4 mucositis in the subsequent 16 pts the induction dose of vosaroxin was reduced to 70 mg/m^2. 40 subsequent pts received vosaroxin 70mg/m2 in induction..

Results: 62 pts (55 AML, 7 high-risk MDS) with a median age of 69 years (range, 60 - 78) have been enrolled. They included 23 (37%) with diploid, 22 (35%) with adverse, and 17 (27%) pts with miscellaneous cytogenetics. 18 (33%) pts with AML had antecedent hematological disorders (AHD) including 9 (16%) with MDS, 5 (9%) with MDS/MPN, and 1 (2%) with CLL. Four pts with AHD had received prior therapy including 5-azacytidine (n=1), decitabine (n=1), ruxolitinib+5-azacytidine (n=1), lenalidomide (n=1). Additionally, 10 (16%) pts had therapy-related disease. Median bone marrow blast %, median white blood cell, hemoglobin, & platelet counts were 36% (9-97), 3.6x10^9/L (0.4 - 57.0), 9.4 g/dL (6.8 - 13.1), and 33x10^9/L (7 - 333), respectively. All 62 pts were evaluable for response. The overall response rate was 74% including CR in 31 (50%), CRp in 10 (16%), and CRi in 5 (8%). Minimal residual disease by 19 color flow-cytometry was not detectable in 21 of 38 (55%) evaluable responders. The median number of cycles to response was 1 (1-4). Response by baseline characteristics is shown in Table 1.

Table 1. Response by baseline characteristics.

Parameter	Category	N	Overall response(CR, CRp, CRi)	CR
Age	60-74≥75	5012	74%75%	52%42%
Cytogenetics	Diploid	23	83%	57%
	-5/-7/other adverse	22	64%	36%
	Miscellaneous	17	76%	59%
MutationStatus	TP53IDH2	1311	69%91%	46%82%
	IDH1	9	33%	33%
	RAS	11	64%	18%

Eleven (18%) pts have proceeded to allogeneic stem cell transplant. The median follow-up is 11.0 months (2.8 - 27.5). The main therapy related grade ≥3 toxicities were mucositis in 11 (18%) and liver enzyme elevation in 8 (13%) pts.

The median overall survival (OS) for all pts is 9.8 months. Four-week and 8-week mortality for all pts were 0 and 13%, respectively. The induction dose of vosaroxin was 90 mg/m^2 in 22 pts and 70 mg/m^2 in 40 pts. The lower dose of vosaroxin was associated with reduced 8-week mortality (8% *versus* 23%), similar overall response (75% *versus* 73%), and improved OS (Figure 1).
Summary/Conclusions: Combination of vosaroxin and decitabine is effective in older pts with AML and high-risk MDS. The lower dose of vosaroxin 70 mg/m2 on days 1 and 4 is associated with improved outcomes.

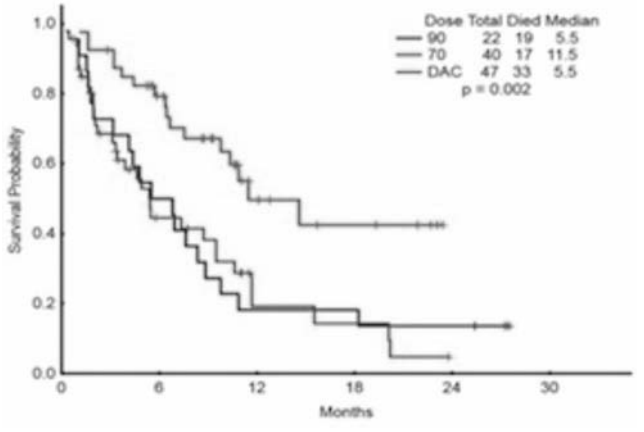

Figure 1. OS with vosaroxin and decitabine compared to historical cohort of pts treated with single agent decitabine at MDACC.

Myeloproliferative neoplasms - Biology

S506

GENETIC VARIATION AT HBS1L-MYB AND GFI1B-GTF3C5 INFLUENCES WHETHER JAK2 V617F MUTATED MPN PRESENT WITH ET OR PV

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Background: Evidence from epidemiological, familial and genetic studies indicate that common, low penetrance variants present in the general population contribute to the risk of developing MPN and also contribute to the phenotypic pleiotropy observed in these disorders. In a recent genome wide study we found that genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to JAK2-unmutated MPN. Furthermore, focused analysis of these four variants demonstrated that rs9376092 at HBS1L-MYB and the JAK2 46/1 haplotype specifically influence whether JAK2 V617F mutated cases present with PV or ET.
Aims: It is likely that variation at other genetic loci influence disease phenotype in MPN. Our aim was to identify inherited genetic factors on a genome wide basis that influence whether JAK2 V617F positive MPN patients present with PV or ET.
Methods: We undertook a two stage genome-wide association study. At stage 1,556 ET and 556 PV patients with JAK2 V617F were genotyped using Illumina Human OmniExpressExome v1.2 BeadChips. Following standard quality control, allelic chi-square tests were used to compare ET and PV cases. At stage 2, significant SNPs were genotyped in four additional JAK2 V617F-positive ET/PV cohorts from the UK (n=180), Spain (n=664), Italy (n=547) and Germany (n=74). The final effect size and significance of SNPs was determined by a fixed effects meta-analysis. The relationship with disease subtype was determined by comparison of either ET or PV cases against healthy controls (stage 1; WTCCC2 n=5200) and linear regression was used to assess the relationship with JAK2 V617F mutation burden following normalisation using Blom transformation.
Results: After quality control and excluding the p arm of chromosome 9 due to recurrent aUPD in PV and to a lesser extent in ET, 650,386 SNPs were tested in 499 ET and 505 PV cases at stage 1. Using a combination of significance, clustering of significant linked SNPs and functional evidence, 163 SNPs were tested at stage 2. Meta-analysis of ET *versus* PV cases identified one SNP, rs9399137 43kb upstream of HBS1L and 83kb upstream of MYB, that achieved genome-wide significance (p_{META}=2.91x10^{-9}) without heterogeneity between cohorts (Cochran's Q test, p=0.31). One additional SNP, rs3011271 16kb downstream of GFI1B and 22kb upstream of GTF3C5, with moderate significance for ET *versus* PV (p_{META}=3.00x10^{-5}) and without heterogeneity between cohorts (p=0.18) also reached genome-wide significance (p_{META}=2.36x10^{-8}) when compared to V617F mutation level. In comparison with healthy controls, the HBS1L-MYB SNP predisposes to ET (p=2.01x10^{-6}, OR=1.41) while the GFI1B-GTF3C5 SNP predisposes to PV (p=1.66x10^{-7}, OR=1.49). Stratifying PV cases by 9p aUPD, showed that the association with GFI1B-GTF3C5 is restricted to PV cases with 9p aUPD (p=1.95x10^{-11} in cases with aUPD; p=0.67 in cases without aUPD). We found no evidence that previously reported SNPs at NR3C1 (glucocorticoid receptor), TET2, ATM, SH2B3, ERCC2 or CHEK2 influenced the development or phenotype of JAK2 V617F-mutated MPN.
Summary/Conclusions: On a genome wide level (excluding chromosome 9p), we have found that germline variation in the intergenic regions between HBS1L and MYB exerts the strongest influence on JAK2 V617F mutated MPN phenotype. In addition, variation between GFI1B and GTF3C5 predisposes to PV and in particular to cases with high JAK2 V617F mutation burden associated with 9p aUPD. Our findings indicate a complex interplay between somatic and germline genetics in MPN, and provides pointers towards the functional basis of the phenotypic pleiotropy of these disorders.

S507

TUMOR NECROSIS FACTOR RECEPTOR 2 MAY BE REQUIRED FOR CLONAL DOMINANCE OF JAK2 V617F MUTANT OVER JAK2 WILDTYPE CELLS

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Background: The JAK2^{V617F} mutation is detected in large subsets of patients

with myeloproliferative neoplasms (MPNs). The presence of JAK2^{V617}in a hematopoietic stem (HSC) or progenitor cell confers a proliferative advantage over native JAK2 counterparts. Several inflammatory cytokines are elevated in MPN patients and in murine models of JAK2^{V617F}-driven MPN, including tumor necrosis factor alpha (TNF-α). We have previously shown that JAK2 kinase activity regulates TNF-α expression, which in turn imparts a competitive advantage on JAK2^{V617F} expressing cells over their normal counterparts (Fleischman *et al.* Blood. 2011;118(24):6392-8).

Aims: 1. To identify the cellular source of TNF-α generation in JAK2^{V617} expressing hematopoietic cells.

2. To deliniate whether TNFR1 *vs* TNFR2 mediates TNF effects in MPNs.

Methods: We used FACS/intracellular staining to measure TNF-α expression in murine and human hematopoietic cells. Blocking antibodies or shRNA knockdown were used to specifically probe the role of TNFR1 *vs* TNFR2 for MPN colony formation. Colonies were genotyped by FACS (GFP) or DNA sequencing. TNFR1/R2 null hematopoietic cells were used in competitive repopulation experiments to interogate the functional relevance of TNFR1 *vs* TNFR2 for MPN pathogenesis.

Results: TNF-α was low in unstimulated hematopoietic cells. However, when cells were treated with lipopolysaccharide (LPS), TNF-α expression was 16-fold higher in HSCs of MF patients compared to normal controls ($p<0.005$), with significant but less pronounced differences in more mature populations. In a murine model of JAK2^{V617F} driven MPN, where JAK2^{V617F} cells are identified by GFP, TNF-α expression was 3-fold higher in JAK2^{V617F}-expressing hematopoietic stem cells (HSCs; GFP$^+$) compared to GFP$^-$ controls ($p<0.005$), irrespective of LPS stimulation. We initially treated JAK2^{V617F} mice with etanercept, a soluble TNF receptor fusion protein that binds and inactivates TNF-α, but found that etanercept did not significantly restrain JAK2^{V617F}-associated WBC or hematocrit increases over a 10-week period, despite suppression of plasma TNF-α activity. As TNF-α may activate pro-apoptotic (predominantly through TNFR1) and pro-survival pathways (predominantly through TNFR2), we reasoned that global (etanercept) blockade of TNF-α may not shift the balance in favor of normal hematopoiesis. To investigate this we sorted primitive (Lin$^-$, cKit$^+$) cells from mice with JAK2^{V617F}-induced MPN by TNF receptor expression. TNFR2$^+$ cells showed significantly increased colony formation compared to TNFR1$^+$ cells, demonstrating that TNFR2 expression is associated with increased clonogenic potential. Next we treated these primitive cells with specific antibodies blocking TNFR1 or TNFR2 and assessed colony formation. Blocking TNFR2 resulted in decreased colony formation, while blocking TNFR1 increased colony formation. Analogous results were seen in CD34$^+$ cells from MF patients. Additionally in samples heterozygous for JAK2V617F, TNFR2-blocking antibodies selected JAK2 wild type over V617F mutant colonies. Preliminary results from murine TNFR1 and TNFR2 null models and shRNA knockdown of TNFR1 and TNFR2 suggest that lack of TNFR2 but not TNFR1 favors outgrowth of wild type cells.

Summary/Conclusions: Our data suggest that TNF-α generated by primitive MPN cells promotes their survival through activation of TNFR2, suggesting that selective inhibition of TNFR2 may shift the equilibrium from MPN toward normal hematopoiesis and that TNFR2 blockage may be useful to treat MF and other myeloproliferative neoplasms.

S508

THE JAK2V617F MUTATION IS A POTENTIAL TARGET FOR CANCER IMMUNE THERAPY

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Background: More than 50% of patients with Philadelphia chromosome negative chronic myeloproliferative neoplasia (MPN) harbor the *JAK2*V617F mutation. This is an acquired somatic mutation, and is found exclusively in myeloid malignancies, rendering it a cancer specific antigen and thus an attractive target for cancer immune therapy.

Aims: By this study we wish to clarify if the *JAK2*V617F mutation is recognized by the immune system, hereby proving the potential of cancer immune therapy as a whole new treatment modality for the *JAK2*V617F mutated MPNs.

Methods: We used the database www.syfpeithi.de to identify epitopes in the mutated JAK2 peptide with high affinity for HLA-A2. We chose the HLA-A2 restricted nonamer peptide JAK201 containing the V617F valine to phenylalanine substitution as the most promising candidate. A specific T cell culture was established from peripheral blood mononuclear cells (PBMCs) from an HLA-A2 positive healthy donor by stimulating with autologous dendritic cells, JAK201 peptide and cytokine as previously described[1]. Enzyme Linked ImmunoSPOT (ELISPOT), intracellular cytokine staining (ICS) and Cr51 cytotoxicity assays were used to analyze the reactivity of the specific T cells. The T cells were either stimulated with JAK201 peptide, T2 cells, HLA-A2 or HLA-A3 positive K562 cells or non-HLA K562 cells. T2 cells are unable to present endogenous peptides and thus only present exogenous peptide – in our experiments the JAK201 peptide. The HLA-A2 positive cancer cell lines UKE1 and SET2, which both harbor the *JAK2*V617F mutation, were used as target cells for the analyses of the T cells' reactivity against *JAK2*V617F mutated cancer cells.

Results: First we analyzed the capacity of the specific T cells to release TNF-α and IFN-γ upon stimulation with JAK201 peptide. Release of both cytokines upon stimulation with JAK201 peptide was confirmed by ELISPOT (Figure 1A and B) and ICS (data not shown). Next we showed, that the specific T cells were able to kill T2 cells pulsed with JAK201 peptide (data not shown). K562-A3 cells pulsed with JAK201 peptide were not killed, whereas K562-A2 cells were killed, demonstrating that killing by the specific T cells is HLA-A2 restricted (Figure 1D). Next we wanted to investigate, if stimulation of T cells with cancer cells carrying the *JAK2*V617F mutation induces cytokine release and killing of the cancer cells. We thus stimulated T cells with the HLA-A2 and *JAK2*V617F positive cancer cell line UKE1. The JAK201 specific T cells released IFN-γ (Figure 1C) and TNF-α (data not shown) in response to stimulation with UKE1. The target cells were stimulated with IFN-γ 48 hours before assaying to increase their antigen presentation, and the T cells showed an enhanced release of IFN-γ upon stimulation with IFN-γ treated UKE1 cells (Figure 1C). Furthermore, T cells were able to kill UKE1 cells in a cytotoxicity assay, and in line with the above, we demonstrated an increased killing of the UKE1 cells after treatment with IFN-γ (Figure 1E). Initially the HLA-A2 and *JAK2*V617F positive cancer cell line SET2 was not killed in a cytotoxicity assay, but after stimulation with IFN-γ, the SET2 cells were readily killed by the JAK201 specific T cells (data not shown). Finally, transfection with *JAK2*V617F siRNA into UKE1 cells abrogated T cell mediated killing, whereas mock transfected UKE1 cells were killed by the T cells (Figure 1F). By this experiment we demonstrated, that killing of target cells is dependent upon the presence of the *JAK2*V617F mutation.

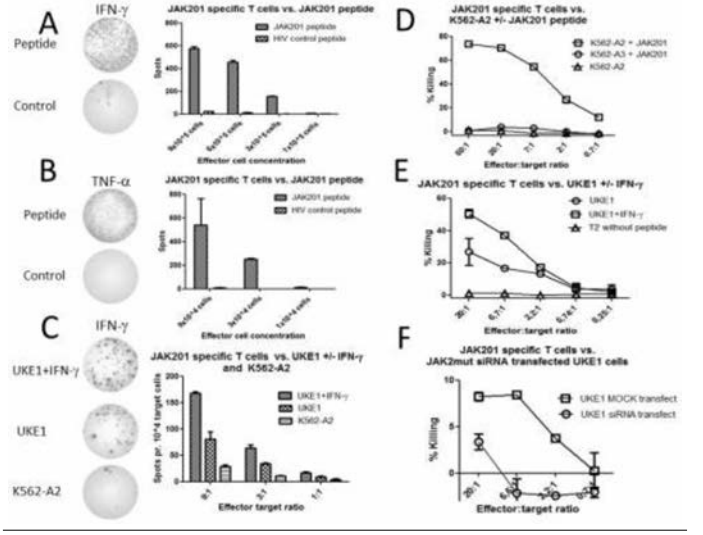

Figure 1.

Summary/Conclusions: By establishing a T cell culture specific for the *JAK2V*617F mutation we have shown that the immune system is able to effectively target cells carrying the *JAK2*V617F mutation. Thus cancer immune therapy in the form of adoptive T cell therapy and/or vaccination could prove to be new treatment modalities for MPN in the future.

Reference

1. Munir S *et al. Cancer Res.* 2013;73(6):1764-76.

S509

CALRETICULIN MUTANT PROTEINS INDUCE MEGAKARYOCYTIC SIGNALING TO TRANSFORM HEMATOPOIETIC CELLS AND UNDERGO ACCELERATED DEGRADATION AND GOLGI-MEDIATED SECRETION

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Background: Somatic calreticulin (CALR), Janus kinase 2 (JAK2) V617F, and thrombopoietin receptor (MPL) mutations are essentially mutually exclusive in myeloproliferative neoplasms (MPN), suggesting that they activate common oncogenic pathways. CALR mutants are confined to MPNs with aberrant megakaryopoiesis, such as essential thrombocythemia and myelofibrosis. Recent data showed that MPL is essential for CALR mutant-driven MPN. However, the characteristics of CALR mutants and their mechanisms of action are still poorly understood.

Aims: Our study was aimed at the elucidation of MPL-dependent and -independent mechanisms of CALR frameshift mutants to drive megakaryocytic dif-

ferentiation and oncogenic transformation. Furthermore, we investigated the cause of low cellular protein abundance of the CALR mutants.
Methods: The murine myeloid cell line 32D and human HL60 cells stably expressing the most frequent CALR mutants (del52 and ins5) were generated to analyze growth factor-independent growth, as the first steps of cellular transformation, in the presence and absence of MPL expression. Additionally, using brefeldin A, MG132, and tunicamycin treatment as well as generation of further mutants (*i.e.* YFP-fusion proteins), CALR mutant protein degradation and secretion were examined.
Results: In this report, we demonstrate that the most frequent CALR mutants, type 1 (del52) and type 2 (ins5), increase critical megakaryocytic transcription factors such as NF-E2 and GATA1. Interestingly, this occurred in an MPL-independent but AKT-dependent fashion, leading to the upregulation of *Mpl* and CD41 expression. These effects were also confirmed by the observation of spontaneous outgrowth of CALR mutant-transduced 32D cells, showing increased NF-E2, CD41, and *Mpl* expression as well as constitutive STAT5 activation and response to JAK inhibitor treatment. Interestingly, we found high cellular levels of mutated CALR and loss of downstream signaling after blockage of the secretory pathway and protein glycosylation. Hence, we demonstrate that low CALR mutant protein abundance is a result of accelerated secretion as well as ubiquitin- and proteasome-independent degradation. CALR mutant degradation was attenuated by MPL expression.
Summary/Conclusions: Together, our data illustrate how CALR mutants may initiate abnormal megakaryopoiesis, even in the absence of MPL, by a mechanism that most likely involves activated AKT. In addition, our findings show enhanced secretion, MPL-driven protein stabilization, and importance of Golgi signaling of the MPL receptor for CALR-mutant mediated transformation.

S510

EXPRESSION, REGULATION, AND FUNCTIONAL ROLE OF HERMES ADHESION RECEPTOR CD44 IN NEOPLASTIC MAST CELLS IN SYSTEMIC MASTOCYTOSIS

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Background: The Hermes receptor CD44 is a multifunctional adhesion molecule that plays an essential role in the homing and expansion of neoplastic stem- and progenitor cells in various myeloid malignancies. Although mast cells (MC) are known to express CD44, little is known about the regulation and function of this receptor on neoplastic stem cells and MC in patients with systemic mastocytosis (SM).
Aims: The aim of this study was to define the expression and function of CD44 on neoplastic MC.
Methods: CD44 expression on primary neoplastic MC and various human MC lines was analyzed by immunocytochemistry and flow cytometry.
Results: CD34+/CD38- stem cells, CD34+/CD38+ progenitor cells, and KIT+/CD34- MC invariably expressed CD44 in all patients with indolent SM (ISM, 12/12), SM with associated hematologic non-MC-disease (SM-AHNMD, 4/4), aggressive SM (ASM, 3/3), and MC leukemia (MCL, 6/6). In addition, all human MC lines examined, including HMC-1.1, HMC-1.2, ROSA^{KITwt}, and ROSA^{KITD816V}, were found to express cytoplasmic CD44 and cell surface CD44. We next examined the regulation of expression of CD44 in neoplastic MC. Incubation with the MEK inhibitor RDEA119 (0.1-5 µM) or the STAT5 blocker pimozide (2.5-10 µM) for 48 hours resulted in a dose-dependent and significant downregulation of surface expression of CD44 compared to control medium (100%) in all MC lines examined (RDEA119, 2.5 µM: 71±9% surface staining intensity in HMC-1.1; 82±3% in HMC-1.2, 33±13% in ROSA^{KITwt}, and 31±3% in ROSA^{KITD816V}cells; pimozide, 7.5 µM: 59±7% surface staining intensity in HMC-1.1; 68±3% in HMC-1.2, 62±16% in ROSA^{KITwt}, and 80±3% in ROSA^{KITD816V} cells, p<0.05). In contrast, incubation with the demethylating agents decitabine (0.1-5 µM) or azacitidine (0.1-5 µM) for 96 hours resulted in a dose-dependent and significant upregulation of CD44 expression compared to control medium (100%) in all MC lines examined (decitabine, 5 µM; 210±50% surface staining intensity in HMC-1.1; 282±78% in HMC-1.2, 236±56% in ROSA^{KITwt}, and 198±55% in ROSA^{KITD816V} cells; azacytidine, 5 µM: 379±103% surface staining intensity in HMC-1.1; 429±105% in HMC-1.2, 412±135% in ROSA^{KITwt}, and 292±136% in ROSA^{KITD816V} cells; p<0.05). We were also able to detect soluble CD44 in the sera of patients with ISM, SM-AHNMD, ASM, and MCL as well as in the supernatants of neoplastic MC lines. In order to define a functional role for CD44 on MC, an *in vivo* xenotransplantation model using severe combined immunodeficient (SCID) mice, HMC-1.2 cells, and shRNA against CD44, were employed. In this model, the shRNA-mediated knock-down of CD44 in HMC-1.2 cells was found to lead to reduced MC expansion, reduced tumor formation, delayed ulceration, and prolonged survival compared to cells transduced with control shRNA (median survival in the CD44 shRNA group: 110 days *vs* 97 days in the control group; p<0.05). In these experiments, the formation of lung metastasis, quantified by human Alu-sequence-specific qPCR, was found to be particularly decreased (15-fold) in the CD44 knock-down group compared to controls.
Summary/Conclusions: CD44 is a relevant MC homing molecule that is expressed in neoplastic MC as well as in neoplastic stem- and progenitor cells in advanced SM. Our data also suggest that CD44 may serve as an interesting new target of therapy in patients with advanced SM.

Gene therapy, cellular immunotherapy and vaccination

S511

TUMOR-SPECIFIC GLYCOSYLATED CD43 IS A NOVEL AND HIGHLY SPECIFIC TARGET FOR ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME

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Background: Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are high-risk diseases with a poor prognosis. Even with intensive treatment regimens less than 50% of patients can be cured, and for the majority of patients - those over 65 years of age and/or patients with comorbidities - such intensive regimens are not feasible. Novel therapeutic approaches such as immunotherapy directed against a highly specific tumor target are highly needed.
Aims: The aim of our study was to identify novel therapeutic antibodies that are highly specific for AML and to discover novel tumor-specific antigens, widely expressed on AML and MDS but not on healthy hematopoietic and non-hematopoietic cells.
Methods: For this we made use of the oldest human tumor immunology model with proven efficacy available: an allogeneic HSCT patient with a potent graft *versus* AML allo-immune response. From this patient we isolated CD27+ IgG+ memory B lymphocytes and transduced these cells with Bcl-6 and Bcl-xL, thereby generating pre-plasmablast B cell clones that produce abundant antibodies. Supernatants of these B cell clones were used to screen for binding to surface antigens on the AML cell line THP-1.
Results: We identified an IgG1 antibody, AT14-013, that specifically interacted with AML cell lines THP-1, MOLM-13, SH-2 and others, and with leukemic blasts isolated from newly diagnosed AML and MDS patients from our clinic. AT14-013 did not interact with healthy hematopoietic and non-hematopoietic cells. This antibody was of donor origin and was antigen experienced as it contained 26/11 somatic hypermutations in the heavy and light chains, respectively. Target identification using mass spectrometry analysis and epitope mapping strategies with FLAG-tagged truncated variants of CD43 expressed by THP-1 that we created revealed CD43 as the target. CD43 is expressed by all hematopoietic cells, but AT14-013 targeted a specific, sialylated epitope on CD43 that is uniquely and widely expressed on all types of AML, as illustrated by its reactivity with blasts of each of 48 randomly selected AML and MDS patients in our clinic. AT14-013 induced antibody-dependent cell-mediated cytotoxicity and complement dependent cytotoxicity of AML cell lines and primary blasts.
Summary/Conclusions: We have identified onco-sialylated CD43 ($CD43^{os}$) as a novel tumor-specific target that is widely expressed on AML and MDS blasts. Antibodies against this target have high potential as therapeutic antibodies, either as a naked antibody or manufactured into an antibody-drug conjugate, bispecific T cell engager or CAR (chimeric antigen receptor) T cell.

S512

THE HIGHLY CLINICAL EFFICACY AND SAFETY OF THE AUTOLOGOUS ANTI-CD19 CHIMERIC ANTIGEN RECEPTOR T CELLS IMMUNOTHERAPY FOR 26 CASES WITH HEAVILY TREATED REFRACTORY B ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: the patients with refractory B-ALL are almost incurable by chemotherapy or allo-HSCT. Many data has demonstrated that anti-19-CART cell immunotherapy can result in CR and bridge to allo-HSCT for these patients.
Aims: to evaluate the clinical efficacy and safety of the second generation of anti-CD19-CART cells immunotherapy.
Methods: leukapheresis products from the patients themselves were cultured in the serum free medium containing antibodies to CD3, CD28, and IL2, IL7, IL15, IL21, transduced with the lentivirus encoding anti-CD19-CD3ξ-4-1BB CAR (Figure 1) for 7 days. After leukapheresis, patients received chemotherapy containing fludarabine 30mg/ m^2 per day IV and cyclophosphamide 250mg/ m^2 per day IV for 3 days except one patient who had persistent cytopenia. On day 7 or/and day 8, the cultured CART cells were infused. Between Jun. 31 2015 to Feb. 20 2016, total 26 cases with chemotherapy refractory B-ALL were treated with CART. After infusion of CART, the patients were followed until die, or relapse, or at least 15 days. In the included cases, the median age was 9 years old (2-59 years old), the median observation period was 100 days (19-204 days), and the median prior chemotherapy period was 14 months (1-36 months). 19/26 cases were hematological relapse, with 10/19 complicated extramedullary relapse, 7/26 cases remained minimal residual disease detected by flowcytometry (FCM-MRD) over 3 months although by alternative chemotherapy. Before CART therapy, all patients relapsed during chemotherapy or hadn't responded to 1 or more cycles of chemotherapy after relapsed except one patient suffered from severe infective pneumonia because she carried inherited immune related gene deficiency (LYST c.6031A>G/p.I2011V gene mutation) and was intolerated to chemotherapy. Before CART therapy, the median percentage of blasts in BM was 67% (11.5-96%) in the cases with hematological relapse, the FCM-MRD were 0.01-1.53% in the MRD+ group. The median infused total CD19- CAR T cells was $10x10^4$/kg (0.5-$140x10^4$/kg), the median cells transfection efficiency is 19% (1.2- 53.6%), the median cells viability is 85% (43-99.4%).
Results: On day 16 after CART infusion, 15/19 hematological relapse cases achieved CR or CRi (83.3%), 14/15 cases achieved CMR (FCM-MRD negative). 6/7(85.71%) FCM-MRD positive cases achieved CMR (FCM-MRD negative). During the first month, 1 case died from CRS, 2 cases died from leukemia progression. 2/16 who have achieved CR and been follow up for ≥60 days relapsed again, 1 is CD19- relapse. 6/6 cases who have achieved CR and bridged to allo-HSCT remain CCMR to now with median follow up period 40 days (24-106 days) after allo-HSCT. The median appearance of CRS was at day 6 (day 1-11), the median CRS grade was 1 (0-5). All MRD+ patients before CART therapy only developed ≤1 grade of CRS. The IL6 and INFγ elevates in PB and CSF in all detected patients (n=4).

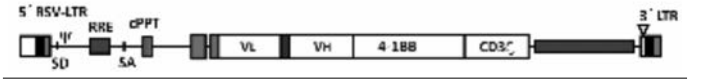

Figure 1.

Summary/Conclusions: Our anti-CD19-CAR T cells immunotherapy can result in high CR/CRi /CMR rate (including extramedullary leukemia) and low TRM for the heavily treated refractory B-ALL, which provide an opportunity to cure these advanced patients by allo-HSCT. The infused CART number in our patients is lower than many reports, which can dramatically reduce the cost of the therapy. The major complication is CRS. CRS grade is associated with the number of malignant B cells in PB. CD19-relapse has been observed.

S513

REGENERATION OF WT1 SPECIFIC CTLS UTILIZING IPSC TECHNOLOGY

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Background: Current cancer immunotherapies mainly aim to activate and expand tumor antigen specific cytotoxic T lymphocytes (CTLs) *in vitro* or *in vivo*, but these methods are not so successuful because of the difficulty in preparing sufficient number of CTLs.
Aims: For successful cancer immunotherapy, we are trying to expand tumor antigen specific cytotoxic T lymphocytes (CTLs) by utilizing the iPS cell technology. When iPSCs are produced from antigen specific T cells, the rearranged configurations of T cell receptor (TCR) genes are inherited to the iPSCs, and when T cells are regenerated from such iPSCs, all of them should come to express the same TCR as the original one. Therefore it will become possible to obtain *de novo* generated tumor specific CTLs almost unlimitedly. In line with this concept, we have succeeded in regenerating MART-1 specific T cells (Vizcardo *et al.,* Cell Stem Cell, 2013).
Methods: We firstly established iPSCs from CTLs specific for WT1, expanded from healthy volunteers. CD4/8 DP cells were generated by culturing these T-iPSCs on Op9/DLL1 cells. By stimulating these DP cells by agonist peptide or anti CD3 Ab, CD8 T cells expressing CD8 alpha-beta heterodimers were formed. They can be expanded more than ten thousand fold by repeated TCR stimulation.
Results: Regenerated CD8 T cells exhibited very high antigen specific killing activity comparable to the original CTLs and were able to kill some leukemia cell lines and primaly leukemia cells which express endogenous WT1 protein and HLA A2402. In *in vivo* leukemia treatment model regenerated CD8 T cells elongated the survival of treated mice.
Summary/Conclusions: We have succeded in regenerating CD8 T cells with high antigen specific cytotoxic activity. This method could bring about a breakthrough in cancer immuno-therapy.

S514

MESENCHYMAL STROMAL CELLS GENERATED FROM POOLED MONONUCLEAR CELLS OF MULTIPLE BONE MARROW DONORS AS A RESCUE THERAPY FOR SEVERE STEROID-REFRACTORY GRAFT *VERSUS* HOST DISEASE: A MULTICENTER SURVEY

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Background: Mesenchymal stromal cells (MSCs) represent a heterogeneous cell population concerning their proliferative, differentiation and allosuppressive potential.
Aims: To circumvent donor-to-donor heterogeneity which may lead to inconsistent results after treatment of acute graft-*versus*-host disease (aGvHD) with mesenchymal stromal cells (MSCs) generated from a single or several individual donors, we developed a novel approach by generating MSCs from pooled bone marrow mononuclear cells (BM-MNCs) of eight healthy "third-party" donors.
Methods: BM-MNCs of 8 healthy "third-party" donors were isolated and frozen separately in cryobags. After thawing, BM-MNCs of 8 donors were pooled and cultured for 14 days in DMEM+5% platelet lysate (PL). Generated MSCs were frozen in 209 cryovials à 1.5x10e6 MSCs, representing the MSC-bank. Several vials were expanded for 12-14 days in DMEM+10% PL till the end of passage 2 and frozen as MSC-end products (MEPs). These products were evaluated for proliferative, differentiation and allosuppressive potential as well as genomic stability. Eighty-one MEPs generated from aliquots of the MSC bank were administered on a compassionate-use basis after approval of the regulatory authorities to 26 patients with steroid-refractory acute GvHD at a target dose of 1-2×10^6 MSCs/kg BW in 7 transplantation centers.
Results: MEPs exhibited typical MSC phenotype, trilineage differentiation potential and replicative senescence after 13 passages, as determined by increased expression of senescence-related markers such as p21 (CDKN1A) and p16 (CDKN2A). Chromosomal analysis of MEPs demonstrated a normal karyotype, whereas FISH analysis demonstrated a normal diploid pattern, indicating their genomic stability. Importantly, MEPs exerted a significantly higher allosuppressive potential than the mean allosuppressive potential of MSCs generated from the same donors individually. Administration of 81 MEPs to 26 patients with severe steroid-resistant aGvHD in 7 stem cell transplant centers who were refractory to many lines of treatment, induced a 77% overall response at the primary endpoint (day 28). Remarkably, although the cohort of patients was highly challenging (92% grade III/IV and only 8% grade II GvHD), after treatment with MEPs the overall survival rate at 2 years follow-up was 71±11% for the entire patient cohort, compared to 51.4±9.0% in GvHD clinical studies, in which MSCs were derived from single donors.
Summary/Conclusions: To our knowledge this is the first serum-free MSC bank generated from pooled BM-MNCs of multiple donors as a source for bulk production of clinical-grade MSCs with a predictable potency. Importantly, clinical data presented in this study demonstrated the *in vivo* safety and efficacy of MEPs, which may provide a novel therapeutic tool for the effective treatment of severe aGVHD.

S515

GENERATION OF CHIMERIC ANTIGEN RECEPTOR - MODIFIED MEMORY STEM CELL CD8+ T LYMPHOCYTES FROM NAIVE PRECURSORS BY MODULATION OF WNT/BETA-CATENIN PATHWAY OR INHIBITION OF AKT-SIGNALING

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Background: Adoptive cellular therapy (ACT) of chimeric antigen receptor (CAR)-reprogrammed T cells has advanced as a personalized and effective immunotherapy for leukemia and solid tumors. However, ACT, especially to solid tumors is often hampered by limited T cell engraftment and limited capability of terminally differentiated, high avidity effector T cells (T_{EFF}) to establish sustained antitumor immunity. Recently, long-living stem cell memory T cells (T_{SCM}) with an enhanced capacity for self-renewal and plasticity to differentiate into central memory (T_{CM}), effector memory (T_{EM}) and T_{EFF} could be shown to elicit potent antitumor responses, prolonged survival and memory. Moreover, modulating the Wnt/ß-catenin or PI3K-Akt-mTOR signaling pathways in T cells using inhibitors of glycogen-sythase-kinase-3β (TWS119) or Akt (inhibitor VIII) have emerged as promising approaches to block $CD8^+$ effector T cell differentiation and to facilitate the *in vitro* generation of T_{SCM} and T_{CM}.
Aims: In the present proof of concept study, we therefore investigated the generation of CD19 CAR expressing $CD8^+$ T_{SCM} from naive $CD8^+$ T lymphocytes by modulating T-cell differentiation using TWS119 or Akt inhibitor VIII to be used for ACT.
Methods: Naive $CD8^+CD45RA^+$ T cells isolated from PBMC by MACS® were polyclonally stimulated with CD3/CD28 Dynabeads in the presence of various cytokines and retrovirally transduced with a second generation CD19 CAR three days after activation. To promote a $T_{SCM/CM}$ phenotype transduced cells were either polyclonally restimulated or co-cultured with $CD19^+$ B-ALL (NALM16) together with TWS119 or Akt inhibitor VIII. CD19 CAR expression was determined by flow cytometry. IFN-γ ELISPOT and ^{51}chromium-release-cytotoxicity assays were used to analyze effector functions of CD19 CAR expressing T_{SCM} and T_{CM}. Adoptive transfer studies of redirected T cells into NALM-16 engrafted NSG mice were performed to evaluate antileukemic responses *in vivo*.
Results: Upon repetitive restimulation and TWS119/Akt inhibitor VIII treatment we obtained strong expansion of T cells with a $T_{SCM/CM}$ phenotype. In contrast, this effect was less pronounced by naive T cells cultured in the sole presence of interleukin (IL)-12 followed by IL-2, IL-7, IL-15, and IL-21, confirming that both Wnt/ß-catenin and PI3K-Akt-mTOR pathways play a key role in T cell differentiation. In addition, $CD8^+CD45RA^+CD45RO^-CD95^+CD62L^+CCR7^+T_{SCM/CM}$ showed high expression levels of CD19 CAR, elicited strong IFN-γ release and cytolytic activity to $CD19^+$ NALM-16 cells when compared to mock transduced controls. This effect was also seen in CD19 CAR positive total $CD8^+$T cells although less pronounced. First studies to evaluate the therapeutic efficacy of CD19-CAR redirected $T_{SCM/CM}$ revealed that upon adoptive transfer of 1-5x10^6T cells into NSG mice engrafted with NALM-16 B-ALL significant regression of leukemia could be observed in spleen and bone marrow. Again, antileukemic responses were also seen with CD19 CAR transduced T_{EM} and T_{EFF} but $T_{SCM/CM}$ appeared to be more effective when applied at lower numbers (1 x10^6 cells/mouse). Further studies are in progress to elucidate the functional properties of CD19 CAR $T_{SCM/CM}$ in more detail.
Summary/Conclusions: In conclusion, these proof of concept studies demonstrate that *ex vivo* generated $T_{SCM/CM}$ redirected to acute leukemia by retroviral transfer of optimized leukemia- or tumor-reactive CARs may be a promising approach to improve ACT.

Platelet disorders 1

S516

DISREGULATED MEGAKARYOCYTE MIGRATION AND MATURATION IS POSSIBLY DUE TO IMPAIRED BONE MARROW SYMPATHETIC NERVETHROUGH NESTIN+ MESENCHYMAL STEM CELL IN CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: Impaired megakaryocyte maturation and reduced platelet production is a most important mechanism responsible for immune thrombocytopenia (ITP).Megakaryocyte (MK) maturation in the bone marrow microenvironment (BMME) is accompanied by increased expression of CXCR4 andits migration from the endosteal niche to the vascularniche, where MKs intrude proplatelets into the bone marrow (BM)sinus to release platelets.Thrombopoietin(TPO) is the major physiological regulator of the proliferation and differentiation of MKs,whereas MK migration is regulated by sympathetic nerves and the chemokine CXCL12, which is mainly produced by nestin+ mesenchymal stem cells (MSCs) in the BM.There is no information regarding MK migration or BMME in ITP.
Aims: We sought to investigate the migration and maturation status of MKsin chronic ITP, to measure the sympathetic nervous response, which regulates the migration and maturation of MKs, and to assess the variationin nestin+ MSCs, which produce the potent chemokine CXCL12 under the regulation and protection of sympathetic nerves.
Methods: BM samples were obtained from chronic ITP patients and healthy haematopoietic stem cell donors.The distribution of MKs, sympathetic nerve fibres, Schwann cells and nestin+ MSCs in BMME was analysed by immunohistochemistry and/or immunofluorescence, and quantitative analyses were conducted using confocal microscopy. BM nestin+ MSCs were sorted by flowcytometry. The quantity and apoptosis of nestin+ MSCs and the surface markers CXCR4 and β3-adrenergic receptor (adrb3)on nestin+ MSCs and CXCL12+ nestin+ MSCs were further analysed by flowcytometry.The expression levels of CXCL12 and adrb3 in nestin+ MSCs wereanalysed by real-time quantitative PCR.
Results: The percentage of CD41+ MKs adjacent to the CD34+ BM sinus and CXCR4+CD41+MKs in the BM were both strikingly reduced in chronic ITP patients compared with normal controls.The concentration of CXCL12 was also significantly lower in the BM of ITP patients,and the percentage of CD41+ MKs in the vascular niche and of CXCR4+CD41+MKs correlated with the concentration of BMCXCL12. The percentage of nestin+ MSCs among CD45-CD31-CD235a- stromal cellsin normal controls was 3.8 times higher than that in chronic ITP patients.Consistently, the percentage of live nestin+ MSCs in normal controls was also significantly increased, whereas apoptosis of nestin+ MSCs in ITP patients was significantly increased compared with normal controls. The proportion of CXCL12+nestin+ MSCs in normal controls was much higher than that in ITP patients, which was correlated with the concentration of CXCL12 in the BM.Sympathetic nerve fibres and Schwann cells adjacent todistinctive nestin+ MSCs and vasculature weremarkedlyreduced in the BMof ITP patients, andthe neurotransmitternorepinephrine and adrb3 in nestin+ MSCs were both significantly reduced in ITP patients.
Summary/Conclusions: This isthe first report to describe BMME and megakaryocyte migration in chronic ITP. Our research suggests that reduced activity of BM sympathetic nervesis associated with impaired MK migration and maturation, resulting in reduced plateletproduction. With reducedBM sympathetic nerves innervation, nestin+ MSCs arefunctionally compromised and reduced due to increased apoptosis, enhancing the dysregulatedmigration and maturation of MKsvia diminished CXCL12 signalling through CXCR4 on MKs. Protection of sympathetic nerves that prevent MSCapoptosis may provide a potentially powerful strategy to promotethrombopoiesis.

S517

FINAL SAFETY AND EFFICACY RESULTS FROM THE EXTEND STUDY: TREATMENT WITH ELTROMBOPAG (EPAG) IN ADULTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP)

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Background: EPAG increased platelets and reduced bleeding in 6-week and 6-month placebo-controlled trials in previously treated cITP patients. Adult patients with cITP initially enrolled in 4 EPAG studies could continue treatment in the open-label extension study, EXTEND.
Aims: To present the final long-term safety and efficacy results from EXTEND (Jun 2006 to Jul 2015).
Methods: EPAG was started at 50 mg and titrated to 25-75 mg/day or less often, based on platelet counts. Maintenance dosing continued after minimization of concomitant ITP medication and optimization of EPAG dosing. Patients who received 2 years of EPAG and transitioned off due to commercial availability of EPAG were considered to have completed EXTEND, whether or not they continued treatment with commercial EPAG.
Results: Of 302 patients enrolled, 67% were females and 38% were splenectomized; 45% (n=135) completed and 55% (n=167) withdrew. The most common reasons for withdrawal were adverse events (AEs; 14%), patient decision (13%), lack of efficacy (11%), and other (13%). The overall median duration of exposure was 2.4 years (range, 2 days to 8.8 years) and mean average daily dose was 50.2 (range, 1-75) mg/day. Median platelet counts increased to 50 Gi/L by Week 2. Overall, 86% (259/302) of patients achieved platelets 50 Gi/L in the absence of rescue therapy and 61% achieved platelets 50 Gi/L for 50% of on-treatment assessments; 126/248 (51%) patients maintained continuous platelet counts ≥50 Gi/L for at least 31 weeks (Figure 1). Incidence of bleeding symptoms (WHO grades 1-4) decreased from baseline (57%; 171/302) to 1 year (16%; 13/80). Proportionately more patients with platelet counts <10 Gi/L had grades 2-4 bleeding compared with those with higher platelet counts (59% *vs* ≤40%). Of 101 patients receiving concomitant ITP treatment at baseline, 34 stopped at least one ITP medication, and 39 had a sustained reduction or permanently stopped at least one ITP medication taken at baseline. The most frequently discontinued/reduced ITP medications were corticosteroids (n=34), danazol (n=5), azathioprine (n=4), and other (n=1). AEs occurred in 277 (92%) patients. Serious AEs (SAEs) occurred in 96 (32%) patients, and 24 (8%) patients had SAEs considered possibly drug-related. Drug-related SAEs occurring in 2 patients were cataracts (n=8), increased alanine aminotransferase (ALT) (n=4), deep vein thrombosis (n=2), increased aspartate aminotransferase (n=2), increased bilirubin (n=2), myocardial infarction (n=2), and pneumonia (n=2). AEs leading to withdrawal occurred in 41 (14%) patients. 28 (9%) patients experienced SAEs. The most frequent AEs leading to withdrawal were increased ALT (n=5), increased bilirubin (n=4), cataracts (n=4), and DVT (n=3). Nineteen patients (6%) reported thromboembolic events (TEEs), and 37 reported hepatobiliary laboratory abnormalities (HBLAs). Ocular-related events started on-therapy occurred in 80 (26%) patients; the most frequently occurring events were cataract (n=28; 9%) and conjunctivitis (n=12; 4%). At baseline, 192 patients had at least one cataract risk factor.

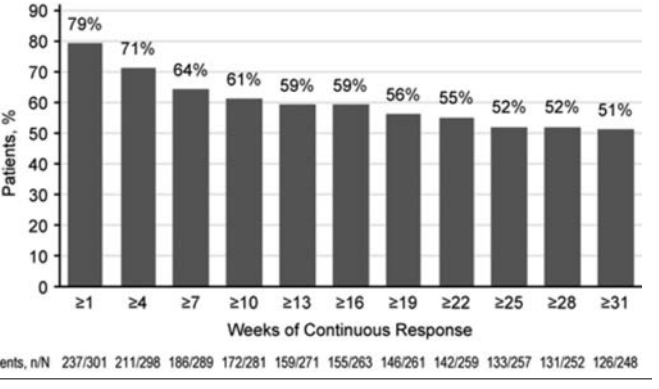

Figure 1. Maximum continuous weeks of maintaining platelets ≥50 Gi/L without rescue therapy.

Summary/Conclusions: Sustained platelet increases and reduced bleeding symptoms were observed in EPAG-treated patients with cITP throughout the study. Concomitant ITP medications were reduced without requiring rescue medications. EPAG was well tolerated with exposures 6 years. Monitoring of HBLA and cataracts is appropriate, even with long-term treatment. *Funding:* Study (NCT00351468) is/remains sponsored by GlaxoSmithKline; EPAG is an asset of Novartis AG as of March 2, 2015.

S518

IDENTIFYING THE GENETIC BASIS OF RARE BLEEDING AND PLATELET DISORDERS USING SYSTEMATIC PHENOTYPING AND GENOME SEQUENCING

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Background: The majority of rare bleeding, thrombotic and platelet disorders (BPDs) do not have an identified genetic basis. Whilst whole genomic sequencing is now an affordable approach, small family sizes, variable penetrance and phenotypic variability are barriers to identifying the responsible genetic variants.

We have used a systematic phenotyping approach combined with novel clustering analyses to identify implicated genes and candidate causal mutations called by next generation sequencing (NGS).
Aims: Detection of novel genetic causes of BPDs.
Methods: A total of 848/1247 index cases and 78/87 affected relatives have been sequenced/phenotyped. The Human Phenotype Ontology (HPO) has been expanded to better capture clinical and laboratory data. Genes known to harbour variants responsible for BPDs have been screened. New algorithms have been developed to identify patients with similar phenotypes and a potentially shared genetic basis of disease.
Results: In 115 cases a definitive or likely genetic explanation has been identified and in 13 a partial genetic explanation. Four genes harbouring variants responsible for platelet abnormalities including *DIAPH1*, *SRC* and *TRPM7* have been identified. Variants responsible for atypical presentations of previously known syndromes, including *MYH9*-related disease and Hermansky-Pudalk syndrome, have also been identified. Finally, we have shown that large numbers of cases are explained by variants in recently reported genes, *e.g.* 27 by variants in *ACTN1*, eight by variants in the 5'UTR of *ANKRD26* and two by variants in *STIM1*. In total a long list of 45 genes have been identified as harbouring variants responsible or possibly responsible for BPDs in our collection and many are under further investigation.
Summary/Conclusions: A systematic method of detailed phenotyping and NGS has formed the basis of screening and clustering analysis. We have identified genes harbouring variants responsible for BPDs and have confirmed previous findings. Syndromic phenotypes have been better defined and a large number of candidate variants remain to be explored. Having used WGS as the main method of DNA sequencing will now allow these methods to be extended to regulatory regions of the genome.

S519

LONG-TERM HEMATOLOGIC, BIOMARKER, AND BONE RESPONSE TO ORAL ELIGLUSTAT IN PATIENTS WITH GAUCHER DISEASE TYPE 1: RESULTS FROM A PHASE 2 AND TWO PHASE 3 TRIALS

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Background: Gaucher disease is caused by deficient activity of lysosomal acid β-glucosidase, leading to glucosylceramide accumulation in macrophages and resultant hepatosplenomegaly, pancytopenia, and skeletal disease. Despite well-characterized symptoms and treatment availability, disease awareness is low. Diagnostic delays are common and can result in irreversible disease manifestations. As thrombocytopenia, anemia, and splenomegaly are common in Gaucher patients, hematologists often identify, evaluate, and manage the disease. Eliglustat, a substrate reduction therapy, is a first-line oral treatment for adults with Gaucher disease type 1 (GD1) who have a compatible CYP2D6 metabolizer phenotype (>90% of patients).
Aims: Summarize hematologic, bone, and Gaucher biomarker outcomes (chitotriosidase and others) in 3 clinical trials of eliglustat in adults with GD1.
Methods: Data were evaluated from 3 Sanofi Genzyme-sponsored trials: Phase 2 in treatment-naïve patients (NCT00358150, N=26); ENGAGE, a randomized, placebo-controlled Phase 3 trial in treatment-naïve patients (NCT00891202, N=40); and ENCORE, a Phase 3 imiglucerase-controlled trial in patients previously stabilized on ≥3 years of enzyme replacement therapy (NCT00943111, N=159).
Results: Primary endpoints were met in all 3 trials (Lukina *Blood* 2010; Mistry *JAMA* 2015; *Cox Lancet* 2015). In the Phase 2 study after 4 years, mean hemoglobin increased by 2.3±1.5 g/dL relative to baseline (BL) (BL=11.3±1.5 g/dL), mean platelet count increased by 95% (BL=69±21x10^9/L), spleen and liver volumes decreased, and mean lumbar spine bone mineral density went from the osteopenic to the normal range with corresponding reductions in biomarkers. In ENGAGE after 18 months, mean hemoglobin levels, platelet counts and spleen and liver volumes improved in placebo patients switched to eliglustat and continued to improve in patients continuing on eliglustat, with corresponding improvements in mean bone marrow burden (BMB) score and biomarkers. In former placebo patients, mean hemoglobin increased by 0.79±0.82 g/dL (BL=12.2±2.0 g/dL), mean platelet count increased by 40%±37 (BL=71.5±25.2x10^9/L) and mean BMB score decreased by 0.9 after 9 months on eliglustat. In patients on eliglustat for 18 months, mean hemoglobin increased by 1.02±0.84 g/dL (BL=12.1±1.8 g/dL), mean platelet count increased by 58%±41 (BL=75±14x10^9/L), and mean BMB score decreased by 2.2. In ENCORE after 2 years, stability with respect to hemoglobin, platelets, spleen, liver, bone, and biomarkers was maintained in former imiglucerase patients switching to eliglustat and in patients continuing on eliglustat. In former imiglucerase patients, 100% maintained stable hemoglobin (mean change: 0.04±0.78 g/dL) and 90% maintained stable platelets after 1 year of eliglustat (mean change: 1.46±31.7x10^9/L). In patients continuing on eliglustat, 97% maintained stable hemoglobin (mean change: 0.10±0.77 g/dL) and 94% maintained stable platelets (mean change: 2.27±17.6x10^9/L) after 2 years. Mean bone values, all in the normal range at baseline, remained normal after 1-2 years of eliglustat. In all 3 trials, eliglustat was generally well tolerated; 90% of patients overall elected to continue on eliglustat. Most adverse events were non-serious, considered unrelated to eliglustat, and mild or moderate in severity.
Summary/Conclusions: Long-term use of eliglustat in adults with GD1 was associated with continued improvements in clinical parameters in previously untreated patients and clinical stability in patients previously stabilized on enzyme replacement therapy.

S520

ROMIPLOSTIM IN SPLENECTOMIZED (SPLNX) AND NONSPLENECTOMIZED (NONSPLNX) PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background: ITP is an autoimmune disorder with increased platelet destruction and insufficient platelet production. Romiplostim, a thrombopoietin receptor agonist, improves ITP outcomes compared with control (placebo or standard of care). Splenectomy removes a major site of sequestration of antibody-coated platelets, which might alter responsiveness to romiplostim or increase the risk of thrombosis or other complications. The efficacy and safety of romiplostim in splnx *versus* nonsplnx patients are not fully characterized.
Aims: This analysis evaluated safety and efficacy for splnx *vs* nonsplnx patients across 13 completed clinical studies of romiplostim in adults with ITP.
Methods: Data up to June 2014 were pooled. Informed consent was obtained in each ITP study. Safety was analyzed after ≥1 dose of romiplostim or control. Adverse event (AE) rates were adjusted for time of exposure. Efficacy included platelet response (any ≥50x10^9/L) and sustained platelet response (≥50x10^9/L ≥9 of 12 consecutive weeks). Four dose-finding studies that employed off-label doses were excluded from efficacy analyses.
Results: Safety was analyzed for 1111 patients (395 splnx; 716 nonsplnx). At baseline, splnx (*vs* nonsplnx) patients had longer median ITP duration (8.7 [95%CI: 7.7, 9.7] *vs* 1.6 [1.4, 2.0] yr), lower median platelet count (14.0 [12.0, 15.3] *vs* 19.3 [18.0, 21.0] x10^9/L), and a higher proportion with >3 prior ITP treatments (38% [33.2%, 43.0%] *vs* 12% [9.5%, 14.3%]). Splnx patients used more rescue medications (263.4 [95%CI: 251.5, 275.7] *vs* 153.3 [125.3, 138.8] per 100 pt-yr). Exposure-adjusted AE rates are provided in the Table 1. AE rates per 100 pt-yr in the control group for both splnx (1861.1 [95%CI: 1616.9, 2132.2]) and nonsplnx (1052.6 [989.3, 1119.0]) patients were higher than in the respective romiplostim group. Efficacy data were analyzed for 1024 patients (376 splnx; 648 nonsplnx). Median platelet counts increased with romiplostim and platelet responses were stable over time in both subgroups. For romiplostim, rates of platelet response (≥50x10^9/L at least once) were 82% (95%CI: 78%, 86%) for splnx and 91% (89%, 93%) for nonsplnx patients (p<.0001), and rates of sustained platelet response (≥50x10^9/L ≥9 of 12 consecutive weeks) were 68% (63%, 72%) and 80% (77%, 83%), respectively (p<.0001).

Table 1. Duration-adjusted AE Rate per 100 pt-yr (95% CI).

	Splnx (702.0 pt-yr)	Nonsplnx (1129.7 pt-yr)
Any AE	1226.4 (1200.6, 1252.5)	851.9 (835.0, 869.1)
Hemorrhage AEs	266.1 (254.2, 278.4)	140.8 (134.0, 147.9)
Infection AEs	156.7 (147.6, 166.2)	124.8 (118.4, 131.5)
Thrombotic AEs	6.3 (4.6, 8.4)	4.6 (3.4, 6.0)
Reticulin AEs*	0.4 (0.2, 7.4)	0.6 (0.2, 1.3)
Any serious AE	68.1 (62.1, 74.5)	44.1 (40.3, 48.1)
Any fatal AE	1.6 (0.8, 2.8)	2.7 (1.9, 3.9)
Any treatment-related AE	123.1 (115.0, 131.6)	82.1 (76.9, 87.6)

**AEs reported as bone marrow reticulin fibrosis, myelofibrosis, or reticulin increase across 12 studies; excluded 1 ITP study specifically designed for bone marrow assessment (reported separately).*

Summary/Conclusions: Removing a major site of platelet sequestration increased neither responsiveness nor toxicity of the thrombopoietin receptor agonist, romiplostim. In splnx patients, platelet response rates were lower, use of rescue medications was higher, and exposure-adjusted rates of hemorrhage AEs and infection AEs were higher. Differences between splnx and nonsplnx patients in disease duration/severity may have influenced concomitant treatments and safety/efficacy results. In conclusion, romiplostim safety generally was comparable between splnx and nonsplnx patients and platelet response rates were high in both populations.

Stem cell transplantation - Clinical 1

S521

PD-1 GENOTYPE OF THE DONOR AND ACUTE GRAFT-*VERSUS*-HOST DISEASE AFTER HLA-IDENTICAL SIBLING DONOR STEM CELL TRANSPLANTATION

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Background: Both T cell receptor (TCR) and co-signalling molecules are required for T cell activation. Programmed death 1 molecule (PD-1) triggers an immune checkpoint and co-inhibitory signalling that leads to peripheral tolerance. Some PD-1 polymorphisms (SNPs) have been described and associated with autoimmune disease or cancer predisposition. Monoclonal antibodies target co-inhibitory molecules enhance the immune response against malignancies.
Aims: Establish the relevance of the PD-1 SNPs on the clinical outcome after allogeneic-hematopoietic stem cell transplantation (allo-HSCT).
Methods: We accomplished a retrospective analysis of the SNPs PD-1.1G/A (promoter) and PD-1.3G/A (4 intron) genotypes of the donor in a cohort of 1485 HLA-identical sibling transplants (SIB) from Spanish transplant centers performed between 1991 and 2013. T-cell depleted graft recipes were not included. PD-1 genotyping determined by allelic discrimination assays and detection system Life Technologies on DNA samples obtained from peripheral blood. Written informed consent was obtained. Statistical cumulative incidence was estimated for aGVHD, relapse and transplant related mortality (TRM) according to PD-1 donor's genotype. TRM was defined as death due other causes but relapse. Overall survival (OS) and relapse-free survival (RFS) were analyzed through Kaplan Meier method comparing curves by the log-rank test. Multivariate analysis was performed by Cox regression model.
Results: PD-1 allele and haplotype frequencies were comparable to other Caucasian populations. The haplotype PD-1.1G/PD-1.3G was the predominant (86.3%). There were statistical differences on age and gender demographic features in donors genotyped as PD-1AG or AA. The remaining clinical variables were comparable between these both genotype groups and between PD-1.3AA or PD-1.3AG/GG cohorts as well, except for the high proportion of myeloablative transplants on the last one. The aGVHD grades II-IV cumulative incidence was higher in recipes receiving grafts from homozygous PD-1.1G (p: 0.027) and PD-1.3A (p: 0.003) donors. Both genotypes were independent risk factors in multivariate analysis for grades II-IV aGVHD (p:0.033; HR2.2; 95%CI: 1.1 to 4.8 and p<0.001; HR4.5; 95%CI: 2 to 10.1 respectively). PD-1.3AA remained as independent risk factors for sever III-IV aGVHD (p<0.001; HR7.22; 95%CI: 2.6 to 19.7). The cumulative incidence for aGVHD grades II-IV was 64% in patients with "high risk" genotypes (PD-1.1GG/PD1.3AA), 32.6% for PD-1.1GG/PD-1.3AG or GG genotypes and 18% in those with of at least one A-allele at PD-1.1 position (p:0.001 and p:0.029). Despite the increased risk of aGVHD, we did not detect any influence of the PD-1 genotype and the secondary clinical endpoints (chronic GVHD, TRM, OS and relapse). We inferred the relative frequencies of genotypes and haplotypes for CTLA-4/PD-1 combinations so, on 883 donors we found that the most frequent matched observed (42.6%) involves the A alleles at both CTLA-4 SNPs coexisting with G allele at PD-1.1 and PD-1.3, without any association between CTLA-4 and PD-1 SNPs.
Summary/Conclusions: PD-1 genotypes carrying with the "high risk" alleles are independent risk factors for grades III- IV aGVHD. Other genotypes seem to be protective as it has been describe in other autoimmune mediated diseases. There were no statistically differences with the other clinical endpoints, suggesting that PD-1 should be relevant in the initial process of allorecognition, but not on prevention of relapses. These preliminary results should be confirmed by other studies. Financed by PI11/01690 and PI14/01646 and MTV3/120210.

S522

ACUTE GRAFT *VERSUS* HOST DISEASE GRADE I : FINAL ANALYSIS OF A GITMO RANDOMIZED TRIAL OF PREDNISOLONE *VS* NO TREATMENT

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Background: Management of patients with grade I acute graft *versus* host disease (GvHD) (a skin rash covering less than 15% of the body surface), is a matter of individual preference: some would treat with steroids, most clinicians would probably not treat. We hypothesized that treatment of grade I GvHD would significantly reduce the proportion of patients progressing to grade II GvHD.
Aims: To test whether steroid treatment of acute GvHD grade I, protects patients from progressing to grade II GvHD.
Methods: Nine Centers of the Italian Group for Bone Marrow Transplantation (GITMO), randomized 171 patients with acute grade I skin GvHD, to an *observation arm (n=85) or to* a *treatment arm (n=86)*- prednisolone 1 mg/kg/day for 5 days and then taper and discontinue on day +30. Patients in the *observation* arm, could be treated when GvHD grade II developed, at whatever interval from randomization. The primary end point of the study was progression to acute GvHD grade II. Secondary end points were: transplant related mortality (TRM), survival, infections and chronic GvHD.The two groups were balanced for diagnosis (p=0.9), disease phase (p=0.4) and donor type (p=0.4) The median age for the *observation* arm, was borderline higher than the *treatment arm (*46 *vs* 38 years, p=0.08).
Results: The cumulative incidence of progression to acute GvHD grade II, was 50% in the *observation* and 35% in the *treatment* arm (p=0.02). This difference was 58% *vs* 35%, (p=0.05) for HLA identical siblings (n=70) and 45% *vs* 36% for alternative donors (n=101) (p=0.3). Grade III-IV GvHD was diagnosed in 11 *observation vs* 12 *treatment* patients (13% *vs* 14%, p=0.8); the incidence was comparable in the two arms, also when stratified in HLA identical siblings (17% *vs* 18%) or alternative donor grafts (10% *vs* 11%). Moderate/severe chronic GvHD was comparable in the two groups (17% *vs* 19%). Patients in the *observation* arm had less bacterial infections (11 *vs* 23, p=0.05) less CMV reactivation episodes (66 *vs* 95, p=0.01), and comparable fungal infections (6 *vs* 8, p=0.4). Severe adverse events (SAE) were recorded less frequently in the *observation* arm (n=18) than in the *treatment* arm (n=31)(p=0.1). Cumulative incidence of transplant related mortality (TRM) was 20% (*observation*) *vs* 26% (*treatment*) (p=0.2). Relapse related death (RRD) was 25% *vs* 21%. Actuarial 1 year survival was 86% (*observation*) *vs* 82% (*treatment*) (p=0.3). Main causes of death in *observation/treatment* arms, were as follows : acute GvHD 7% -12%; chronic GvHD 4%>2%; infection, 6%>11%; and leukemia relapse 21% -17%.
Summary/Conclusions: In conclusion, steroid treatment of grade I GvHD prevents progression to grade II GvHD, however, it does not protect patients against GvHD grade III-IV, nor against chronic GvHD, and exposes patients to a higher risk of infections. For this reason there is no beneficial effect on TRM, relapse related death and survival. Based on this prospective trial, patients with grade I GvHD should be left untreated, untill GvHD resolves spontaneously or progresses to grade II.

S523

INFUSION OF BPX-501 (DONOR T CELLS TRANSDUCED WITH THE IC9 SUICIDE GENE) AFTER A/B T-CELL DEPLETED HAPLO-HSCT IN CHILDREN WITH ACUTE LEUKEMIA: PRELIMINARY RESULTS OF A PHASE I-II TRIAL

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Background: We recently conducted a prospective study (NCT01810120) which showed that haploHSCT after depletion of α/β T cells is a suitable and effective option for those children in need of an allograft and lacking an immediately available HLA-identical donor. However, recovery of adaptive T-cell immunity remains suboptimal and some patients experienced either relapse of the original disease or severe viral infections.
Aims: In light of these considerations, strategies aimed at accelerating early recovery of adaptive T-cell immunity are desirable.
Methods: We designed a phase I/II trial aimed at testing the safety and efficacy of post-transplant infusion of donor-derived T cells transduced with the new iC9 suicide gene (BPX-501) in children with either malignant or non-malignant disorders (NCT02065869); enrollment started in December 2014. Cells are administered within 14±4 days after haploHSCT. The phase I portion of the trial consisted of a classical 3+3 design with 3 cohorts, receiving escalating doses of BPX-501 cells of $2.5x10^5$, $5x10^5$, and $1x10^6$ cells/kg, respectively. Patients included in the phase II portion received the highest tolerated/recommended dose identified during the phase I portion of the study. As of February 15th 2016, 13 children with acute leukemia either in first or second complete remission (CR1/CR2) have been transplanted; 7 were males and 6 were females.

Median age at HSCT was 6.5 years (range 0.9-16.1); 10 pts had acute lymphoblastic leukemia (ALL) and 3 acute myeloid leukemia (AML). All pts transplanted in CR1 had either poor cytogenetic/molecular characteristics or high levels of minimal residual disease (MRD) at the end of induction therapy. All children received >10x10^6 CD34+ cells/Kg and <1x10^5 αβ+ T cells/Kg. A myeloablative regimen was given to all children, who also received as prophylaxis of graft-*versus*-host disease (GVHD) anti-thymocyte globulin (12 mg/kg over 3 days, from -5 to -3). Rituximab (200 mg/m^2) was administered on day -1 to further prevent EBV-related lymphoproliferative disorders. No pharmacological GvHD prophylaxis was employed after HSCT.

Results: Sustained primary engraftment occurred in all pts. The median time to infusion of BPX-501 cells was 16 days (range 13-18) after HSCT; median cell viability was 91% (range 65-97). Only one child developed gut and liver acute GVHD requiring infusion of AP1903, which controlled the disease. Five pts experienced skin-only grade I-II acute GVHD, this leading to a cumulative incidence (CI) of 38%. None of the patients at risk developed chronic GVHD. No patient died for transplant-related complications. One pt relapsed at 4 month after HSCT, the CI of disease recurrence being 12%. With a median follow-up of 6 months (range 3-12), the Kaplan Meier estimate of leukemia-free survival (LFS) is 88% (95% CI 38.7-98.1) compared to 76.6% (95% CI 65.4-84.5) in the 77 CR1/CR2 ALL/AML patients included in our previous study (Figure 1). BPX-501 cells progressively expanded over time after the infusion and are persisting, contributing to the recovery of adaptive T-cell immunity.

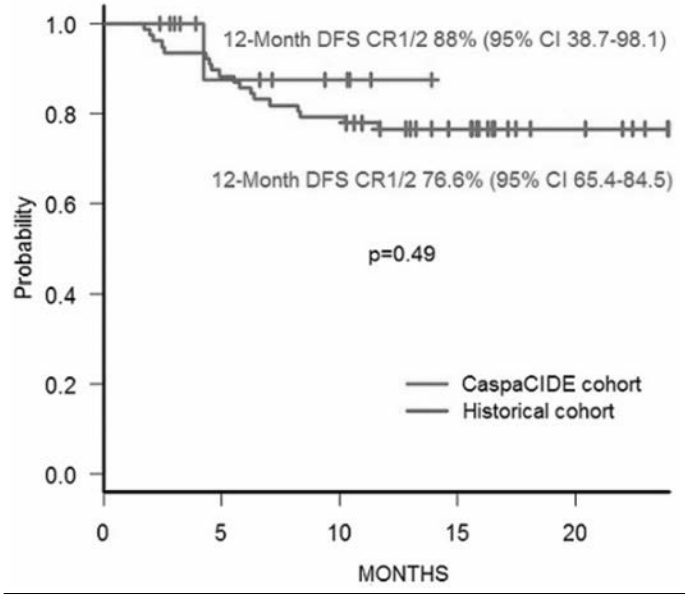

Figure 1.

Summary/Conclusions: These data indicate that the infusion of BPX-501 cells in children with acute leukemia given selectively manipulated haploHSCT results in the absence of transplantation-related mortality and chronic GVHD. Although the median observation time is still limited, the CI of disease recurrence is promising.

S524

TRENDS IN THE USE OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: A REPORT FROM THE ACUTE LEUKEMIA WORKING PARTY OF EBMT

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Background: Indications for hematopoietic stem cell transplantation (HSCT) for adults with acute lymphoblastic leukemia (ALL) evolve over time and vary among countries as well as the methodology of the procedures.

Aims: The goal of this study was to assess general trends in the number of various types of HSCTs performed between years 2001 and 2012 in Europe.

Methods: Data reported to the European Society for Blood and Marrow Transplantation (EBMT) registry were used for this analysis. In addition, we evaluated HSCT rates with respect to the incidence of ALL in selected European countries.

Results: Altogether, 11602 first allogeneic (n=9938) or autologous (n=1664) HSCTs were performed in the period 2001-2012. Comparing years 2010-2012 and 2001-2003, the number of alloHSCTs performed in first CR increased by 108%, most prominently for transplantations from unrelated (219%) and mismatched related donors (89%). The number of HSCTs from matched sibling donors increased by 43%, while the number of autoHSCTs decreased by 71% (Table 1). The increase of the use of alloHSCT, irrespective of the disease stage, was stronger for Ph-pos (108%) than Ph-neg ALL (21%) while similar for B- and T-ALL. Among patients aged >55 years, the number of alloHSCT increased by 386% while among younger adults (18-55 years), by 48%. Between 2001 and 2003, peripheral blood was used as source of stem cells in 69% cases of alloHSCT, compared to 93% between 2010-2012 (p<0.0001). The use of bone marrow decreased from 30% to 6%, respectively (p<0.0001). The proportion of alloHSCT with reduced-intensity conditioning (RIC) increased from 6% to 19% (p<0.0001). Among myeloablative transplantations, regimens based on total body irradiation were the preferable option (app. 80% over the whole study period, p=NS). In contrast, among RIC regimens, the use of chemotherapy predominated (75% between 2010-2012, p=0.006). In most of analyzed individual countries, the estimated rates of alloHSCT (no. HSCT per 100 newly diagnosed ALL) for patients in CR1 increased over time. However, the values for a period 2010-2012 varied strongly, being highest in the Netherlands (42.2), followed by the UK (32.3) and France (32.2) while lowest in Russia (0.8). HSCT rates correlated with socio-economic status of a country as defined by the Human Development Index (R=0.71, p=0.046).

	YEAR			
Type of HSCT	2001-2003	2004-2006	2007-2009	2010-2012
Total alloHSCT	1005	1344	1811	2093
MSD-HSCT	604 (60%)	736 (55%)	819 (45%)	862 (41%)
URD-HSCT	363 (36%)	581 (43%)	956 (53%)	1159 (55%)
MMRD-HSCT	38 (4%)	27 (2%)	36 (2%)	72 (3%)
AutoHSCT	459	327	163	134

MSD, matched sibling donor; URD, unrelated donor;
MMRD, mismatched related donor
% refer to proportions of various donor types among alloHSCT

Table 1. Numbers of HSCT procedures for ALL in first CR by donor type.

Summary/Conclusions: Results of our analysis indicate a continuous trend for an increased use of alloHSCT for adults with ALL, which may be attributed to increasing availability of unrelated donors. However, it may also be speculated that the introduction of tyrosine kinase inhibitors allowed higher proportion of patients with Ph-pos ALL proceeding to transplantation. Finally, the implementation of RIC regimens contributed to wider use of alloHSCT among older adults. Limitations of the analysis include any assumptions made regarding ALL incidence for the specified time period and possible variation in reporting to the EBMT registry from different countries over time.

S525

FIRST ANALYSIS OF THE MULTICENTRE, BLINDED, PLACEBO-CONTROLLED PRE-GVHD CLINICAL-TRIAL FOR PREDICTION AND PRE-EMPTIVE TREATMENT OF ACUTE GVHD IN 210 OF 260 PATIENTS

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Background: The success of allogeneic stem cell transplantation (HSCT) is limited by acute graft-*versus*-host disease (aGvHD), one of the major and life-limiting complications. A proteomic urine pattern "aGvHD_MS17" capable to predict aGvHD grade II or more has been developed (Weissinger *et al.,* 2007 and 2014). In 2008 a multicenter, randomized, placebo-controlled, double blind clinical trial to evaluate the proteomic-based prediction of aGvHD as well as possibilities of pre-emptive therapy was initiated. Patients after the first allogeneic HSCT could be included in this trial. Ten centers in Germany contributed 267 patients to this trial and 90 were randomized according to the positivity of proteomic urine pattern aGvHD-MS17 to receive either prednisolone or placebo. To date, data of 210 (89 randomized) patients have been analysed for incidence of acute GvHD grade II or more and overall survival for the first year post HSCT.

Aims: The aim of this clinical trial was to evaluate the predicitve potential of

aGvHD_MS17 for the development of aGvHD grade II to IV. In additioan we analysed the possibility of 2mg/kg body weigth prednisolon for pre-emtptive therapy of aGvHD diagnosed by the positivity of aGvHD_MS17.
Methods: Urine was collected on days +7, +14, +21,+28, +35, +50 and +80 (all +/-3 days) after allogeneic HSCT frozen, shipped to Hannover and analysed within 72h as described (Weissinger *et al.*, 2007). Positivity of aGvHD_MS17 was achieved, when the dimensionless classification factor (CF) was +0.1 or more. Upon positivity of a sample for aGvHD_MS17, patients were randomized and received either prednisolone (2mg/kg BW for 5 days) or placebo. The majority of the patients had acute leukaemia prior to transplantation (n=120), were not in CR/CP (n=126; 60%) and were transplanted from matched (n=184; 84%). One hundred fifty one (71%) received reduced intensity conditioning regimens and the majority (84%; n=177) received immunosuppressive antibodies as GVHD-prophylaxis prior to transplantation and a calcineurin-inhibitor (CSA/MTX n=78; CSA/MMF n=93) based GvHD-prophylaxis afterwards.
Results: Prospective and blinded evaluation of aGvHD_MS17 revealed in this first analysis the correct classification of patients developing aGvHD grade II or more with a sensitivity of 71.17% and a specificity of 80%. The best CF (criterion) for the prediction of acute GvHD was at 0.16, which is between the previously published discriminatory CF of 0.2 (Weissinger *et al.,* Blood 2007) and 0.11 (Weissinger *et al.,* Leukemia 2014). The aGvHD-MS17-pattern turns positive about 7 days (range: 1 to 21) prior to the prior to clinical or biopsy based diagnosis of aGvHD. Patients in the aGvHD-MS17-positve group developed aGvHD grades II to IV in 49.5% while 19% of patients with aGvHD-MS17 negative samples developed acute GvHD grade II to IV. Interestingly, aGvHD_MS17 positivity between day +5 to +35 indicated pending aGvHD II-IV very accurately. Pattern-negative patients developed aGvHD II-IV later namely between days +20 and +40. Both groups reach a plateau around day +50. Patients with aGvHD_MS17-positive samples have a 2.76 fold risk of developing severe acute GvHD ($p<0.0001$). In addition, overall survival is significantly different for patients with samples positive for aGvHD_MS17, 40% of those die within the 1st year after HSCT (day +80 and +250). In contrast, 90% of patients who never had aGvHD_MS17-positive samples survive the first year ($p<0.0001$).
Summary/Conclusions: Our first analysis of the Pre-GvHD-trial implicates that aGvHD_MS17 is highly reproducible in the early prediction of aGvHD development, especially shortly after HSCT (up to day +25). Additionally, univariate analysis suggests that aGvHD_MS17 accurately separates patients with high and low overall survival after allogeneic HSCT.

POSTER SESSIONS II

Acute lymphoblastic leukemia - Biology 3

P526

IN VIVO ROLE OF ACTIVATION INDUCED CYTIDINE DEAMINASE (AID) EXPRESSION IN THE DEVELOPMENT OF PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: AID is essential for somatic hypermutation and class switch recombination in mature B-cells, while its role in precursor B-cells is controversially discussed. Since recently there is *in vitro* experimental evidence that AID is upregulated in precursor B-cells after exposure to LPS and contributes to the clonal evolution of pB-ALL.
Aims: Our rational was to establish an *in vivo* model to investigate the effect of AID in precursor B-cells independent of BCR-signaling and/or Rag1 induced oncogenic alterations. In this study we elucidate its role *in vivo* using a $Rag1^{-/-}$ tumor-prone model, which is AID deficient or AID competent. Therefore we are able to study the relevance of the *Aicda* gene in a dose dependent manner in pro-B cells.
Methods: The $p19Arf^{-/-}/Rag1^{-/-}$ mouse model (Hauer *et al.*, 2011) was crossed back on an AID deficient background ($p19Arf/Rag1/AID^{-/-}$). Healthy and leukemic mice were characterized by FACS analysis, immunohistochemistry, and molecular genetics. Preleukemic pro-B-cells were analyzed for IL7 sensitivity. Three pB-ALL tumors were further subjected to whole exome sequencing on a HiSeq2500 (Illumina) platform.
Results: $P19Arf/Rag1^{-/-}$ deficient mice develop pB-ALL at an incidence of 26% with evidence of AID expression. Surprisingly, additional loss of AID in these mice accelerates the pB-ALL incidence to 96% (43/45, median disease onset 25 weeks). Moreover we show that this effect is AID dose dependent, since AID heterozygous animals on the same background displayed a significant disease reduction (83%, 15/18, median disease onset 33 weeks). The leukemias displayed blast infiltration through spleen and peripheral blood with a cell surface phenotype of $CD19^+B220^+ckit^+IgM^-CD25^-$ and were able to engraft in secondary recipients with a phenotype identical to the primary disease. Whole exome sequencing of murine tumors additionally revealed an accumulation of recurrent somatic Jak3 mutations (R653H, V670A), highlighting the relevance of the IL7R signaling in these cells. AID expression was verified in preleukemic pro-B cells ($CD19^+B220^+ckit^+IgM^-$) of $p19Arf/Rag1^{-/-}$ and $P19Arf/Rag1^{-/-}AID^{+/-}$ animals by qRT-PCR. The importance of an intact IL7-signaling pathway was further shown by an increased sensitivity to IL-7 withdrawal in AID deficient preleukemic pro-B cells of these mice.
Summary/Conclusions: This work provides evidence for an *in vivo* role of AID in precursor-B cells and that absent AID expression as well as overexpression of AID can promote leukemia.

P527

UNFAVORABLE PROGNOSTIC VALUE OF BCR-ABL1-LIKE IS INDEPENDENT OF AN INTEGRATED GENETIC RISK SCORE IN PEDIATRIC BCP-ALL

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Background: Pediatric B cell precursor acute lymphoblastic leukemia (BCP-ALL) is characterized by cytogenetic aberrations and deletions of B cell development genes. Patients with cytogenetic aberrations *ETV6-RUNX1*, high hyperdiploidy (51-65 chromosomes) and *TCF3-PBX1* have a good prognosis, whereas *BCR-ABL1*, *MLL*-rearranged, iAMP21 and haploid/near-triploid BCP-ALLs have a poor prognosis. Cases without these established chromosomal abnormalities are called B-other ALL and have an intermediate prognosis. Within the B-other group a novel high-risk subtype, *BCR-ABL1*-like, was discovered based on similar gene expression to *BCR-ABL1*-positive BCP-ALL and a similar poor outcome (Den Boer *et al.* 2009; Mullighan *et al.* 2009). Recently, Moorman *et al.* developed an integrated genetic risk score combining cytogenetic data and deletion profile of B cell differentiation genes which was predictive for outcome of pediatric BCP-ALL patients treated on UK protocols (Moorman *et al.* 2014).
Aims: We aimed to evaluate the independent prognostic value of the integrated genetic risk score in B-other and *BCR-ABL1*-like ALL of a Dutch/German pediatric cohort.

Methods: This study comprised 170 children with BCP-ALL including 78 previously described *BCR-ABL1*-like cases identified by hierarchical clustering and 92 non-*BCR-ABL1*-like B-other cases (Van der Veer *et al.* 2013). Patients with Down syndrome were excluded because of increased risk for treatment-related toxicity. Copy number of 8 commonly deleted genes was measured by the SALSA MLPA kit P335, a ratio <0.75 was considered a deletion. Using the described MLPA profiles (Moorman *et al.* 2014) the genetic risk score was determined for each sample. Briefly, genetic good risk is defined by no deletion of *IKZF1*, *CDKN2A/B*, *PAR1*, *BTG1*, *EBF1*, *PAX5*, *ETV6* or *RB1*; isolated deletions of *ETV6*, *PAX5* or *BTG1*; *ETV6* deletions with a single additional deletion of *BTG1*, *PAX5* or *CDKN2A/B*. Genetic poor risk is defined by any deletion of *IKZF1*, *PAR1*, *EBF1* or *RB1,* and all profiles not mentioned above. Event-free survival (EFS) was estimated using the actuarial Kaplan-Meier method, survival data between groups were compared using Cox regression.
Results: Among 170 cases negative for sentinel cytogenetic aberrations, we identified 126 genetic poor risk and 44 genetic good risk cases. The 5-year EFS in the genetic poor risk cases was 74% compared with 83% in the genetic good risk group (p=0.1). Genetic poor risk was more frequent in *BCR-ABL1*-like than in B-other (86% *vs* 64%, Fisher p=0.001). The prognosis of B-other/genetic poor risk (5-yr EFS 81%, p=0.3) and *BCR-ABL1*-like/genetic good risk (5-yr EFS 73%, p=0.3) cases was worse, but not significantly worse, from that observed for B-other/genetic good risk (5-yr EFS 86%), whereas the *BCR-ABL1*-like/genetic poor risk cases had poorer outcome (5-yr EFS 68%, p=0.03; Figure 1). *IKZF1* deletion, known to be associated with poor outcome, occurred in 54% of *BCR-ABL1*-like/genetic poor risk and 36% of B-other/genetic poor risk cases. After removing *IKZF1*-deleted cases, outcome of the *BCR-ABL1*-like/genetic poor risk cases remained poor (5-yr EFS 57%, p=0.01), while outcome of the B-other/genetic poor risk cases (87%) did not differ from that observed for B-other/genetic good risk cases (86%). In multivariate analyses including subtype and genetic risk score, the *BCR-ABL1*-like subtype was independently prognostic in the total group of *BCR-ABL1*-like and B-other cases (HR 1.8, p=0.06) and upon exclusion of *IKZF1*-deleted cases (HR 3.0, p=0.01) whereas genetic risk score was not independently prognostic of these subtypes. About 80% of both *BCR-ABL1*-like and B-other genetic poor risk *IKZF1*-wild type cases carried *CDKN2A/B* and/or *PAX5* aberration, however intragenic amplification of *PAX5* was more frequent among *BCR-ABL1*-like than B-other genetic poor risk group (16% *vs* 3%, p=0.08).

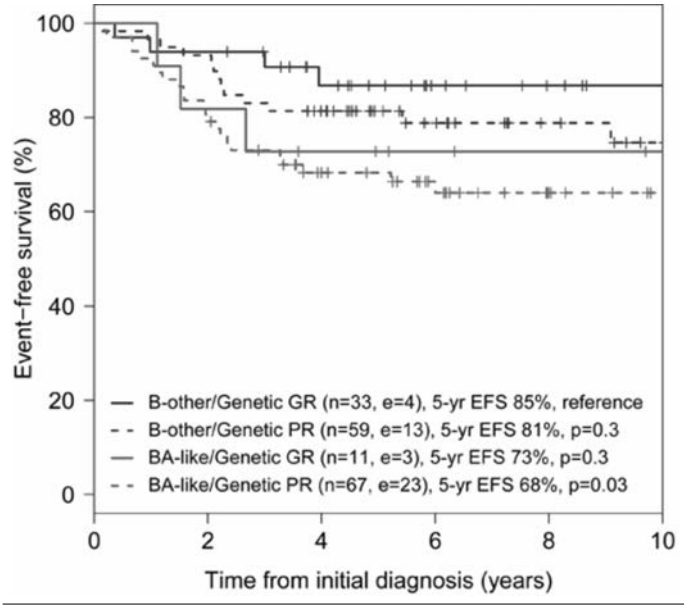

Figure 1.

Summary/Conclusions: The genetic risk score developed in the UK identified a group of *BCR-ABL1*-like patients at higher risk of treatment failure in a Dutch/German pediatric BCP-ALL cohort. The outcome of B-other/genetic poor risk and *BCR-ABL1*-like/genetic good risk cases showed a trend for a worse outcome compared with B-other/genetic good risk. The genetic poor risk group largely overlapped with the *BCR-ABL1*-like group, and only the latter independently predicted for an adverse clinical outcome.

P528

THE HISTONE DEACETYLASE INHIBITOR GIVINOSTAT (ITF2357) HAS A POTENT ANTI-TUMOR ACTIVITY AGAINST CRLF2 REARRANGED LEUKEMIA

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Background: The cure rate for B Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) approaches 90% with current treatment regimens, however only a third of patients with relapse are cured. Therefore, there is an urgent need to focus on subgroups of patients with hallmarks of bad prognosis that could benefit from novel therapeutic approaches. Alterations of *Cytokine Receptor-like Factor 2 (CRLF2)*, a negative prognostic factor in pediatric BCP-ALL, have been identified in up to 10% of patients representing half of the high risk Ph-like ALL and of Down Syndrome-associated BCP-ALL. Rearrangements of *CRLF2* resulting in its overexpression are associated with activating mutations of the JAK-STAT pathway, causing the hyperactivation of JAK/STAT and PI3K/mTOR signaling. Inhibition of CRLF2/JAK2 signaling has the potential to become a therapeutic targeted intervention for this subgroup of poor prognostic patients. Previous studies have shown that the HDAC inhibitor Givinostat/ITF2357 has potent anti-tumor activity against JAK2V617F mutated myeloproliferative neoplasms (MPN), for which it has already a clinic application and established safety profile.
Aims: We studied the *in vitro* and *in vivo* efficacy of Givinostat in cases with *CRLF2* rearrangements.
Methods: The effect of Givinostat in proliferation and apoptosis was tested on MHH-CALL4 and MUTZ5 cell lines positive for exon 16 *JAK2* mutations and in patient derived CRLF2-rearranged blasts expanded in xenograft mouse models (PDX) and co-cultured on OP9 stroma.
Results: Givinostat inhibited proliferation and induced apoptosis of BCP-ALL *CRLF2*- rearranged MHH-CALL4 and MUTZ5 cell lines, with IC50 values lower than those for the SET2 cell line bearing JAK2V617F mutation, both for proliferation (IC50: 0.08±0.05μM *vs* 0.14±0.03μM) and apoptosis (IC50: 0.17±0.03μM *vs* 0.22±0.04μM). Consistently, after 72 hours of co-culture on OP9 stroma, Givinostat (0.2μM) was able to kill up to >90% of PDX cells (Annexin V/Sytox negative), in contrast with the vehicle-treated samples which showed 25-60% of blasts still alive after treatment. Moreover, in primary samples from diagnostic BM, CyTOF analyses showed that CD10+/CRLF2+ blasts were preferentially killed by the drug whereas the normal residue remained unaffected. At low doses (0.2 μM), Givinostat downregulated genes of the JAK/STAT pathway (*STAT5A*, *JAK2*, *IL7Rα*, *CRLF2, BCL2L1* and *cMYC)* and inhibited the basal and ligand induced signaling, reducing the phoshporylation of STAT5 in all tested samples (mean fold decrease of pSTAT5: 2.4±0.6). The down modulation of CRLF2 protein was also observed by flow cytometry (mean fold decrease 3.55±1.38). Furthermore, Givinostat augmented the effect of chemotherapy in inhibiting proliferation and inducing apoptosis in CRLF2 rearranged cell lines and in primografts, *in vitro*. After 72 hours, the combined treatment reached considerable reduction in viable blasts than single treatments (*e.g.* 6.3-35.3% viable cells in chemotherapy-treated samples *vs* 1.4-4.3% of combination). *In vivo*, Givinostat significantly reduced engraftment of human blasts in xenograft models of CRLF2 positive BCP-ALL.
Summary/Conclusions: In conclusion the HDAC inhibitor Givinostat efficiently kills CRLF2 rearranged leukemia by inhibiting JAK/STAT pathway and synergizes with current chemotherapy. Givinostat may represent a novel and effective tool, in combination with current chemotherapy, to treat this subsets of ALL with poor prognosis and chemotherapy-related toxicity. These data strongly argue for the translation of Givinostat in combination with conventional therapy into human trials.

P529

INSIGHTS INTO THE MOLECULAR EVOLUTION OF BCR-ABLP190 MEDIATED PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Although, the genetic aberration associated with BCR-ABL^{p190}-positive leukemia is already well characterized, the link between the BCR-ABL^{p190} fusion protein and precursor B-cell acute lymphoblastic leukemia (pB-ALL) development is only insufficiently understood.
Aims: By applying a combination of tyrosine kinase inhibitors (TKI) and chemotherapy, significant improvements have been made in fighting the poor prognosis of Ph$^+$ ALL, but there is still no long term survival achieved in 30-50% of pediatric and adult ALL cases. Thus for the development of individual disease based treatments, further insights are needed to identify the underlying mechanism that shape BCR-ABL^{p190} pB-ALL development.
Methods: We generated a mouse model, restricting BCR-ABL^{p190} expression to hematopoietic stem/progenitor cells (HS/PCs), by placing the *BCR-ABL*^{p190}

cDNA under the control of the Sca1 promoter (Sca1-BCR-ABL^{p190}). This model was further crossed back with Pax5$^{+/-}$ mice (Sca1-BCR-ABL^{p190}+*Pax5*$^{+/-}$). PB-ALL tumors of both strains were subjected to whole exome and genome sequencing (HiSeq2500 - Illumina). Reporter gene assays containing copies of a CD19 promoter derived high affinity binding site were used to assess the transcriptional functionality of the detected Pax5 variants.
Results: Here we show that pB-ALL development can be established in mice (13%, 5 out of 36, starting at 13 months), when BCR-ABL^{p190} oncogene expression is restricted to HS/PCs (Sca1-BCR-ABL^{p190}). These leukemias are a phenocopy of the human disease with respect to clinical, pathology and genomic lesions. All pB-ALLs displayed clonal immature BCR rearrangement and the cell surface phenotype (CD19^{-}B220^{+}IgM^{-}), suggested a reduction of Pax5 activity. Therefore, Sca1-BCR-ABL^{p190}+*Pax5*$^{+/-}$ mice were generated, which almost exclusively developed pB-ALL (CD19^{-}B220^{+}IgM^{-}Mac1$^{+/-}$) with shorter latencies (93%, 27 out of 29, first disease onset at 6 months). In order to identify the second hit related to pB-ALL disease we next performed whole exome (n=13) and whole genome (n=3) sequencing of Sca1-BCR-ABL^{p190}+*Pax5*$^{+/-}$ tumors, which further revealed alterations of the remaining wild-type *Pax5* allele (6/13 point mutations; 1/3 deletions). All point mutations were shown to confer reduced transcriptional Pax5 activity by luciferase assay.
Summary/Conclusions: Thus, we shed further light on Ph+ ALL disease evolution on a molecular level, by presenting first proof that expression of the BCR-ABL^{p190} oncogene is not necessary in the leukemic cell, but is sufficient in HS/PCs to induce pB-ALL. Moreover we show an essential function of Pax5 in BCR-ABL^{p190} mediated pB-ALL development.

P530

THE ROLE OF CNOT3 AND THE ENTIRE CCR4-NOT COMPLEX IN TUMOR DEVELOPMENT

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Background: CNOT3 is a subunit of the evolutionary conserved CCR4-NOT complex, mediating RNA degradation through deadenylation of mRNA. We recently discovered loss-of-function mutations in CNOT3 in patients with T-cell acute lymphoblastic leukemia (T-ALL) and CNOT3 has also been found to be mutated in chronic lymphocytic leukemia. These finding suggests a role for CNOT3 as tumor suppressor gene. However, the role of CNOT3 in normal and malignant T-cell development remains largely unknown.
Aims: We used mouse and fly (Drosophila melanogaster) models to investigate the role of CNOT3/Not3 and other members of the CCR4-NOT complex in normal T-cell development and in tumor development.
Methods: RNAi mediated down-regulation of *Not3* and other subunits of the CCR4-NOT complex was performed in the fly eye cancer model (based on NOTCH hyper-activation). In mice, we conditionally deleted *Cnot3* in lymphoid lineage cells by crossing *Cnot3*$^{fl/fl}$ mice with the *CD2*-iCre strain.
Results: In the fly model, reduction of *Not3* expression resulted in a significant increase in tumor incidence. The knockdown of other subunits of the CCR4-NOT complex (Not1, Not2, Twin and Pop2) also enhanced tumor formation, indicating that the entire complex and its deadenylase activity are required for tumor suppression. Overexpression of *Not3* suppressed formation of eye tumors. Gene expression profiling (RNA-seq) showed that suppression of *Not3* expression leads to the stabilization of a set of transcripts that might support tumor growth and development. In the mouse, we confirmed that CNOT3 and other subunits of the CCR4–NOT complex are expressed in normal T-cells during T-cell development. A single *Cnot3* allele was sufficient to support normal T-cell development. However, in mice with deletion of both *Cnot3* alleles, CD4+ and CD8+ cells were greatly reduced in spleen, bone marrow and peripheral blood. Analyses of thymi revealed that cells were blocked at the DN2-DN3 developmental stages. Thus, CNOT3 is essential for the normal development and differentiation of T cells.
Summary/Conclusions: Our results show that CNOT3 is important for proper T-cell development and establish the entire CCR4-NOT complex as a tumor suppressor complex.

P531

CAPTURE-BASED NEXT GENERATION SEQUENCING (NGS) IDENTIFIES ALSO RARE IG/TCR REARRANGEMENTS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: Minimal Residual Disease (MRD) has been demonstrated as the most important prognostic factor in Acute Lymphoblastic Leukemia (ALL). To date, allele-specific oligonucleotide quantitative PCR (ASO-QPCR) has been considered the gold standard for MRD analysis. This approach requires the identification of clonal markers at diagnosis starting from PCR amplification of the most frequent IG/TCR gene rearrangements. However, in a proportion of cases it fails to identify clonality and suitable markers for MRD evaluation. Recently, a Next Generation Sequencing (NGS) approach, similarly based on selected rearrangements amplification, has been described to identify clonal markers at diagnosis and to monitor MRD in lymphoid malignancies.
Aims: To apply a novel capture-based NGS approach for the identification of IG/TCR clonal markers at diagnosis possibly overcoming limits of an amplicon-based approach.
Methods: A capture-based NGS panel was designed targeting coding V, D and J genes in the IG/TCR loci. Libraries were prepared using Nextera Rapid Capture Enrichment protocol (Illumina) and pair-end sequenced on the MiSeq platform (v3 chemistry, 2x300bp, Illumina). NGS experiments were performed on 10 diagnostic bone marrow samples of adult ALL patients (6 B- and 4 T-lineage) already studied for clonality following the EuroMRD guidelines within the NILG 09/00 clinical trial (ClinicalTrials.gov Id: NCT02067143). We also included 2 Human Umbilical Vein Endothelial Cells (HUVEC) and 2 mesenchimal cord blood cells as negative controls for IG/TCR rearrangements. NGS data were analyzed by the Vidjil software (Giraud, Saison *et al.,* BMC Genomics 2014, http://www.vidjil.org).
Results: In the 10 studied diagnostic samples, capture NGS detected all the fifty clonal rearrangements previously identified by standard clonality assessment and Sanger sequencing. Only one IGH rearrangement was not revealed because of low coverage of the V, D and J genes involved in this specific rearrangement. Furthermore, this novel approach allowed to identify 24 additional clonalities that were subsequently confirmed: (i) 3 corresponded to low represented clones that were finally revealed by standard method only after an additional amplification round and sequencing; (ii) 10 corresponded to oligoclonal rearrangements in which Sanger sequencing did not allow the discrimination of single clones that was only possible thanks to single sequences deriving from NGS; (iii) 11 were characterized by uncommon V/DJ combination that were amplified and sequenced only after NGS based oligonucleotide design. HUVEC and mesenchimal cells capture NGS data analysis did not reveal any rearrangement in IG/TCR genes.
Summary/Conclusions: Our capture-based NGS approach for IG/TCR loci allowed to identify known as well as low represented and uncommon rearrangements not previously isolated by conventional methods. This technology offers the opportunity to identify at diagnosis minor leukemia clones so preventing unexpected relapse in patients proved negative for major clones. Furthermore, capture NGS can be of high value in patients with rare rearrangements in which standard procedures could not identify molecular markers suitable for MRD evaluation. In fact these patients could not benefit from a MRD driven treatment. In the future, we aim to retrospectively apply this technology on samples where standard clonality assessment failed in order to redefine patients molecular MRD risk classification and to correlate the new assignment with clinical outcome.

P532

MIR-126 DRIVEN LEUKEMIA: A MURINE MODEL TO UNDERSTAND HUMAN B-ALL PATHOGENESIS

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Background: Deregulated miR activity can contribute to hematologic malignancy. We have shown that miR-126 regulates HSC quiescence, and its ectopic expression induces a monoclonal leukemia in mice. By engineering a Doxycycline(doxy)-repressible miR-126 overexpression cassette (Tet.126), we showed that spontaneous B-ALL development was prevented, and full-blown disease regressed when miR-126 was turned off.
Aims: We sought to further investigate the mechanism behind miR-126-driven leukemia in our mouse model and explore its relevance in human B-ALL.
Methods: We performed RNAseq of murine B-ALL blasts before and after turning miR-126 off. We then functionally validated involved pathways by phosphoflow and performed *in vivo* rescue experiments by lentiviral (LV) overexpression of candidate factors. We then transduced human B-ALL blasts with miR-126 reporters-, knockdown- or overexpressing L*Vs* and transplanted them into NSG mice for characterization.
Results: Transcripts changing in our murine B-ALL model upon miR-126 withdrawal clustered into 3 main categories: (1) up-regulation of p53-dependent pathways and negative cell cycle regulators; (2) induction of B-cell differentiation genes including the transcription factors Myb, Mafb, Ebf1, Ikzf1/3, Pbx1 and pre-BCR-type signaling; (3) down-regulation of oncogenic/prosurvival pathways

typically associated with stem and progenitor cells (Kit, Wnt, Thy1, Jak/Stat, Bcl2). Further proof of miR-126-dependent activation of Stat5 and Akt pathways, two key signaling axes in leukemia, was obtained by phosphoflow analysis. To explore the functional relevance of these pathways in our mouse B-ALL model, we transduced Tet.126 ALL blasts with LV expressing dominant negative p53 (GSE56), Ikaros (IK6) or BCRABL (p210), engrafted them in mice and turned off miR-126 expression by doxy when mice had full-blown leukemia. Doxy treatment cured mice transplanted with Tet.126 ALL. However, GSE56 delayed early blast clearance, and this effect was further enhanced when combined with IK6 or BCRABL, confirming a relevant role of these pathways in our model. We are currently knocking out direct miR-126 targets by Crispr/Cas9 technology in B-ALL blasts in order to identify the components that render this model fully independent from miR-126 expression. To test the relevance of the model for human B-ALL, we focused on Philadelphia (Ph)+ B-ALL, which share some of the molecular features encountered in our model. Most patient Ph+B-ALL expressed to various levels miR-126. To measure biological miR-126 activity, we transduced primary blasts (n=10 Ph+ALL) with a miR-126 reporter LV and successfully engrafted 7 of them in NSG mice. In all 7 diseases, we detected substantial miR-126 activity that was stable and disease-specific over serial transplantation, suggesting a fine-tuned regulation of miR-126 expression in B-ALL. We then over-expressed or knocked down miR-126 in primary Ph+B-ALL. Both perturbations negatively affected engraftment in NSG mice and increased apoptosis, further confirming that human B-ALL depend on a precisely set level of miR-126 expression. Interestingly, miR-126 knock-down and Imatinib treatment showed a less than additive effect on B-ALL burden in the mice (n=2 different diseases), suggesting that miR-126, at least in part, maintains active kinase signaling in human Ph+B-ALL. Our murine miR-126 B-ALL model shows similarities to Ph+B-ALL.

Summary/Conclusions: We propose that miR-126 constitutes part of the armamentarium of B-ALL cells to counteract apoptosis and differentiation thereby permanently maintaining them in an otherwise unstable progenitor cell state.

Acute lymphoblastic leukemia - Clinical 2

P533

MUTATIONS IN TP53 AND JAK2 GENES ARE ASSOCIATED WITH POOR PROGNOSIS IN PEDIATRIC AND ADULT PATIENTS WITH B-CELL PRECURSOR ALL

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Background: B-cell precursor Acute Lymphoblastic Leukemia (B-ALL) is a malignancy of lymphoid progenitor B-cells, characterized by a marked heterogeneity. The identification of hidden genomic lesions that could be associated with treatment failure and relapse is still a challenge.

Aims: To determine the frequency and prognostic impact of mutations in a panel of genes in both children and adults with B-ALL, treated according to PETHEMA and SEHOP protocols.

Methods: The mutational status of *TP53, JAK2, PAX5, LEF1, CRLF2* and *IL7R,* genes was investigated in 340 newly diagnosed patients with B-ALL, 211 were children while 129 were adults. Mutation analyses were performed using oligonucleotide primer plates designed as part of the IRON-II collaborative network applying 454 massively parallel amplicon next-generation sequencing technology (454 Life Sciences, Branford, CT, USA). 19 exons (E) were sequenced by case: *TP53* (E4-E11), *JAK2* (E12-E16), *PAX5* (E2-E3), *LEF1* (E2-E3), *CRLF2* (E6) and *IL7R* (E5).The variant analysis was performed using Amplicon Variant Analyzer (AVA-Roche 454) and Sequence Pilot (JSI Medical Systems) software. A cut-off 2% was applied to define variants.

Results: A mutation rate of 12.1% (41 of 340) was identified. Eleven (26.8%) patients with mutations concomitantly harbored 2 or 3 mutations. The mutations were more frequent in adult than in children (20.2% *vs* 7.1%, p<0.0001). *TP53* was the most frequently mutated gene (4.1%), while mutations in *CRLF2* (2.9%), *JAK2* (3.5%), *PAX5* (2.4%), *IL7R* (0.3%) and *LEF1* (0.6%) were less frequent. All mutations observed in the childhood cohort of B-ALL, were exclusively observed in the subgroup of patients without *ETV6-RUNX1* translocation (p=0.045) while in the adults all mutations were exclusively detected in *BCR-ABL1* negative cases (p<0.0001). In the whole cohort of patients, the presence of mutations in *TP53* (*TP53*mut) (p=0.007) and *JAK2* (*JAK2*mut) (p=0.003) was associated with poor response to frontline therapy. The univariate survival analysis in whole B-ALL cohort showed that, *CRLF2*mut was associated with shorter RFS (p=0.023) and EFS (p=0.027), *JAK2*mut with a decreased RFS (p<0.0001) and EFS (p=0.001), while *TP53*mut was associated with poorer OS (p<0.0001), RFS (p=0.001) and EFS (p<0.0001). In the multivariate analysis, *TP53*mut was an independent risk factor associated with shorter OS (HR, 3.4; p=0.032). *TP53*mut (RFS:HR, 3.2; p=0.04 and EFS:HR, 3.2; p=0.04) and *JAK2*mut (RFS: HR,4.0;p=0.01 and EFS: HR,3.9;p=0.01) were independent markers of poor prognosis for RFS and EFS respectively. In the group of children with B-ALL, a significantly shorter OS (p=0.002), RFS (p=0.04), and EFS (p=0.009) was observed in patients with *TP53*mut. *TP53*mut was an independent risk factor associated with significantly shorter RFS (HR, 4.5; p=0.04). In adult B-ALL patients, *TP53*mut was associated with a shorter OS (p=0.02) and RFS (p=0.03), and *JAK2*mut was related with poorer RFS (p=0.002) and EFS (p=0.03).The presence of *TP53*mut was an independent risk factor associated with significantly shorter OS (HR, 2.3; p=0.03). Moreover, *TP53*mut (HR, 5.9; p=0.03) and *JAK2*mut (HR, 5.6; p=0.04) retained their independent prognostic significance in multivariate analysis regarding for RFS.

Summary/Conclusions: The mutations in *TP53* and *JAK2* genes could be considered as a novel biomarkers associated with poor prognostic in B-ALL patients.

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P534

NBN GENE VARIABILITY IS ASSOCIATED WITH INCREASED RISK OF CNS RELAPSE AMONG CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: *NBN* gene encodes nibrin which is a part of the MRN complex responsible for DNA double-strand brakes (DSB) repair. Homozygous mutation in *NBN* gene leads to rare autosomal recessive immunodeficiency associated with chromosomal instability and increased predisposition to lymphoproliferative disorders, called Nijmegen breakage syndrome (NBS). Although the role of homozygous deletion (657del5) of *NBN* gene in ALL development has been established, the impact of heterozygous variants of *NBN* gene on clinical course of childhood ALL remains undefined.
Aims: To determine clinical course and biological features of childhood ALL in homo- and heterozygous carriers of 657del5 mutation in *NBN* gene.
Methods: In total, 593 children (median age 6.48±4.64 years, median follow-up 3.31 years) with newly diagnosed BCP-ALL treated in 15 centers of the Polish Pediatric Leukemia/Lymphoma Study Group were enrolled in the study. We detected 657del5 mutation in *NBN* gene using PCR with fluorescent primer and capillary electrophoresis. Positive results were subsequently confirmed by Sanger's sequencing. Targeted copy number screening of selected 23 loci was performed on available DNA samples (n=366) using the P335-B2 SALSA MLPA kit (MRC-Holland, Netherlands). *IKZF1* deletions were additionally identified using multiplex-PCR with breakpoint-specific primers. Survival analysis with estimation of the probability of overall survival (OS), event-free survival (EFS) and relapse-free survival (RFS) was performed with respect to cytogenetic and molecular features of ALL, clinical characteristics at diagnosis (WBC, age, gender, risk group, CNS involvement) as well as response to treatment (steroidoresistance and minimal residual disease at day 15).
Results: In total, we identified n=23 patients (3.88%) with 657del5 in *NBN* gene, four of them (17.39%) harbouring biallelic deletions (Nijmegen breakage syndrome) while the remaining n=19 were heterozygous carriers (82.61%). Carriers of 657del5 in *NBN* had shorter EFS compared to wild type patients (median 2.05 years, IQR: 1.52-3.82 years *vs* 3.16 years, IQR:1.89-4.79 years; p=0.082). No significant differences in OS were observed. Interestingly, we found that CNS relapse of BCP-ALL was significantly more frequent in 657del5 carriers as compare to wild-type patients (17.65% *vs* 1.87%; p=0.002). In the multivariate analysis we found that steroidoresistance (OR=15.82, 95%CI 2.44-102.37, p=0.004) and being a carrier of 657del5 in *NBN* (OR=38.46, 95%CI 5.69-259.22, p<0.001) are independent risk factors increasing the risk of CNS relapse.
Summary/Conclusions: We report for the first time the association between *NBN* gene variants and biological and clinical features of childhood ALL. In our study cohort, carriers of 657del5 in *NBN* gene who were diagnosed with BCP-ALL had increased risk of CNS relapse regardless CNS involvement at diagnosis. Carriers of 657del5 have worse prognosis (shorter EFS) comparing to wild-type patients.

P535

TARGETED LOCUS AMPLIFICATION & NEXT GENERATION SEQUENCING FOR THE DETECTION OF RECURRENT AND NOVEL GENE FUSIONS FOR IMPROVED TREATMENT DECISIONS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Despite developments in targeted and whole genome gene sequencing, the robust detection of all genetic variation, including structural variants, in and around genes of interest and in an allele-specific manner remains a challenge. Targeted Locus Amplification (TLA) [de Vree *et al.*, Nature Biotechnology 2014;32:1019-25] is a strategy to selectively amplify and sequence entire genes on the basis of the crosslinking of physically proximal sequences. Unlike other targeted re-sequencing methods, TLA works without detailed prior locus information, as one or a few primer pairs are sufficient for sequencing tens to hundreds of kilobases of surrounding DNA. This enables robust detection of single nucleotide variants, structural variants and gene fusions in genes of interest.
Aims: We describe the use of TLA and NGS to detect fusion genes and sequence mutations relevant for stratification of B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Genomic profiling of BCP-ALL in the last few years has substantially extended the number of risk factors that can be used for risk stratification. However, conventional tests provide incomplete sequence information and can therefore miss clinically relevant information. In addition the opportunities TLA presents in the detection of breakpoint sequences promise to empower breakpoint specific minimal residual disease tests.
Methods: A total of 31 primer sets targeting 19 recurrently affected genes were designed and multiplexed, including the 'classical' players *MLL*, *RUNX1*, *TCF3*, and *IKZF1*, the tyrosine kinase genes *ABL1*, *ABL2*, *PDGFRB*, *CSF1R*, *JAK1*, *JAK2*, *JAK3*, *FLT3*, and *TYK2*, and the cytokine signaling genes *CRLF2*, *EPOR*, *IL7R*, *TSLP*, *SH2B3*, and *IL2RB*. Primer sets were chosen such that the most relevant regions were sufficiently covered. Viable cells from 47 selected BCP-ALL samples were analysed. TLA prep was performed, TLA amplicons were library prepped using Nextera and sequenced on an Illumina NextSeq.
Results: All 20 rearrangements known to be present in these samples were detected by TLA, including rearrangements in *ETV6-RUNX1* (n=5), *MLL* (n=2), *TCF3-PBX1* (n=3), *CRLF2* (n=3), *EBF1-PDGFRB* (n=2), *BCR-ABL1* (n=1), *RCSD1-ABL2* (n=1), *SSBP2-CSF1R* (n=1) and iAMP21 (n=2). For 14 of the fusions sequencing depth was sufficient to extract breakpoint-spanning sequences directly. For two cases with known *JAK2* fusions with an unknown partner, the fusion gene was identified (*TERF2* and *BCR*), as was the case for an unknown ABL1 fusion (*FOXP1*). New fusions were identified in 8 cases, including previously described *IGH@-EPOR*, *TCF3-ZNF384*, *MLL,* and *CRLF2* fusions, and novel gene fusion of *TCF3-CD163L1* and *HDAC9-FLT3.* In addition we identified multiple deletion breakpoints in *IKZF1*, and sequence mutations in *JAK2*.
Summary/Conclusions: We conclude that TLA is an effective method for the reliable detection of sequence mutations and structural variations that are relevant for disease prognosis and/or improved treatment decisions.

P536

TARGETING BET FAMILY PROTEINS IMPROVES THE THERAPEUTIC EFFICACY OF BCL-2 INHIBITION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: More effective and less toxic therapies are required for the treatment of T-cell acute lymphoblastic leukemia (T-ALL). Recently, we and others reported promising therapeutic activity for ABT-199, a highly specific inhibitor of the anti-apoptotic BCL-2 protein, in immature subtypes of human T-ALL. Nevertheless, ABT-199 sensitivity is variable between different T-ALL patient samples and the emergence of therapy resistance together with the occurrence of dose-limiting toxicities provides a rationale for the evaluation of ABT-199 as part of combination therapies. Indeed, previous studies have shown that ABT-199 can synergize with conventional chemotherapeutic agents in human T-ALL.
Aims: In this study, we aimed to identify novel synergistic drug combinations with the BH3 mimetic ABT-199 in the context of human T-ALL.
Methods: A drug-screening platform was used to identify promising drug combinations of ABT-199 with a panel of 21 drugs, including approved anticancer drugs and emerging investigational and preclinical compounds, in a panel of primary T-ALLs. Subsequently, *in vitro* synergism was analyzed by determination of the combination index (CI) based on cell viability. Moreover, *in vivo* drug treatment assays were performed using xenografts of luciferase-labeled T-ALL cells. Finally, microarray-based gene expression analysis, RT-qPCR, Western blot and co-immunoprecipitation assays were used to obtain additional insights in the molecular mechanisms of drug synergism.
Results: A drug screening in 6 primary human T-ALLs (5 diagnostic and one relapse specimen) revealed strong anti-leukemic effects for the combination of ABT-199 with BET bromodomain inhibitors, including JQ1 and OTX-015. *In vitro* drug synergism between these molecules was confirmed in a panel of 13 human T-ALL cell lines and the degree of synergism was significantly correlated with BCL-2 expression levels. Furthermore, *in vivo* drug treatment experiments using xenografts of luciferase-positive LOUCY cells confirmed the synergistic activity of the JQ1/ABT-199 combination therapy as evaluated by bioluminescence, percentage of leukemic blasts in blood or bone marrow and spleen size.

Finally, to better understand the molecular mechanism by which BET bromodomain inhibition enhances the anti-leukemic properties of ABT-199, we defined the transcriptional consequences of JQ1 in synergistic T-ALL cell lines. Notably, *BCL2L11* expression was rapidly induced after short-term JQ1 exposure. As a result, BET bromodomain inhibition triggered an enhanced binding of BIM to BCL-2 in synergistic T-ALL cell lines, providing a putative explanation for the improved therapeutic efficacy of ABT-199 and JQ1 in human T-ALL.
Summary/Conclusions: BET bromodomain inhibition can sensitize BCL-2-positive human T-ALL to ABT-199 treatment by interfering with the balance of pro- and anti-apoptotic BCL-2 family members.

P537

PATIENT-REPORTED OUTCOMES FROM A GLOBAL PHASE 3 RANDOMIZED CONTROLLED TRIAL OF INOTUZUMAB OZOGAMICIN *VERSUS* STANDARD CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, has demonstrated superior clinical activity *versus* standard care (SC; intensive chemotherapy) for relapsed/refractory acute lymphoblastic leukemia (ALL) in the ongoing phase 3 INO-VATE trial (DeAngelo, European Hematology Society 2015 meeting [abstract: LB2073])
Aims: To assess quality of life (QoL), functioning, and symptoms in patients with relapsed/refractory ALL receiving InO *versus* those receiving SC, based on patient-reported outcomes (PROs) from this phase 3 trial.
Methods: Patients were randomized to InO (max 1.8 mg/m^2/cycle [≤6 cycles]) or SC (fludarabine/cytarabine [ara-C]/granulocyte colony-stimulating factor, ara-C+mitoxantrone, or high-dose ara-C [≤4 cycles]) and completed the European Organization for Research and Treatment of Cancer Quality of Life Core Questionnaire (EORTC QLQ-C30) and EuroQoL 5 Dimensions questionnaire (EQ-5D) at baseline, day 1 of each cycle, and end of treatment. Treatment differences in PRO measures over time were assessed in the intent-to-treat population using longitudinal mixed-effects models with random intercepts and slopes. Analyses were supportive and no multiplicity adjustments were made. Informed consent was obtained from all patients.
Results: 141 and 138 patients were randomized to InO or SC; PROs completion rates were 85% and 64%, respectively. Overall, patients receiving InO reported numerically better quality of life (QoL), functioning and symptom scores at each cycle *versus* SC, with statistically significant differences in least squares mean Physical and Role Functioning (7.6 and 11.5, respectively; $P<0.02$). Mean treatment differences in favor of InO in EQ-VAS, Global Health Status/QoL, Social Functioning, Dyspnea, Appetite Loss, and Fatigue exceeded or approached 5 (generally considered the minimally important difference [MID] to be clinically meaningful), although without statistical significance. Other dimensions directionally favored InO, except Emotional Functioning, Constipation, and Pain, but none approached MID.
Summary/Conclusions: This is the first time that PROs are reported in a large randomized controlled trial of ALL. QoL, functioning, and symptoms in relapsed/refractory ALL patients receiving InO appeared generally better than those receiving SC. These data support the favorable benefit risk ratio of InO for relapsed/refractory ALL treatment, with superior clinical efficacy that does not compromise patients' QoL.

P538

DELETIONS OF IKZF1 GENE, BUT NOT GENETIC RISK GROUPS, DEFINED BY COPY-NUMBER ALTERATIONS, ARE PROGNOSTICALLY SIGNIFICANT FOR PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA ON ALL-MB 2008 PROTOCOL

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Background: Recently *A. Moorman et al* introduced new stratification strategy for pediatric B-cell precursor ALL (BCP-ALL) based on cytogenetic risk groups and copy-number alterations (CNAs) detected by single multiplex ligation probe amplification (MLPA) kit (*Blood. 2014; 124(9):1434-1444*).
Aims: To test refined diagnostic strategy on independent cohort of childhood BCP-ALL enrolled into Russian multicenter trial ALL-MB 2008.
Methods: 142 BCP-ALL patients with median age of 3.1 years (range 1-16 years) were included in the current study based on availability of genomic DNA obtained at the time of diagnosis. Median of follow-up period was 4.2 years. Ph-positive ALL was revealed in 2 patients; both of them were allocated into high risk group (HRG) and included in the study. MRD was evaluated by multicolor flow cytometry with sensitivity non-less than 0.01%. CNAs were estimated by SALSA MLPA P335 ALL-IKZF1 probemix, all revealed *IKZF1* deletions were confirmed by SALSA MLPA P202 IKZF1 (IKAROS) probemix (both MRC-Holland, The Netherlands). Informed consent was obtained in all cases.
Results: *IKZF1* deletions were found in 15 (10.6%) patients, deletions of *CDKN2A/B* was detected in 44 (31.0%) cases, *BTG1* deletions in 12 (8.4%), *EBF1* in 2 (1.4%), *PAX5* in 46 (32.4%), *ETV6* in 39 (27.5%), *RB1* in 11 (7.7%), *PAR1* region was deleted in 9 (6.3%) cases. Based on cytogenetic risk and CNA group assessment 111 (78.2%) patients allocated to the genetic good risk (GGR) group, 31 (21.8%) to the genetic poor risk (GPR) group. Patients referred to the GGR group had higher EFS than patients in GPR group (0.89±0.03 *vs* 0.59±0.11, p=0.002) and lower cumulative incidence of relapse (CIR) (0.07±0.03 *vs* 0.39±0.11, p<0.001). Although in multivariate analysis the relation between genetic risk group stratification and EFS as well as relapse risk was not statistically significant. While *IKZF1* deletions (HR 5.214 (95% CI 2.045-13.292), p=0.001), WBC count at diagnosis more than $50*10^9$/L (HR 3.273 (95% CI 1.264-8.476), p=0.015) and M3 status of bone marrow (BM M3) at day 15 (HR 2.711 (95% CI 1.612-4.559), p<0.001) were significantly associated with decreased EFS. Presence of *IKZF1* deletions also led to higher relapse risk (HR 15.175 (95% CI 3.676-62.643), p<0.001) and it was independent of other risk factors. After that we estimated the role of *IKZF1* deletions, solely. Patients with *IKZF1* deletions were older (9.7 *vs* 3.2 years, p=0.007) and more frequently stratified to HRG (53.3% *vs* 5.5%, p<0.001) than the 127 cases without *IKZF1* deletions. *IKZF1* deletions were associated with delayed blast clearance, assessed by BM M3 status at day 15 (p=0.003), lack of hematological remission at day 36 (p<0.001). As a result, the *IKZF1*-positive cases more often had MRD levels ≥10% at day 15 (45.4% *vs* 12.4%, p=0.014), ≥0.1% at day 36 (66.7% *vs* 13.1%, p<0.001) and ≥0.01% at day 85 (41.7% *vs* 6.6%) than those with normal status of *IKZF1. IKZF1* deletions led to significantly worse outcome in the whole cohort of patients (EFS 0.30±0.15 *vs* 0.89±0.03, p<0.001, CIR 0.67±0.18 *vs* 0.07±0.02 P<0.001). Additionally, *IKZF1* deletions retained their prognostic significance in different subsets of patients: in the 'B-other' group, in BM M3 as well as in BM M1+M2 groups at day 15, in MRD-defined risk groups at day 15, day 36 and day 85, in ALL-MB 2008 ImRG patients, but not in SRG or HRG. Notably, all 2 SRG patients with *IKZF1* deletions remained in the first CCR.
Summary/Conclusions: We could not confirm that stratification for genetic risk groups was independent of other factors. It might be protocol-dependent. In contrast, *IKZF1* deletions led to unfavorable outcome in the whole cohort of patients, various subgroups and appeared to be MRD-independent.

P539

LACK OF PROGNOSTIC SIGNIFICANCE OF CD20 EXPRESSION IN ADULTS WITH PHILADELPHIA CHROMOSOME-NEGATIVE, B-CELL PRECURSOR ALL TREATED ACCORDING TO MRD-ORIENTED PROTOCOLS

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Background: Several studies have reported a poorer prognosis for patients with Ph-negative, B-cell precursor (BCP) ALL expressing CD20 in ≥20% blasts. This has led to perform phase II and III studies demonstrating an improvement in prognosis with the addition of anti-CD20 monoclonal antibodies to the initial treatment. However, little is known about the prognostic value of CD20 expression in BCP ALL patients treated according to MRD-oriented protocols.
Aims: To analyze the frequency and prognostic impact of CD20 expression in adult patients (18 - 59 yr) with Ph-negative, BCP ALL treated with the MRD-oriented protocols ALL-HR-03 and ALL-HR-11 (for patients with high risk ALL) and ALL-SR-08 (for patients with standard risk ALL) from the Spanish PETHEMA Group.
Methods: Immunophenotypic reports of adult patients with Ph-negative, BCP

ALL included in the above referred protocols were reviewed. CD20 expression ≥20% was considered to define CD20 positivity (CD20+) as this cut-off level has been selected in some studies performing treatment with antiCD20 MoAb. MRD was assessed by multiparameter flow cytometry. High-risk (HR) Ph-negative, BCP ALL patients were defined according to one of the following criteria: age (≥30 years), WBC count (>30x10^9/L) or cytogenetics (11q23/MLL gene rearrangements), whereas patients lacking all these features were considered as standard-risk (SR) ALL. HR-ALL patients included in ALL-HR-03 and ALL-HR-11 protocols received intensive induction and consolidation therapy followed by allogeneic HSCT if the MRD clearance was poor, whereas SR-ALL cases received a pediatric-based protocol, allogeneic HSCT being also indicated if persistent MRD positivity.

Results: Overall 308/362 patients included in the three protocols were evaluable for CD20 expression, of whom 111 (36%) were CD20+. Compared with patients with CD20 level ≤20%, CD20+ cases showed a trend towards lower WBC count (X[SD] 33[76] *vs* 46[77]x10^9/L, P=0.05) and a higher frequency of common+pre-B phenotypes (107/111 *vs* 148/197, P<0.001). No differences in outcomes (CR rate, CIR, DFS and OS) were observed on comparison of CD20+ patients with the other cases, neither for the whole series nor for the different risk groups (Table 1). Similar results were observed after censoring the follow-up of patients at the time of HSCT. The lack of prognostic impact was observed after stratification (cut-off) for age (30 yr.) and WBC count (30x10^9/L).

Table 1.

	CD20	CR rate	4-yr CIR (CI95%)	4-yr DFS (CI95%)	4-yr OS (CI95%)
Whole series (n=308)	≥20% (n=111)	103 (93%)	35% (24;47)	40% (28;52)	50% (39;61)
	<20% (n=197)	180 (91%)	44% (35;53)	42% (33;51)	48% (39;57)
HR ALL (n=250)	≥20% (n=92)	84 (91%)	36% (24;48)	35% (22;48)	43% (31;55)
	<20% (n=158)	144 (91%)	47% (37;56)	36% (26;46)	40% (31;49)
SR ALL (n=58)	≥20% (n=19)	19 (100%)	39% (0;68)	61% (22;100)	88% (65;100)
	<20% (n=39)	36 (92%)	32% (8;50)	68% (47;89)	82% (67;97)

Summary/Conclusions: One third of adult patients (18-59 yr) with Ph-negative, BCP ALL treated with MRD-oriented PETHEMA protocols expressed CD20 ≥20%. CD20 expression ≥20% did not show prognostic significance in this study.

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Acute myeloid leukemia - Biology 2

P540

PROTEIN PHOSPHATASE 4 REGULATORY SUBUNIT 2 (PPP4R2) IS RECURRENTLY DELETED IN ACUTE MYELOID LEUKEMIA (AML) AND REQUIRED FOR EFFICIENT DNA DOUBLE STRAND BREAK REPAIR

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Background: We have previously identified a commonly deleted region (CDR) at chromosomal band 3p14.1-p13 in cytogenetically normal (CN) and complex karyotype (CK) AML. This CDR has also been described in solid tumors like cervix and prostate cancer. Within the CDR, *PPP4R2* is one of eight affected genes. As part of the protein phosphatase 4 (PPP4) complex, PPP4R2 was shown to be involved in the DNA repair of double-strand breaks (DSBs) by dephosphorylation of DNA damage response (DDR) proteins like phosphorylated histone H2AX (γH2AX) or KRAB-domain associated protein 1 (pKAP1) in osteosarcoma or cervix carcinoma cells.

Aims: To investigate the role of *PPP4R2* in hematopoiesis and leukemogenesis.

Methods: Gene expression of *PPP4R2* was analyzed by qRT-PCR in a cohort of AML patients (pts, n=63), that is 3p CDR within CN-AML (n=10), 3p CDR within CK-AML (n=26), CN-AML (n=24), CK-AML (n=3), and healthy bone marrow (BM) controls (n=8). Lentiviral transduction with *PPP4R2*-ORF was used to overexpress (OE) *PPP4R2* in the human myeloid leukemic cell line MEG-01 with a homozygous deletion of *PPP4R2*. Knockdown (KD) of *Ppp4r2* was performed by lentiviral delivery of *Ppp4r2*-shRNA in murine pre-leukemic MLL-AF9 and normal Lineage negative (Lin^-) BM cells. We determined the effects on proliferation by MTS-Assay, clonogenic growth in methylcellulose, and apoptosis and DDR by flow cytometry and Western blot analysis.

Results: Compared to healthy BM, *PPP4R2* expression was significantly lower in AML pts with lowest expression levels in CN-AML 3p CDR cases and CK-AML 3p CDR cases (p=0.007; p<0.0001, respectively). *PPP4R2* OE in MEG-01 cells considerably reduced proliferation (p=0.007), as well as colony forming potential (p=0.07). On the other hand, apoptosis caused by DSBs upon ionizing radiation (IR) was not induced in *PPP4R2* OE MEG-01 cells, suggesting a restoration of DDR. In line, *PPP4R2* OE in MEG-01 cells showed a significant decrease of pKAP1 (S824), reduced γH2AX (S139) [0.5 h post (p.) IR: p=0.01, p=0.4; 2 h p. IR: p=0.0001, p=0.8, respectively] and less P53 (S15) activation. Next, we determined loss-of-function effects upon *Ppp4r2* KD in murine MLL-AF9 transformed BM cells. In this leukemia model, *Ppp4r2* KD significantly decreased cell proliferation (p<0.0001) as well as colony forming potential (p=0.0002), but enhanced repopulating capacity (p=0.04). IR caused enhanced apoptosis in *Ppp4r2* KD cells (48 h p. IR: p=0.009) that was accompanied by significant accumulation of pKAP1, γH2AX (0.5 h p. IR: p=0.005, p=0.0002; 2 h p. IR: p=0.004, p<0.0001, respectively), and activated P53, indicating constitutive DNA damage and less efficient DNA repair. Furthermore, we assessed the effect of *Ppp4r2* KD in Lin^- BM cells. *Ppp4r2* KD reduced proliferation (p=0.3), but not clonogenic growth (p=0.6). In response to IR, *Ppp4r2* KD in Lin^- BM cells resulted in a higher amount of apoptotic cells (48 h p. IR: p=0.07), which was associated with elevated DDR protein pKAP1 and γH2AX levels (0.5 h p. IR: p=0.02, p=0.6, respectively).

Summary/Conclusions: As candidate gene of a CDR on 3p in AML pts, *PPP4R2* displayed lowest expression in CN-AML with 3p CDR. Furthermore, we identified *PPP4R2* as a critical regulator of DDR in both normal hematopoietic and leukemic cells via deregulation of the PPP4 complex. While impaired DNA repair and constitutive DNA damage might contribute to the pathogenesis of AML, further studies are warranted to determine potential additive effects of *PPP4R2* deletion to leukemogenesis.

P541

PRELEUKEMIC CLONES IN NPM1-MUTATED AML MAY BE ASSOCIATED WITH EVOLUTION TO MYELODYSPLASTIC SYNDROME (MDS) OR MYELOPROLIFERATIVE NEOPLASM (MPN)

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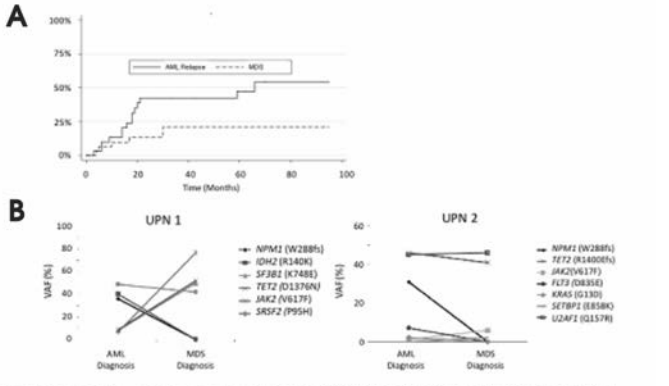
Background: Nucleophosmin 1 gene (*NPM1*) mutations account for 30% of AML and 50% of cytogenetically normal (CN) AML. *NPM1* mutated AML without *FLT3*-ITD (NPM 1^+FLT3 ITD^-) is classified in the favorable risk group. Recent studies, including from our group, showed that most *NPM1*$^+$*FLT3*-ITD^-AML also carry mutations in other genes such as *DNMT3A, IDH1, IDH2, TET2* muta-

tions which may constitute "preleukemic" clones, as suggested by the fact that those mutations often persist in patients with undetectable *NPM1* minimal residual disease (MRD) after intensive chemotherapy (Peterlin Haematologica 2015, Corces-Zimmerman PNAS 2014). On the other hand, a recent report showed that virtually all relapses in *NPM1*$^+$AML carried *NPM1* mutation, arguing against the fact that relapse can emerge from those preleukemic clones, independently of the presence of *NPM1* mutation (Hills NEJM 2016).

Aims: To better understand the genetic mechanisms underlying disease evolution in patients with *de novo* CN *NPM1*$^+$ *FLT3*-ITD$^-$AML in complete remission (CR).

Methods: Over a 10-year period, 34 *de novo* CN *NPM1*$^+$ *FLT3*-ITD$^-$AML (median age 55 years) were treated with intensive chemotherapy at our center, and 32 achieved CR. 12 (37%) remained in hematological CR with undetectable *NPM1*-MRD, 14 (44%) relapsed with the same *NPM1* mutation, and 6 (19%) developed MDS or MPN while still in molecular CR for NPM1 (Figure 1A). 12 of the relapsing patients and all 6 patients with MDS or MPN evolution had sequential molecular analysis using NGS of PCR-amplified exons of a panel of the 26 genes most frequently mutated in myeloid malignancies.

Results: The 6 patients who developed MDS or MPN with undetectable *NPM1*-MRD, after a median of 14 months (range: 4-30), were all aged >55 years, and included 2 RARS, 2 RCMD, 1 CMML type 1 and 1 primary myelofibrosis (PMF). In all 6 cases, a "preleukemic" clone with at least 1 mutation in *TET2 (n=4), JAK2 (n=2), ASXL1 (n=1), IDH2 (n=3)* or spliceosome genes (SRSF2, SF3B1, U2AF1, (n=3)) was found at AML diagnosis, and was still present (with stable or increasing variant allele frequency) at the time of MDS or MPN diagnosis (Figure 1B). In the 12 relapsing patients, "preleukemic" mutations were also present at AML diagnosis in 10/12 cases. However, they possibly differed between the 2 groups: At AML diagnosis, *TET2* mutation was identified in 4/6 (66%) patients who developed MDS or MPN, *versus* 2/12 (16%) patients who relapsed, p=0.1). Conversely, 8/12 patients who relapsed had mutations in *DNMT3A* or *IDH1*, *versus* 0/6 patients who developed MDS or MPN (p=0.025). At AML relapse, mutations co-occurring with *NPM1*mutation were identical to those observed at AML diagnosis.



a. Cumulative incidence of relapse and evolution to MDS/MPN in 32 patients with *NPM1*$^+$, *FLT3*-ITD$^-$ cytogenetically normal AML in first CR.
b. Evolution of Variant Allele Frequency in mutated genes at AML diagnosis and MDS/MPN diagnosis in 2 patients who developed PMF (UPN 1, left) or MDS (UPN 2, right), with undetectable *NPM1* MRD.

Figure 1.

Summary/Conclusions: Our study suggests that a significant proportion of patients with *de novo NPM1*$^+$*FLT3*-ITD$^-$ CN-AML (19% in our series) may subsequently develop MDS or MPN in the absence of *NPM1* mutation recurrence, and that this type of hematological evolution seems to be related to the persistence and expansion of preleukemic clones. The high incidence of such evolution may be related to the relatively advanced age of our patient cohort, due to our hospital unit recruitment (mostly elderly AML). Interestingly, in most patients with AML relapse, preleukemic clones were also observed at AML diagnosis but with a different mutational pattern, possibly pointing out different pathophysiological mechanisms. If those findings are confirmed in a larger number of patients, *NPM1*$^+$ AML patients may have to be closely followed-up after CR achievement even when *NPM1* MRD remains undetectable, due to the relatively high risk of MDS or MPN, at least in elderly patients.

P542

CLINICAL, HEMATOLOGICAL, BIOLOGIC AND MOLECULAR CHARACTERISTICS IN PATIENTS WHO DEVELOP ACUTE MYELOID LEUKEMIA FROM CHRONIC MYELOMONOCYTIC LEUKEMIA

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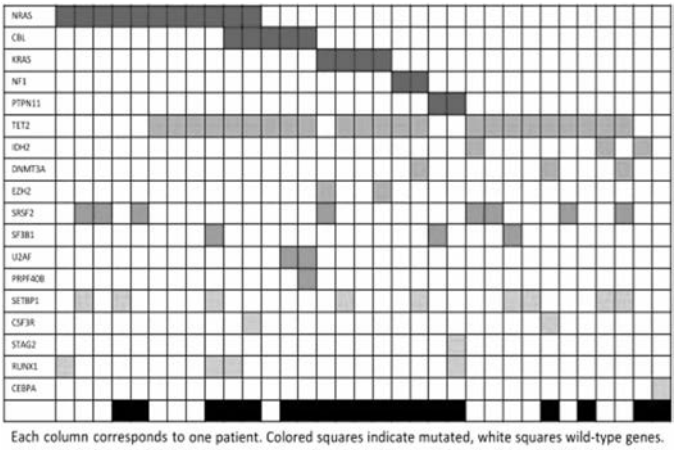
Background: Little is known about factors contributing to the transformation of CMML into secondary AML. In the "Austrian Biodatabase for Chronic Myelomonocytic Leukemia (ABCMML)" we retrospectively and prospectively collect clinical, biologic and molecular information of patients with CMML from different centers in a real life setting. Up to now 355 patients have been included, cytogenetic data are available in 186 patients, targeted next-generation sequencing (NGS) data in 138 patients, and *in vitro* culture data in 120 patients, respectively.

Aims: Our aim was to characterize the clinical, molecular and biologic features of patients with CMML who develop AML.

Methods: Clinical, hematological and cytogenetic data were obtained from patients records. For molecular characterization we used NGS with amplicon-based target enrichment of 39 CMML associated genes. Only mutations with an allele burden of 10% or higher were considered positive in this analysis. Since increased spontaneous myeloid colony formation is an *in vitro* characteristic of RAS pathway hyperactivation *(Wang et al., Blood 2010)* colony-forming units granulocyte/macrophage (CFU-GM) growth in the absence of exogenous cytokines was assessed using semisolid cultures as previously described (*Geissler et al., J Exp Med 1996)*. Transformation into AML was defined as a blast cell percentage of at least 20% in the peripheral blood and/or bone marrow.

Results: Of the 355 CMML patients studied, we identified 47 who had already CMML derived AML at the time of inclusion (group A), 80 patients who later had documented transformation into AML (group B) and 228 patients, in whom no transformation was recorded (group C).

Characteristics (median values, ranges) [p-values] of CMML patients who developed AML (group A+B) *vs* CMML patients without AML (group C) were: age (years): 70 (34-88) *vs* 73 (45-92) [0.14]; WBC (G/l): 14.0 (0.7-360) *vs* 14.2 (1.6-156) [0.818]; hemoglobin (g/dl): 9.8 (6.0-16.7) *vs* 11.0 (5.1-12.2) [0.000]; platelets (G/l): 89 (5.0-435) *vs* 90 (2.0-709) [0.435]; PB blasts (%): 14 (0-79) *vs* 0 (0-18) [0.000]; LDH (U/l): 294 (106-3685) *vs* 259 (102-1912) [0.013]; OS (months): 23.1 *vs* 38.7 [0.004]; CFU-GM per 10^5 PBMNC: 333 (0-4533) *vs* 13 (0-1009) [0.002]; (range in normal individuals 3.5 – 8.5). Thus, CMML patients who developed AML had a significantly inferior survival, lower hemoglobin value, higher LDH, and higher spontaneous CFU-GM formation than patients who did not develop secondary AML. The incidence of RAS pathway mutations in group A+B (67%; 22/33) was significantly higher than in group C (37%; 39/105), [p=0.003]. The detailed pattern of mutations in RAS pathway, epigenetic regulator and spliceosome components in CMML patients who developed AML (group A+B) is shown in Figure 1.



Each column corresponds to one patient. Colored squares indicate mutated, white squares wild-type genes. The colores of mutant genes indicate the most affected functional categories. Red, green, blue and yellow represent the RAS pathway, epigenetic regulators, spliceosome, and other components, respectively. At the bottom of the graph black squares indicate patients who had AML at the time of sampling and open squares patients who later developed AML.

Figure 1. Mutation status of genes in CMML patients who developed AML.

RAS pathway mutations were found in 15 of 19 (79%) CMML patients in whom molecular profiles were studied at the time of AML and in 7 of 14 (50%) CMML patients who later developed AML. The incidence of TET2 mutations was 70% (23/33) in group A+B which was not significantly different from the incidence in

group C (63%; 66/105), [p=0.474]. There was also no significant difference in the incidence of mutations with regard to other epigenetic modulators and to components of the spliceosome. Other mutations were rare and were also not significantly different. The most common cytogenetic abnormalities were+8 (12%), -7/del(7q) (6%), and –Y (4%), respectively, without significant differences in the two groups.
Summary/Conclusions: Our data show that patients with CMML who transform into AML have an unfavorable prognosis. In the majority of them the transformation process seems to be associated with hyperactivation of the RAS pathway. In order to improve outcome RAS pathway inhibitors should be studied in this high risk cohort.

P543

MOLECULAR CLASSIFICATION AND COMPARATIVE ANALYSIS OF ACUTE MYELOID LEUKEMIA MULTI-OMICS DATA USING THE INTERACTIVE HEMAP RESOURCE

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Background: Collections of genome-wide data can facilitate the characterization of disease states. The current challenge is to unify pre-existing patient molecular data to allow i) subtype-specific mechanisms to be distinguished from common mechanisms that underlie the departure from healthy states and ii) to evaluate disease contexts for promising drug targets.
Aims: The first goal in this study was to determine the robustness of molecular stratifications in acute myeloid leukemia (AML) by comparing them across different datasets and clinical annotations. We further focused on characterization of acute myeloid leukemia (AML) cases with normal or complex karyotypes and develop methodology for *in silico* evaluation of drug specificity.
Methods: The unsupervised dimensionality reduction method t-Distributed Stochastic Neighbor Embedding (t-SNE) was used to stratify a heterogeneous collection of 1,713 AML transcriptomes. We developed bioinformatics methodology for inclusion of new samples and unsupervised discovery of their cytogenetic type. Additional multi-omics datasets of 99 patients from The Cancer Genome Atlas were integrated to gain insight into disease biology. *In silico* screening of drug target expression was combined with chemical screen data of target specificity to compare two FLT3 inhibitors that are in ALL and AML clinical trials (Lestaurtinib and Tandutinib, respectively). A larger collection of 9,544 transcriptomes from 36 hematological malignancies and healthy blood cell type controls was utilized for this purpose.
Results: The current clinical classification of AML distinguishes between fusion-gene-positive categories. Unsupervised clustering in the t-SNE space yielded a performance comparable to that of robust and reproducible classifiers. Furthermore, we found additional clusters using t-SNE maps that corresponded to a distinct mutational pattern (CEBPA, NPM1 or RUNX1/TP53). CEBPA-mutated cases were found to exhibit elevated expression of S-adenosylmethionine-dependent methyltransferase pathway and we could show that this leads to globally elevated DNA methylation levels genome-wide. Expression patterns of targets inhibited by Lestauritinib revealed that it has the potential to act on considerably more genes (in addition to *FLT3*), of which several targets with low Kd values are also expressed in normal cell types, thus having potential to lead to side effects. Comparison of AML and other malignancies showed that the more specific Tandutinib may also be a promising drug for lymphoma trials, based on the multiple low Kd targets associated with this disease context.
Summary/Conclusions: Our results revealed molecular phenotypes that distinguish normal karyotype AML samples. The generated interactive resource, Hemap, is available online for investigating the molecular states of hematological malignancies and expediting therapeutic innovations. The FLT3 inhibitor currently evaluated in AML clinical trials, Tandutinib showed highly AML-specific expression profile of its low Kd targets and has potential for drug repositioning in lymphoma.

P544

SOMATIC DDX41 P.R525H MUTATION CAUSES GROWTH DEFECT IN HEMATOPOIETIC CELLS

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Background: The *DDX41* gene, encoding a DEAD-box type ATP-dependent RNA helicase, is rarely but reproducibly mutated in myeloid diseases. The acquired mutation in *DDX41* is highly concentrated at c.G1574A (p.R525H) in the conserved motif VI located at the C-terminus of the RecA-like helicase core domain where ATP interacts and is hydrolysed. Therefore, it is likely that the p.R525H mutation in the DDX41 protein perturbs the ATPase activity in a dominant-negative manner.
Aims: This study was aimed at elucidating the molecular functions of DDX41 protein and the role of p.R525H DDX41 mutant in leukemogenesis.
Methods: In this study, we first screened for the *DDX41* mutation of CD34-positive tumour cells based on mRNA-sequencing and identified the p.R525H mutation in three cases among 23 patients. Then we analyzed changes in gene expressions and phenotype of hematopoietic cells transduced with the DDX41 mutant.
Results: We initially found that the patients harboring the p.R525H DDX41 mutation commonly exhibited AML with peripheral blood cytopenias and low blast counts, suggesting that the mutation inhibits growth and differentiation of hematopoietic cells. To address molecular functions of DDX41, we clarified the localization of DDX41 protein. Ectopically expressed Myc-tagged DDX41 was mostly nuclear regardless of the p.R525H mutation. In addition, the majority of the endogenous DDX41 protein was also mostly nuclear. In the nucleus, DDX41 protein was localized both in the nucleoplasm and in the nucleolus, implying that DDX41 plays a role in the nucleoplasm as well as in the nucleolus. We then transduced cord blood-derived CD34-positive cells with DDX41 wild type (WT) or p.R525H. After a 30-day culture, p.R525H cells showed decreased proliferation compared with WT cells, with a certain ribosomopathy phenotype as suggested by GSEA analysis. This finding and the highly conserved DEAD-box type RNA helicase domain of DDX41 led us to speculate that the enzyme might be involved in the pre-rRNA processing. Northern blot analysis probing internal spacer (ITS) 1 and 2 of pre-rRNA showed that signals of 47S and 41S pre-rRNAs were increased, whereas the 21S signal was decreased in THP-1 cells expressing DDX41 p.R525H. Although the precise phase at which DDX41 takes part in the pre-rRNA processing has not been elucidated yet, this series of experiments suggests a role for DDX41in the trimming of 5' external spacer and/or ITS2. Recent studies on ribosomopathies including 5q- syndrome revealed an activation of the MDM2-p53 pathway in the pathogenesis of the diseases. Although p53 activation was not evident in the p.R525H DDX41 expressing cells, GSEA instead revealed a negative enrichment of cell cycle-promoting genes regulated by the RB-E2F axis in p.R525H DDX41 cells. Cell cycle inhibition through E2F suppression was also detected in patient-derived samples. In our study, we further found increased RPL5 and RPL11 bound to MDM2 in p.R525H DDX41 cells. It is likely that the mutant DDX41 increased free ribosomal proteins that are not incorporated into the 60S ribosome and that these proteins eventually formed a complex with MDM2, thus the degradation of RB by MDM2 might be compromised.
Summary/Conclusions: In summary, we propose a mechanism of growth defect in haematopoietic cells triggered by p.R525H DDX41 occurring in the following order: (i) p.R525H mutant inhibits pre-rRNA processing;, (ii) compromised ribosomal biogenesis as a result of impaired rRNA synthesis causes a release of ribosomal proteins that bind to MDM2; (iii) MDM2-mediated RB degradation is suppressed, thus eventually activating the RB pathway and resulting in the inhibition of E2F activity. Considering late occurrence of AML in patients harbouring the mutation, it might require age-dependent epigenetic alterations or other somatic changes for this mutation to fully transform haematopoietic cells.

P545

WHOLE EXOME SEQUENCING REVEALS NOVEL CANDIDATE GENES IN FAMILIAL MDS/AML

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Background: Myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) are related heterogeneous haematopoietic stem cell clonal disorders characterised by defective haematopoiesis and premature mortality in many patients. The majority of MDS and AML cases are sporadic but there are also rare cases (<5%) where two or more affected individuals are found within the same family (familial MDS/AML). Familial MDS/AML patients represent a high-risk population who require unique follow-up and treatment strategies to achieve cancer risk reduction, prevention and best management. To date, heterozygous germline mutations in 10 disease genes (*ACD, ANKRD26, CEBPA, DDX41, ETV6, GATA2, RUNX1, SRP72, TERC* and *TERT*) have been associated with the inherited predisposition in these families, but explain only 50-60% of cases.
Aims: To identify new germ-line mutations in families with MDS/AML of unknown aetiology
Methods: Bone marrow or peripheral blood samples were collected from 78 families, where two or more members have been diagnosed with a haematological disease (AML, MDS, aplastic anaemia or thrombocytopenia) but including at least one with MDS or AML. Each index case of these families was tested for mutation in the 10 known disease genes using deep targeted sequencing.

Whole exome sequencing (WES) was performed in families with still unknown aetiology. Exome libraries were prepared using Illumina Nextera reagents and sequenced on the HiSeq2000. Germline variant calling was performed using GATK and all functional variants were selected. We used the following criteria to enrich for genes with pathogenic relevance: (i) recurrent genes (those with mutations in at least two families); (ii) novel variants or genetic lesions, not described in dbSNP137, ExAC, HAPMAP or the 1000 Genomes projects; (iii) functional annotation based on both PolyPhen scores (>0.850) and the MutationTaster algorithm (*disease causing*) to assess pathogenicity.
Results: 46 of the 78 families in our cohort (59%) have mutations in 8 of the 10 known loci (*ACD, CEBPA, DDX41, GATA2, RUNX1, SRP72, TERC* and *TERT*). In 32 families of unknown aetiology, whole exome sequencing (WES) was performed and a mean of 422 non-synonymous variants were found per sample. The chosen criteria have allowed us to limit the search to the most relevant germline mutations in our cohort, reducing the number of new candidate disease genes to nine with shared nonsynonymous variants: *ATP13A4, COL2A1, DNAJC10, GLDC, LRP2, MAN1C1, PDCD4, SEC23B* and *TFIP11*.
Summary/Conclusions: We have identified nine new potential candidate disease genes in familial MDS/AML. However, a single unifying candidate gene has not been identified, suggesting genetic heterogeneity. Further studies will establish which of these can be assigned as true 'familial MDS/AML' disease genes.

P546

CLONAL EVOLUTION IN NPM1 MUTATED ACUTE MYELOID LEUKEMIA (AML)

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Background: Mutations in the *nucleophosmin 1* (*NPM1*) gene are considered a key event in the pathogenesis of AML. Recently, we gained insights into clonal architecture and clonal evolution of *NPM1 mutated (NPM1*^{mut}*)* AML. While *DNMT3A* mutations (*DNMT3A*^{mut}) showed the highest stability suggesting *DNMT3A*^{mut} to be an early event in leukemogenesis, we also observed an increase in genomic complexity at the time of relapse.
Aims: To validate our previous results on clonal evolution in a larger cohort of *NPM1*^{mut} AML patients (pts), to define relapse specific mutation patterns as well as to identify the impact on gene expression.
Methods: Paired samples at diagnosis and relapse from 129 *NPM1*^{mut} AML pts were assessed for additional mutations by comprehensive mutation analysis (*FLT3*-ITD, *FLT3*-TKD, *DNMT3A*, *IDH1/2*, *NRAS, ASXL1*, *TP53*, *MLL*-PTD). In addition, AML cases with loss of *NPM1*^{mut} at the time of relapse and with persisting *NPM1*^{mut} were subjected to whole exome sequencing (WES; n=20) and RNA-Seq was performed on selected paired *NPM1*^{mut} loss diagnosis and relapse cases (n=6).
Results: At diagnosis, concurrent gene mutations were found at the following incidence: *FLT3*-ITD 32% (40/126), *FLT3*-TKD^{mut}19% (22/118), *DNMT3A*^{mut} 69% (78/113), *NRAS*^{mut} 19% (22/117), *IDH1*^{mut} 21% (26/123) and *IDH2*^{mut} 18% (22/118). None of the pts analyzed exhibited a *TP53*, *MLL*-PTD or *ASXL1* mutation. At relapse, we found a significant shift in the genetic pattern of 74 pts (59%) with the most frequent changes seen for *FLT3*-ITD, which was lost in 10 and newly acquired in 24 pts. Additional changes affected *FLT3*-TKD^{mut}, *DNMT3A*^{mut}, *NRAS*^{mut}, *IDH1*^{mut}, *IDH2*^{mut}, *ASXL1*^{mut} and *TP53*^{mut}. Notably, gain of *MLL*-PTD (n=4) was restricted to pts with loss of *NPM1*^{mut} at the time of relapse. Based on these findings we calculated the following stabilities for the most frequent mutations: *FLT3-TKD*^{mut}27%, *NRAS*^{mut}45%, *FLT3-ITD* 75%, *IDH1*^{mut} 88% and *IDH2*^{mut} 90%, and *DNMT3A*^{mut} 97%, thereby confirming our previous findings for *DNMT3A*^{mut}. Similarly, *NPM1*^{mut} was lost at relapse in 10 pts (8%) of which 9 pts showed a stable *DNMT3A*^{mut} at relapse. By WES we could confirm persistence of mutations known to be involved in clonal hematopoiesis, such as *DNMT3A* and *TET2* mutations. These were usually seen during all analyzed time points (diagnosis, remission and relapse). Otherwise, we found distinct mutational patterns at the time of relapse compared to the time of diagnosis for *NPM1*^{mut} loss cases, while mutational profiles of cases without *NPM1*^{mut} loss at relapse remained more stable. For example, while the mutational pattern of diagnostic samples showed an enrichment for mutations affecting MYC signaling, at the time of relapse in *NPM1*^{mut} loss cases there was a significant enrichment of mutations in the MAPK signaling cascade. RNA-Seq for selected diagnosis/relapse pairs further supported a switch in signaling cascades at the time of relapse in *NPM1*^{mut} loss pts with differentially expressed genes being significantly enriched for MAPK signaling genes.
Summary/Conclusions: In total, 59% of *NPM1*^{mut} AML pts showed clonal evolution at the time of relapse. In accordance with our previous findings, *DNMT3A*^{mut} demonstrated the highest stability constituting early events that persist in preleukemic hematopoietic stem cells. While some relapse cases evolve from clones already forming the tumor bulk at diagnosis, *NPM1*^{mut} loss relapse cases possess almost no relationship to the paired diagnosis sample, apart from preleukemic mutations, and demonstrate a switch in major signaling cascades.

P547

POOR PROGNOSIS MONOSOMIC AND TRISOMIC ACUTE MYELOID LEUKEMIA ASSOCIATES WITH CHECKPOINTS AND CDC20 DEREGULATION: A NOVEL LEUKEMOGENIC MECHANISM AND THERAPEUTIC TARGETS

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Background: Chromosome number alteration, aneuploidy, causes unfitness in eukaryotic cells and is generally not sufficient to induce malignant transformation. However, it is a hallmark of cancer and 10-15% of acute myeloid leukemia cases are aneuploid.
Aims: Aim of the study was to investigate the molecular mechanisms activated by A-AML cells to tolerate an abnormal chromosome number and overcome the unfitness barrier, in order to define potential druggable targets.
Methods: We performed whole exome sequencing of 42 aneuploid (A-) AML (isolated trisomy and monosomy, complex and monosomal karyotype) and 34 euploid (E-) AML (100 bp, paired-end, Illumina platform). Variants were called by MuTect and Varscan 2.0. Gene expression profiling was obtained from bone marrow cells of 22 A-AML and 27 E-AML with 80-100% blasts (HTA 2.0, Affymetrix). Data were analyzed using Transcriptome Analysis Console 3.0 (Affymetrix) and Gene Set Enrichment Analysis (Broad Institute).
Results: In a cohort of 166 AML patients (80 A-AML and 86 E-AML) treated at our Institute, aneuploidy was associated with poor overall survival (median survival: 13 and 26 months in A-AML and E-AML, respectively; p<.01). A-AML showed reduced expression of RAD50 and ATR compared with E-AML (p<.001), suggestive of an impaired DNA damage response and checkpoint arrest, despite normal levels of CHK1 and CHK2 kinases. These defects hampered the activation of p53, the guardian of ploidy and its transcriptional program, which were disrupted both at functional and genomic levels. A-AML was not only enriched for *TP53* mutations, which were common in complex and monosomal karyotype cases and absent in E-AML, but also for genomic lesions targeting p53-related genes, including those involved in p53 activation (*APAK*, *FATS*, *PIAS4*) and stability (*USP10*, *DDX31*) and known p53 targets (*RBM38*, *DDR1*). Overall, 42% A-AML had a mutation in *TP53* or its related genes, compared with 15% E-AML (p=.01). Moreover, a gene expression signature of p53 downregulation was enriched in A-AML, irrespective of the mutational status (p<.05). Upregulation of PLK1 contributed to functional inactivation of p53 in A-AML (p<.01). PLK1 also forced entry and progression through mitosis in A-AML by cooperating with overexpression of CDC20 (p<.001), which is sufficient to overcome the spindle-assembly checkpoint, leading to the formation of daughter cells with an aberrant chromosome number. The spindle assembly machinery and additional cell cycle-related genes involved in DNA replication, centrosome dynamics, chromatid cohesion and chromosome segregation were frequently mutated (p<.05) or deregulated at transcriptional level (p<.01) in A-AML, suggesting a role in promoting and maintaining genomic instability, which was highlighted by a higher number of genomic alterations in A-AML. The mutations mostly targeted chromatin modifiers, splicing, DNA methylation and signaling genes. In parallel, a KRAS transcriptional signature was upregulated in A-AML (p<.05), irrespective of the mutational status, to sustain cell survival and proliferation.
Summary/Conclusions: We depicted here for the first time the complex molecular mechanisms promoting and maintaining the aneuploid phenotype in AML, which include deregulated checkpoint response and cell cycle machinery, inactivated p53 and hyperactivated KRAS pathway. This evidence suggests that pharmacological reactivation of p53, inhibition of cell cycle checkpoints and KRAS signaling, which are under investigation, may be valuable therapeutic strategies for aggressive A-AML.
GS & AP: equal contribution. Supported by: FP7 NGS-PTL project, ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi).

P548

IN ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPE CHROMOTHRIPSIS IS LINKED TO TP53 ALTERATION AND CELL CYCLE IMPAIRMENT

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Background: The single catastrophic event "chromothripsis", uncovered by

genome sequencing, is a phenomenon of genomic instability. It is characterized by extensive genomic rearrangements reflected by an oscillating pattern of DNA copy number levels in one or few chromosomes as assessed by *e.g.* single-nucleotide polymorphism (SNP) microarray analysis. The prevalence of chromothripsis across tumor entities is very heterogeneous, and despite several studies linking chromothripsis with *TP53* alterations, the underlying mechanisms are still largely unknown. In acute myeloid leukemia (AML), *TP53* alterations (loss and/or mutation) are almost exclusively found in cases with complex karyotype (CK-AML).

Aims: Our aim was to gain further insight into the role of chromothripsis in AML.

Methods: We performed integrative analysis using genomic SNP microarray profiling, global gene expression profiling as well as *TP53* mutation screening in 103 CK-AML and correlated our findings with clinical data of 55 age-adjusted intensively treated study patients (HD98A, AMLSG 07-04, and AMLSG 06-04).

Results: SNP profiling identified rearrangement patterns consistent with chromothripsis (defined as at least ten switches between two or three copy-number states on an individual chromosome) in 36 of 103 CK-AML (35%). The most frequent affected chromosome (chr) was chr 7 (14%) followed by chr 3, 5, 17, 21 (11% each), and 6, 8, 10, 11, and 12 (5% each). Cases with chromothripsis were characterized by a higher degree of genomic complexity, as measured by total number of copy number alterations per case (mean±SD 23±10 *versus* 11±10, P<.0001), and by the association with specific genomic alterations, that is, monosomy 5 or losses of 5q (-5/5q-) (P=.003), -7/7q- (P=.02), -16/16q- (P=.009), and the cytogenetic category "monosomal karyotype" (P=.0002). Genetically, as previously reported chromothripsis was correlated with *TP53* alteration (30/36 chromothripsis-positive CK-AML *vs* 37/67 chromothripsis-negative CK-AML, P=.005). Clinically, CK-AML patients with chromothripsis were slightly older (median age, 62 *versus* 58 years, P=.02) and showed in trend lower bone marrow blast counts (median 45% *versus* 80%, P=.07). Whereas *TP53* alteration predicted for lower complete remission (CR) rate (*TP53*^{altered} 25% *vs TP53*^{unaltered} CK-AML 68%, P=.005), chromothripsis had no impact on CR, but predicted for inferior survival; the 2-year estimated survival rates for chromothripsis-positive and chromothripsis-negative patients were as follows: event-free survival (EFS), 0% *versus* 12% (P=.01); relapse-free survival (RFS), 0% *versus* 35% (P=.06); and overall survival (OS), 0% *versus* 26% (P=.02), respectively. Since chromothripsis is associated with *TP53* alteration, which is the most important prognostic factor in CK-AML outweighing all other known prognostic variables in multivariable analysis, chromothripsis does however not provide additional independent prognostic information. On the other hand, gene expression profiling comparing chromothripsis-positive *vs* –negative *TP53*^{altered} CK-AML cases showed a distinct gene signature pointing to mechanisms underlying chromothripsis. For example, in addition to *TP53* alteration chromothripsis-positive cases showed a significant deregulation of genes associated with genomic instability (*e.g.* Fanconi anemia pathway genes), mitotic regulation (*e.g. CCNA1*) as well as transcriptional regulation (*e.g. CEBPA*).

Summary/Conclusions: Detailed molecular profiling links chromothripsis to impaired *TP53* in CK-AML and suggests an addition pathogenic basis for the occurrence of chromothripsis.

P549

PHYSIOPATHOLOGICAL MECHANISM OF LEUKEMIC PREDISPOSITION IN ANKRD26-RD PATIENTS

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Background: Familial thrombocytopenia 2 (THC2) is a rare autosomal dominant platelet disorder, associated with a predisposition to develop haematological malignancies and is caused by different mutations in the *ANKRD26* 5' UTR. This gene is coding for a membrane-associated protein that is predicted to interact with signaling effectors including cytokine receptors, and that behaves as a scaffold between different protein partners.

In megakaryocytes (MKs), the mutations in the 5' UTR eliminates the DNA binding of two transcription factors, RUNX1 and FLI1, causing the loss of transcriptional repression of *ANKRD26* along differentiation; this results in an abnormal expression of the protein in mature megakaryocytes inducing an over-activation of the TPO/MPL/MAPK signaling pathway responsible for a defect in platelet formation and explaining the thrombocytopenia.

Aims: To study the mechanisms responsible of the pre-leukemic state, we generated iPSC lines from isolated CD34+ cells of three patients harbouring three different *ANKRD26* mutations; in addition, they were transduced with a short hairpin RNA (shRNA) targeting *ANKRD26* (shANKRD26).

Methods: The iPSCs were successfully differentiated toward an haematopoietic fate with a co-culture system (OP9 cells) and a feeder-free system: the derived MKs showed the same defects observed in patient primary cells, that were rescued by the shANKRD26, therefore validating the relevance of the iPSC model.

Results: Next, we used this model to study the granulo-monocytic (GM) compartment: we found an increased expression of *ANKRD26* in the GM progenitors derived from patients iPSC and primary cells, but also an increase in their number and proliferation. We observed an over-activation of the JAK/STAT, MAPK and AKT signalling pathways in presence of G-CSF, suggesting a possible interaction between ANKRD26 and the G-CSF receptor (CSFR3). Those results were confirmed in the cell line UT7/G-CSFR. This cell line constitutively overexpresses ANKRD26, and its knock down by the shRNA markedly decreased proliferation in the presence of G-CSF. In contrast we did not observe any difference in the proliferation rate in presence of GM-CSF, a feature that suggests a functional link between ANKRD26 and the G-CSFR, but not with the GM-CSF receptor. In addition, we observed a sensible reduction of the proliferation rate of patients and iPS-derived granulocytic precursors in presence of JAK1/2 inhibitor (ruxolitinib). We also detected an increase of PIM1 expression, a direct target of STAT3/5 and a possible mediator of this lineage over-proliferation (Figure 1).

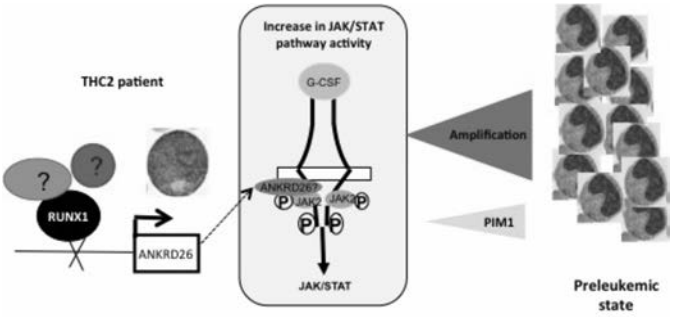

Figure 1.

Summary/Conclusions: In conclusion, this work shows an important role for ANKRD26 in two different signaling pathways, TPO/MPL and G-CSF/G-CSFR, the former responsible for the thrombocytopenia, the latter may be implicated in the pre-leukemic state.

P550

NOTCH SIGNALLING INHIBITION AS A MULTI-TARGET THERAPY TO OVERCOME BONE MARROW MICROENVIRONMENT-MEDIATED DRUG RESISTANCE IN AML

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Background: Several pro-survival proteins promoting resistance to chemotherapy, such as BCl-2, STAT3,NFκB and AKT,are over-expressed in AML cells, thus representing the basis for targeted therapies. However, only multi-target drug strategy may lead to the modulation of the pro-survival protein network, due to the simultaneous activation of alternative pathways.

Notch signalling is a master developmental pathway that controls tumour cell survival by interacting with pro-survival proteins, such as β-catenin, BCL-2, STAT3, NFκB, and AKT, thus representing an ideal target to interfere with all these pathways in different cancer systems.

We recently showed that Notch inhibition was capable of abrogating microenvironment-mediated AML cell chemo-resistance (P550, EHA20); however, little is known about the mechanism involved.

Aims: We studied the mechanisms underlying microenvironmental, Notch-mediated AML chemo-resistance by investigating the contribution of BCl-2, STAT3, NFκB and AKT. Using *in silico* and *in vitro* approaches we analyzed the expression changes of these proteins in *ex-vivo* AML cell samples in condition of pharmacological or genetical Notch down-regulation, as well as in AML cells either cultured alone or co-cultured with human bone marrow mesenchymal stromal cells (hBM-MSCs) in presence of chemoterapeutic agents, such as cytarabine (Ara-C) and Idarabucin.

Methods: Cells were obtained from bone marrow (33) and peripheral blood (22) samples of AML patients. hBM-MSCs were expanded from bone marrow of 20 healthy donors (BM-MSCs) and 20 AML patients (BM-MSCs*). Gene set enrichment analysis (GSEA) were perform using GEOR tools on AML expression array of 304 patients previously deposited in Gene Expression Omnibus (GSE10358). Genetic inhibition of Notch signalling was achieved in AML cell lines (HL-60 and THP1) by infecting cells with lentiviral particles carrying shRNA for either RBP-jK or MALM1, two mediators of Notch signaling. Pharmacological Inhibition of Notch in AML was achieved by using Gamma secretase Inhibitors (GSIs), Notch transcription factors Inhibitor SAHM1, and combination of Notch blocking antibodies.Ara-C, and idarubicin were added to culture supernatants at different concentrations. Cell viability was evaluated by Annexin-V/Propidium Iodide (PI). Protein levels were analyzed by intracellular staining with corresponding fluorophore conjugated antibodies, followed by flow cytometry analysis.

Results: *In silico* Gene set enrichment analysis and flow cytometry analysis showed that AML samples highly expressed Notch1, Jagged1, STAT3, NFκ-B and AKT genes and proteins. Notably, higher levels of Notch1 were found in patients with poor cytogenetic prognosis, while STAT3, NF-κB and AKT were uniformly expressed by AML patients. Protein analysis revealed low levels of

pro-survival proteins AKT, STAT3 and NF-κB in RBP-jk and MALM1 knockdown cells, as compared to control cells infected with non specific shRNA. We then verified that genetic (shRNA) and pharmacological inhibition of Notch, by using either GSIs or Notch receptor blocking antibodies, was capable of sensitizing AML cells, either cultured alone or in presence of hBM-MSCs, to ARA-C or idarubucine. Additionally, we found that hBM-MSC-dependent induction of AML chemoresistance was associated to increase of AKT, NF-κB and STAT3 protein levels in AML cells. Similarly, Notch inhibition with GSIs prevented hBM-MSC-mediated increase of AKT, NF-κB and STAT3, thus restoring sensibility of AML cells to Idarubucin treatment.

Summary/Conclusions: These results suggest that inhibition of Notch signalling is sufficient to reduce protein levels of AKT, STAT3 and NF-κB proteins involved in AML chemoresistance, thus making the pro-survival core network controlled by Notch a potential target for specific Notch targeted therapies.

Acute myeloid leukemia - Biology 3

P551

INTEGRATIVE ANALYSIS OF LINCRNA EXPRESSION IN 922 ACUTE MYELOID LEUKEMIA PATIENTS REVEALS MULTIPLE PROGNOSTIC GENE SIGNATURES

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Background: Acute myeloid leukemia (AML) is a heterogeneous myeloid neoplasm that develops in hematopoietic stem and progenitor cells (HSPCs) with altered ability to self-renew, proliferate and differentiate. The karyotype and recurrent lesions in *NPM1*, *FLT3-ITD* and *CEBPA* are associated with patient remission, relapse and survival and have been integrated into AML prognostic indices such as those developed by the United Kingdom Medical Research Council (MRC) or the European Leukemia Net (ELN). However, the utility of these guidelines for clinical decision making has only been validated in the ~40% of patients younger than 60 years (SEER). The human genome consists of more than three billion nucleotides that encode a relatively small number of coding (~1.5%) and a larger number of non-coding (~75%) transcripts. The association between the coding genome and the biology and pathogenesis of AML and its utility for clinical decision making has been extensively studied. However, the contributions from the large pool of non-coding RNAs to these events is largly unknown.

Aims: Long intergenic non-coding RNAs (lincRNA) are emerging as key regulators of an increasing number of molecular processes and have been shown to regulate stem cell activity and function during myeloid and lymphoid differentiation, while their role in leukemia is poorly understood. Therefore, we aimed to: 1. Comprehensively describe and evaluate the expression of lincRNAs in leukemic cells from widely used, large, clinically annotated AML cohorts, AML cell lines and in normal hematopoietic stem and progenitor cell subsets. 2. Develop and validate lincRNA signatures that are robust predictors of patient survival, not limited to single patient cohorts but significant across multiple large cohorts, and do not require measurement of large numbers of genes.

Methods: Here we analysed expression profiles of 1664 lincRNAs measured using three technologies in 922 patients from three independent patient cohorts including those from the United States of America (The Cancer Genome Atlas), Netherlands (HOVON), Germany (CALGB) and we sourced a fourth independent Australian AML dataset to experimentally validate our findings. We also analysed datasets from the BLUEPRINT epigenome project and the Cancer Cell Line Encyclopedia to compare our findings to expression profiles in normal hematopoietic cells and AML cell lines.

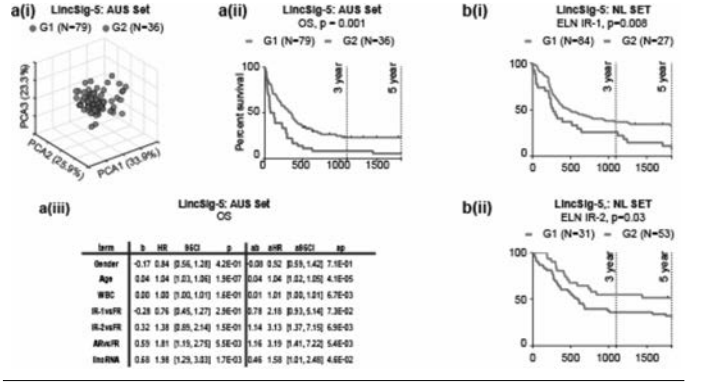

Figure 1.

Results: We developed and validated a novel approach to quantify the expression of 1664 lincRNAs in 736 AML patients from 3 well-characterized patient cohorts representing the largest resource of lincRNA expression profiles available in AML. Interrogation of this database (i) reconfirmed previously known regulatory relationships and uncovered lincRNAs in close proximity to key haematopoietic genes that showed strong expression correlations across AML

datasets, identified that distinct lincRNAs expression profiles are (ii) associated with recognized cytogenetic and mutational subgroups of AML and (iii) expressed in AML and normal HSPC subsets. We also (iv) identified 48 lincRNAs that were significantly correlated with patient outcomes and (v) expression signatures composed of 2-4 lincRNAs that have prognostic value in addtion to current clinical risk algorithms based on the assesment of karyotype and mutations in *CEBPA*, *FLT3* and *NPM1*, (vi) that these signatures can be measured by RT-PCR and used as a standalone tool or in conjunction with the ELN to predict survival across multiple AML cohorts, including patients older than 60 years (Figure 1a and b).
Summary/Conclusions: Taken together, this comprehensive analysis of lincRNA expression in multiple AML patient cohorts and in normal hematopoietic cell subsets not only highlights their value as prognostic markers but also provides a platform to selectively perturb lincRNAs in leukemic cells.

P552

ENANTIOMER-SPECIFIC AND PARACRINE LEUKEMOGENICITY OF MUTANT IDH METABOLITE 2-HYDROXYGLUTARATE

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Background: Canonical mutations in *IDH1* and *IDH2* produce high levels of the R-enantiomer of 2-hydroxyglutarate (R-2HG), which is a competitive inhibitor of αKG-dependent enzymes and a putative oncometabolite. Almost all patients with *IDH1/2* mutations express high levels of intracellular R-2HG, while an increase of the S-enantiomer of 2HG has never been described in AML, glioma, chondrosarcoma or intrahepatic cholangiocarcinoma patients.
Aims: To investigate whether oncometabolites R-2HG and/or S-2HG, have a causative function in leukemogenesis or instead are rather only biomarkers for oncogenic IDH.
Methods: We daily administered R-2HG and S-2HG at a dose of 1mg/mouse *in vivo* in three independent mouse models (HoxA9, MLL-AF9 and an AML patient derived xenotransplantation model) containing only wild-type IDH1 and compared the blood profile, survival, gene expression and differentially methylated regions between the groups.
Results: R-2HG treated mice had significantly higher engraftment levels in peripheral blood than S-2HG and PBS treated mice. R-2HG treated mice developed striking leucocytosis, and had declining hemoglobin and platelets compared to S-2HG or PBS treated mice. Peripheral blood from R-2HG treated mice revealed significantly more immature and less mature myeloid cells after treatment than S-2HG, αKG and PBS treated mice. Furthermore, the R-2HG treated mice died significantly earlier than mice treated with S2HG and PBS in all three models. A question of current investigation is whether R-2HG alone is responsible for the transforming effects of mutant IDH or if the mutant protein contributes additional oncogenic functions. Interestingly, both cohorts, HoxA9+IDH1mut and HoxA9 treated with R-2HG, developed leukemia, albeit with different kinetics. Next, we examined whether the faster disease kinetics of mutant IDH1 compared to R-2HG would be overcome if the R-2HG dose was increased from 1 to 5 mg R-2HG per day. We observed a dose dependent increase of R-2HG in bone marrow cells of treated mice, while the dose of 5 mg R-2HG per day even slightly exceeded R-2HG of cells expressing mutant IDH1. Mice treated with 1 or 5 mg of R-2HG had similar engraftment levels and died with an identical latency, while IDH1 mut mice died significantly earlier. In gene expression data R-2HG treated cells clustered with IDH1 mut cells, while IDH1 wt cells clustered with HoxA9 control cells. However, many genes that were exclusively upregulated in IDH1 mut cells but not in R-2HG treated cells showed that R-2HG treated cells were at an intermediate stage between control/IDH1 wt cells and IDH1 mut cells. The differentially methylated regions (DMRs) between R-2HG treated cells and IDH1 wt cells were well represented in IDH1 mut cells. However, DMRs between IDH1 mut and IDH1 wt cells were not well represented in R-2HG treated cells and these cells clustered with IDH1 wt and not IDH1 mut cells. The higher number of hyper- and hypomethylated regions in IDH1 mut cells compared to R-2HG treated cells, supports an additional function of the mutant protein on transcriptional regulation and DNA methylation beyond the function of the metabolite R-2HG and can explain the earlier disease onset of IDH1 mut cells compared to R-2HG treated cells.
Summary/Conclusions: We show that R-2HG, but not S-2HG, is an oncometabolite that does not require the mutant IDH1 protein to induce hyperleukocytosis and to accelerate disease onset *in vivo*. Thus, circulating R-2HG acts in a paracrine fashion and can drive the expansion of many different leukemic and preleukemic clones that may even express wildtype IDH1, and therefore can be a source of clonal evolution and diversity.

P553

INVOLVEMENT OF THE TRANSCRIPTION FACTOR ASCL2 IN NORMAL AND LEUKEMIC HEMATOPOIESIS

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Background: The transcription factor ASCL2 (Achaete Scute-like 2) is involved in intestinal stem cell biology regulating their stem cell identity while its overexpression induces an intestinal hyperplasia. In addition, it is over-expressed in numerous tumor types. In cancer cells and T follicular cells, it regulates the expression of CXCR4, a chemokine receptor involved in cell migration, homing and proliferation. Conversely, CXCR4 and Wnt signaling regulates ASCL2 expression suggesting that CXCR4 and ASCL2 interact to regulate the metastasis formation. In acute myeloid leukemia (AML), CXCR4 has a predominant role in the regulation of interaction of cells with their environment and its expression is correlated with poor prognosis. However, ASCL2 contribution to AML in poorly understood.
Aims: The aim of this study is to elucidate the role of ASCL2 in normal and leukemic hematopoiesis by analyzing of its expression in AML and normal hematopoietic progenitors and using different cell line models for the overexpression and down regulation.
Methods: To study the function of ASCL2 transcription factor, 135 samples of AML patients were analyzed for the ASCL2 mRNA level. CXCR4+/+ and CXCR4-/- murine leukemic cells were generated by expression of MLL-ENL fusion protein in fetal liver cells for E15 embryos. To examine the effects of loss of function of ASCL2 on leukemic development, murine leukemic cells were transduced with shRNA targeting ASCL2 and engrafted to mice. Finally, we examined the consequences of ASCL2 overexpression by enforcing its expression using lentivirus transduction in CD34+ progenitor cells.
Results: We first observed that 32% (43/135) of AML samples displayed ASCL2 mRNA expression higher than normal progenitor CD34+ cells from bone marrow of control individuals. In addition, the level of ASCL2 mRNA correlates with poor prognosis AML (p<0,001). Normal hematopoietic cells and progenitors show a weak expression of ASCL2, except monocytes and NK cells. In murine leukemic models, we found a high expression of ASCL2 in leukemic stem cells that is dependent of CXCR4 and correlates with expression of the stem cell associated genes HOXB4 and HOXB13. Knocking-down ASCL2 by shRNA in murine leukemic cells induces a sharp decrease in the number of leukemic cells and delayed leukemic progression *in vivo* resulting *in* increased survival of leukemic mice. Enforced expression of ASCL2 in cord blood CD34+ progenitor cells induces a 4-fold decrease in the cloning efficiency in progenitor assays compared to cells transduced with GFP (p<0,02). Surprisingly, a subset of ASCL2 transduced cells were able to sustain colony formation in secondary, third and fourth serial colony assays suggesting that ASCL2 overexpression has differential effect depending on targeted cells.
Summary/Conclusions: Our results indicate that ASCL2 is overexpressed in AML. In AML murine models, ASCL2 down regulation delayed leukemic progression while it may maintains the self-renewal capacity of subsets of cells. Altogether, our results implicate ASCL2 as an important regulator in AML development and suggest that ASCL2 expression is associated with aggressiveness of leukemia.

P554

DIFFERENTIAL DNA METHYLATION IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA IS LINKED TO ALTERED GENE EXPRESSION OF ENHANCER TARGET GENES

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Background: During normal hematopoiesis, DNA methylation is tightly controlled at regulatory elements. Enhancer methylation patterns can distinguish both between different cell identities and maturation stages. In our previous work, we found aberrant DNA methylation in low CpG density regions and in transcription factor genes in AML patients and that dynamic enhancer methylation during normal granulopoiesis correlate with gene expression pattern. However, changes of DNA methylation in enhancer regions occur in leukemia are remaining largely unknown.
Aims: To study the aberrant DNA methylation of enhancers in AML patients in correlations to altered chromatin status and putative target gene expressions.
Methods: In this study a total of 57 cytogenetically normal AML (CN-AML) patients and 12 FACS sorted bone marrow myeloid samples with four differentiation stages from 3 healthy individuals were used to profile DNA methylation changes in AML and granulopoiesis using the Illumina 450k array. H3K27ac ChIP sequencing was performed on 6 CN-AML patients' mononuclear cell samples and analyzed together by integrating data of hypersensitivity sites sequencing (DHSseq) of normal bone marrow CD34+, NB4 and HL60 from ENCODE project and 5 CN-AML patients from recent published paper. Total enhancers were obtained from FANTOM5 project and used for analyzing correlation to putative target genes. Moreover, selected enhancers were edited by CRISPR/Cas9 system in order to test the expression change of target genes.
Results: We found a number of hypermethylated and hypomethylated sites in

AML patients compared to NBM, some of which are specific to AML while others correspond to changes observed during granulopoesis. In addition a core set of the changes observed were independent of specific mutations. Strikingly, the majority of these changes occurred in regulatory regions of the human genome defined as DNA hypersensitivity (DHS) sites. Hypermethylated sites show reduced chromatin accessibility and histone acetylation (H3K27ac) at transcription start sites (TSS) proximal and distal regions from TSS suggesting active promoters and enhancers that are hypermethylated are silenced. Hypomethylated sites occurred more frequently outside of TSS regions with only a subset of hypomethylated sites in distal regions from TSS correlated to increased chromatin accessibility and H3K27ac. To examine if the changes in DNA methylation at enhancer regions are correlated to altered gene expression of target genes in AML we focused on differential methylation of enhancers defined by the FANTOM consortium, which have high confident target gene predictions. An adverse correlation between enhancer methylation changes and putative target gene expression levels was found by integrating transcriptome data from RNA sequencing in the AML patients. Moreover, by using CRISPR/Cas9 system, targeted editing of selected active enhancer elements in AML cell lines HL60 and KG1a lead to decreased expression of target genes.
Summary/Conclusions: This study demonstrates the relationship between abnormal DNA methylation and enhancer activation, which correlates with target gene expression in CN-AML. This suggests that there are unique regulatory events that occur in CN-AML at enhancer regions, which distinguish AML patients from NBM and granulopoietic progenitors. Our results suggest that aberrant DNA hypomethylation at distal regulatory regions in AML can cause opening up of the chromatin, resulting in abnormal transcriptomic alternation of the target genes that potentially contribute to leukemogenesis.

P555

IN VIVO SHRNA SCREEN IDENTIFIES SPLICING FACTOR RBM25 AS A TUMOR SUPPRESSOR IN ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid Leukemia (AML) is a heterogeneous collection of hematological malignancies, which are maintained by a population of so-called leukemic stem cells (LSCs). LSCs accumulate various genetic and epigenetic abnormalities, including aberrations in mRNA splicing. Recent progress suggests that dysregulation of splicing factors contributes to pathogenesis of AML, but little is known about the AML-specific dependency on splicing factors for leukemic cells survival and proliferation.
Aims: The current study uses *in vivo* shRNA screen approach to identify novel splicing factors required for leukemia progression.
Methods: We used a mouse model of human CEBPA mutant AML and a splicing factor shRNA library to screen for novel splicing factor genes essential for AML. Functional study of the best candidate gene RBM25 included *in vivo* competitive bone marrow transplantation assay, survival assay and *in vitro* colony forming unit assay. In the human leukemic cell line U937, RBM25 knockdown cells were assessed proliferation, cell cycle and apoptosis by flow cytometry. Splicing events were detected by SpliceR tool based on RNA-sequencing data on U937 cells carrying RBM25-shRNA or not.
Results: By *in vivo* shRNA screen in CEBPA mutant AML mouse model, we found that splicing factor Rbm25 was important for murine leukemic cells *in vivo* and for human leukemic cells in liquid culture. Rbm25 knockdown accelerated the growth of leukemic cell after transplantation, and shortened the lifespan of leukemic mice. RBM25 knockdown accelerated proliferation and colony formation of human leukemic cells U937 by increasing cell cycle progression and decreasing apoptosis. Using RNA-seq profiling in U937 cells, we identified 368 transcripts that were regulated by RBM25. Particularly, RBM25 knockdown promoted alternative splicing of BIN1 to produce inactive form that block its interaction with MYC, thus the oncogenic MYC pathway was activated. Moreover, by accurate RT-qPCR method, we showed RBM25 regulated Bcl-x splicing to affect apoptosis. RBM25 knockdown caused the accumulation of anti-apoptotic isoform Bcl-xL, and ABT-263 inhibition of Bcl-xL partially reversed the RBM25-mediated tumor progression.
Summary/Conclusions: This study showed that splicing factor Rbm25 knockdown can promote proliferation of both murine and human leukemic cells, and provides detailed mechanistic insights into RBM25-regulated splicing dysregulation in AML. The newly identified tumor suppressor gene RBM25 has great therapeutic potential and clinical values as a prognostic factor.

P556

HIGH MTORC1 ACTIVITY DRIVES GLUCOSE METABOLISM ADDICTION AND SENSITIVITY TO G6PD INHIBITION IN ACUTE MYELOID LEUKEMIA CELLS

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Background: Acute Myeloid Leukemia (AML) are heterogeneous haematological diseases characterized by a clonal expansion of immature progenitors and associated with overall poor prognosis. Many aberrant activation of signalling pathways are found in AML which enhances the proliferation and survival of leukemic cells. Among them, mTORC1 signalling, which controls protein translation, autophagy and metabolism, is activated in almost all AML samples. Metabolic reprogramming is a common phenotype in cancer cells and mainly occurs through the well-known "Warburg effect» which consists in enhanced glucose catabolism even in the presence of oxygen. High rate glycolysis provides tumor cells advantages through rapid production of ATP and intermediates for the synthesis of nucleotides, amino acids, and lipids.
Aims: We investigated in our present study whether mTORC1 signaling could drive the metabolic reprogramming of AML cells, especially toward glucose utilization, and if high mTORC1 activity could lead to the sensitivity of leukemic cells to metabolic targeted therapy.
Methods: We performed analysis in 6 AML cell lines, in primary AML cells obtained from bone marrow or blood samples of 18 patients at diagnosis and in normal CD34+ hematopoietic progenitor cells (HPCs). mTOR signalling was down-regulated with either rapamycin or ShRNAs targeting RAPTOR, mTOR or RICTOR and mTORC1 activity was up-regulated through TSC2 deletion by CRISPR/Cas9. Glucose flux was analyzed using ^{13}C nuclear magnetic resonance.
Results: Using a transcriptomic profiling, we first identified an mTORC1-dependant glucose metabolic signature in AML cells. We also showed that high mTORC1 activity promoted glycolysis and led to glucose addiction in AML cells. Indeed, the level of mTORC1 activity determines the sensitivity of AML cells to glycolysis inhibition: whereas AML cell survival with high mTORC1 activity is dependent on glucose availability, switch-off mTORC1 activity by pharmacological (rapamycin) or genetic invalidation (mTOR or RAPTOR knockdown) leads to glucose-independent cell survival that is mainly sustained by a compensatory increase in mitochondrial oxidative phosphorylation (OXPHOS). Finally, we observed a high flux of glucose through the pentose phosphate pathway (PPP) which supports AML cell growth. Indeed, 6-aminonicotinamide (6-AN), which blocks the activity of the glucose-6-phosphate dehydrogenase (G6PD), the key limiting enzyme of PPP, induced cytotoxicity against AML cells *in vitro* – including in primary samples from AML patients – and *in vivo*, without impact on normal hematopoietic progenitor cells.
Summary/Conclusions: Overall, our results demonstrate that high mTORC1 activity creates a specific vulnerability to G6PD inhibition that may work as a new AML therapy. Given that mTORC1 signaling is deregulated in almost all AML samples, targeted therapy against G6PD may be beneficial for a broad range of patients with AML.

P557

ALVOCIDIB POTENTIATES THE ACTIVITY OF ABT-199 IN NONCLINICAL MODELS OF ACUTE MYELOID LEUKEMIA

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Background: The BCL-2 inhibitor ABT-199 (or venetoclax) has recently received FDA breakthrough therapy designation for use in combination with hypomethylating agents (HMAs) in patients with acute myeloid leukemia (AML). Early stage trials have demonstrated clinical response rates over 70% in combination with HMAs (azacitidine or decitabine) in previously untreated elderly patients with AML. Single-agent activity of ABT-199 was modest in AML, and resistance is developing in combination regimens. One proposed mechanism of resistance is compensatory up-regulation of other BCL-2 family proteins such as MCL-1, which plays a general role in resistance to BCL-2 inhibitors. The CDK9 inhibitor, alvocidib, as a component of a sequential regimen with chemotherapy, demonstrated a superior rate of complete remission in newly diagnosed intermediate and high-risk AML patients over standard induction chemotherapy in a randomized multicenter Phase 2 trial. We and others have demonstrated that alvocidib inhibition of CDK9 can mediate transcriptional repression of anti-apoptotic MCL-1. Further, we and others have shown that alvocidib can increase pro-apoptotic BIM (a pan BH3-only protein) in some cells. BIM is known to neutralize all anti-apoptotic BCL-2 family proteins such as MCL-1 and BCL-2, thus having a similar net effect on mitochondrial outer membrane permeabilization (MOMP)-induced apoptosis as MCL-1 down-regulation. Therefore, we hypothesize that alvocidib and ABT-199 synergize in the treatment of AML by shifting the balance/activity of pro- *versus* anti-apoptotic BCL-2 family members in favor of apoptosis induction.
Aims: These studies sought to investigate the nonclinical activity of the combination of alvocidib with ABT-199 in the context of (drug-resistant) AML.
Methods: CellTiter-Glo was used for all cell viability assays interrogating alvocidib and ABT-199 activity in cell lines, following manufacturer's protocol. RT-PCR was used to measure mRNA expression of MCL-1 and other markers in

response to drug treatment. Protein expression was assessed using standard western blotting techniques. To determine the efficacy of an alvocidib/ABT-199 combination on tumor growth in an *in vivo* model, multiple xenograft mouse models, and *ex vivo* studies with AML patient samples were performed.
Results: In this report, we demonstrate that alvocidib inhibits both mRNA and protein expression of MCL-1 in a time and concentration-dependent fashion in a majority of AML cell lines analyzed. Additionally, in a cell line for which alvocidib did not reduce MCL-1 protein levels (MOLM-13), we observe a potent, dose-dependent increase in BIM protein after 24 hours of alvocidib treatment. Concurrent treatment of alvocidib with ABT-199 in standard 96-hour assays resulted in potent, dose-dependent, synergistic reductions of cell viability in ABT-199-sensitive and resistant cells. ABT-199-sensitive lines, MV4-11 and MOLM-13, exhibited 5- to 10-fold reduction of ABT-199 EC50 values in the low nM range when combined with 80 nM alvocidib. Importantly, ABT-199-resistant lines, OCI-AML3 and THP-1, exhibited at least a 20-fold reduction of ABT-199 EC50 values from near 1 μM to 10-50 nM, when combined with 80 nM alvocidib. Xenografts models, as well as *ex vivo* drug synergy studies with AML patient samples, are currently under way to interrogate the efficacy of this combination.
Summary/Conclusions: Taken together, our data suggest that the combination of alvocidib with ABT-199 could be a novel therapeutic regimen in both ABT-199-sensitive and -resistant AML across a heterogeneous genomic background. Although reduction of MCL-1 by alvocidib may be associated with synergy in some cells, other mechanism(s) are operating as well. We conclude that a CDK9 inhibitor/ABT-199 combination may be a novel approach for the treatment of AML and warrants further pre-clinical and clinical validation. To this effect, animal models are underway, as are plans for clinical trials.

P558

FIRST-IN-CLASS CDK7 INHIBITOR, INDUCES ROBUST APOPTOSIS IN ACUTE MYELOID LEUKEMIA AND DEMONSTRATES DURABLE *IN VIVO* EFFICACY

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Background: CDK7 acts bi-functionally as a CDK-activating kinase (CAK) controlling proliferation and as a transcriptional kinase phosphorylating the P-CTD-RNAPol II, thereby driving efficient transcriptional processes. CDK7 has recently emerged as an attractive gene control target in cancers driven by transcriptional dependencies and regulated by Super-enhancers (SEs) (Kwiatkowski *et al.*, 2014; Chipumuro *et al.*, 2014; Christensen 2014; Wang 2015). Acute Myeloid Leukemias (AML) harbor mutations in genes controlling gene expression, and as a consequence, are exquisitely sensitive to CDK7 inhibition. Employing our SE-platform technology we reveal mechanistic insights underlying the vulnerability of AML to gene control modulation via selective CDK7 inhibition.
Aims: Evaluate CDK7 as an anti-cancer target in AML, using our covalent, potent and highly selective CDK7 inhibitor. Investigate the cellular and mechanistic consequences of targeting CDK7 in AML underlying their susceptibility to CDK7-dependent transcriptional control.
Methods: Characterize CDK7 inhibition *in vitro* and *in vivo* to evaluate potency and efficacy in AML cell lines and xenografts (cell line derived xenografts, CDXs and patient derived xenografts, PDXs). We employ our SE-platform technology and next-generation sequencing to identify key mechanisms targeted in AML with our selective CDK7 inhibitor.
Results: Here we report a first-in-class CDK7 inhibitor that covalently targets a cysteine outside the kinase domain, resulting in sustained, highly selective inhibition. Syros compound, SY-1365, exhibits significant biochemical potency (K_i=17±7nM) and selectivity when profiled against >450 other kinases. In a cancer cell line panel, acute leukemias emerged among the most sensitive to CDK7 inhibition. Moreover, AML cell lines undergo rapid and robust apoptosis within 24 hours. This is preceded by >90% CDK7 target engagement and concomitant loss of P-CTD-RNAPol II suggesting the primary consequence of targeting CDK7 is the impaired transcriptional activity dependent on CDK7. Further investigation of the transcriptional consequences of CDK7 inhibition point to a reliance on key disease relevant transcriptional aberrations including translocations and SE-genes (*e.g. MLL* fusions, *MYB, HOX10A* and *MYC*). Given the covalent mechanism of SY-1365 and PK profile, we evaluated intermittent treatments *in vitro* with subsequent washout of free drug to model dosing regimens *in vivo*. We demonstrate that brief drug exposures maintain a robust irreversible apoptotic response in leukemia cells. In contrast, treated non-transformed cells recover from a transient G2/M arrest followed by re-synthesis of free CDK7 and no apoptosis/cell death. We have extended these findings to *in vivo* experiments whereby intermittent dosing in AML patient derived xenograft models (PDX, AM7577) maintains efficacy (reducing human CD45+ leukemia cells to <1%) with a significant survival advantage. We have established a PD assay by measuring target engagement of CDK7 both in mouse xenografts and human PBMCs to support: 1) establishing a PK-PD-efficacy relationship 2) quantifying target engagement during dose escalation in a Ph1 clinical trial.
Summary/Conclusions: In summary, we describe our first-in-class CDK7 inhibitor, SY-1365 that is potent, highly selective and leads to durable, complete responses in xenograft models of AML. These data support the rationale for advancing compounds with this profile toward clinical development.

P559

IDENTIFICATION OF A NOVEL STAT5 INHIBITOR TO INTERFERE WITH THE ONCOGENIC ACTIVITIES OF STAT5 IN AML

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Background: Among STAT family members, STAT5 has been described to be the most frequently hyper-activated in a variety of hematopoietic cancers and solid tumors as a result of deregulated tyrosine kinase signaling. To date, various tyrosine kinase inhibitors (TKIs) are in the clinic or in clinical trials for treatment of hematopoietic diseases. However, TKI treatment is often accompanied by resistance development, cytotoxicity as a result of poor kinase selectivity, as well as cardiovascular toxicity. Bypassing tyrosine kinases through direct inhibition of STAT5 phosphorylation would be advantageous for therapy development especially in the case of STAT5 regulated cancers. Despite essential roles of STAT5 in solid and hematopoietic cancers, only first generation pharmacologic STAT5 inhibitors are currently available for clinical development.
Aims: The tight association of STAT5 activation with transformation and tumor progression has made STAT5 an attractive molecular target for the development of novel cancer therapeutics. We therefore aim to identify a selective and specific inhibitory compound to interfere with STAT5 signaling in hematopoietic cancers to contribute to the development of a new generation of targeted drugs. On top, we will support functional studies into the mechanistic details of STAT gene activation, which holds the potential to develop even more specific and less toxic compounds for clinical application.
Methods: In collaboration with Prof. Patrick Gunning, University of Toronto, Canada, a library of lead STAT5 inhibitors targeting the SH_2 domain has been established, which was extensively validated *in vitro* on AML model cell lines and primary AML patient samples in regard to their mechanism of binding and action. Therefore, standard techniques, such as Western Blotting, immunoprecipitation, Electrophoretic Mobility Shift Assay (EMSA), qRT-PCR, as well as AnnexinV/PI and PI staining were used. Furthermore, combinatorial effects of the selected STAT5 inhibitor with a library of >1800 experimental or FDA approved drugs, especially TKIs, were evaluated using a luminescent cell viability assay.
Results: We identified a small inhibitory molecule, called AC-4-130, which binds to the SH_2 domain of STAT5, subsequently resulting in the disruption of the reciprocal STAT5-phosphopeptide interactions. The selected compound efficiently blocked kinase-mediated phosphorylation, dimer formation, nuclear translocation, DNA binding and STAT5 mediated target gene expression. Furthermore, AC-4-130 led to a cell cycle blockade in G_0/G_1 and the induction of apoptosis in the model cell lines used. Studies with human AML patient-derived samples similarly showed the induction of apoptotic cell death and an encouragingly low IC_{50} of about 1 μM after efficient blockade of STAT5 activation. A combinatorial drug screen revealed a synergistic effect of AC-4-130 with TKIs, such as Dasatinib and Sunitinib, as well as with drugs standardly used in the clinical treatment of AML patients *e.g.* with the JAK2 inhibitor Ruxolitinib or the chemotherapeutic agent Clofarabine.
Summary/Conclusions: In summary, our findings indicate that AC-4-130 is a potent and selective inhibitor of STAT5. This compound provides a lead structure for further chemical modifications and clinical development, especially in combination with standard treatment, to improve existing therapies and overcome resistance development in hematopoietic malignancies.

P560

IMPAIRED BASE EXCISION REPAIR GLYCOSYLASE/AP ENDONUCLEASE ACTIVITY CONTRIBUTES TO CYTARABINE RESISTANCE IN ACUTE MYELOID LEUKEMIA

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Background: Base excision repair (BER) is the primary DNA repair mechanism dealing with DNA base lesions representing the predominant type of DNA dam-

age in mammalian cells. Deficiencies in BER initiating enzymes - glycosylases and AP endonuclease - have been associated with increased genomic instability and cancer.
Aims: Here we investigated the role of BER in acute myeloid leukemia (AML).
Methods: We determined BER activity in 99 primary AML samples, 34 CD34+ umbilical cord blood cell samples and 27 AML cell lines using the alkaline comet assay and H_2O_2 treatment. Oxidative base lesion levels were determined in 10 AML cell lines using a modified version of the Comet assay with bacterial enzymes Fpg and Endo III as well as using liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS). Using nuclear protein extracts in an oligonucleotide incision assay, we tested the enzymatic activity of glycosylases and AP endonuclease. Nuclear protein levels were determined by western blot. Mutational analysis of BER genes was performed using Sanger sequencing and sensitivity of AML cells to cytarabine treatment was tested using an MTS assay.
Results: We found DNA strand incision of oxidatively damaged bases significantly impaired in primary AML cells as compared to UCB cells (p=0.003) suggesting a deficiency in BER glycosylases and AP endonuclease. In addition, 5/27 AML cell lines showed impaired DNA strand incision activity. We hypothesized that BER deficient cells harbor an increased number of oxidative base lesions as compared to BER proficient cells. Using a modified comet assay and LC-MS/MS we were able to show that increased numbers of unrepaired oxidative base lesions were indeed present in glycosylase deficient AML cells (comet assay: p=0.0001; LC-MS/MS: p=0.03). We then evaluated the activity of oxidative DNA glycosylases/AP endonuclease and found significantly decreased DNA strand incision activity in BER deficient cells as compared to proficient cells (p=0.002) further supporting the fact that these enzymes are impaired in BER deficient cells. Cytarabine is a cornerstone of AML therapy and has recently been shown to induce oxidative damage. Therefore, we tested for a potential effect of BER impairment on sensitivity to treatment with increasing concentrations of cyarabine and found that BER deficient cells are less sensitive to cytarabine treatment as compared to BER proficient cells (p=0.009). In further exploring the causes of BER deficiency, Sanger sequencing of key BER genes showed several single nucleotide polymorphisms but no consistent mutational pattern. However, gene expression profiling revealed decreased expression of nuclear OGG1 and MUTH1 proteins in BER impaired cells.
Summary/Conclusions: Taken together we found impaired BER in a substantial number of primary AML samples and AML cell lines potentially contributing to decreased sensitivity to cytarabine treatment.

P561

DOUBLE INACTIVATION OF TET2 AND TET3 INDUCES HYPOMETHYLATING AGENT-SENSITIVE ACUTE MYELOID LEUKEMIA

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Background: TET enzymes are methylcytosine dioxygenases regulating gene expression by oxidizing methylcytosine. Loss-of-function *TET2* mutations are frequent in myeloid malignancies, but *TET3* gene mutations are rare. Thus, although *TET3* has similar function to *TET2* and its expression is regulated during differentiation in hematopoietic tissue, the role of *TET3* in the pathogenesis of these diseases has not previously been addressed.
Aims: The aim of our study was to investigate the role of extensive loss of hydroxymethylation activities by double inactivation of *TET2* and *TET3* in acute myeloid leukemia (AML).
Methods: Relationship of *TET2* and *TET3* expression in human AML patient samples was analyzed using the TCGA database. To investigate the cooperative and distinct roles of *Tet2* and *Tet3*, *Tet2*^{flox} and *Tet3*^{flox} mice were crossed. Then, neonates baring double floxed alleles and *Mx1-Cre* transgene were injected with pIpC. Genome-wide transcription and methylation profiles in granulocyte-macrophage progenitor cells (GMPs) from 4-6 weeks old *Tet2*/*Tet3*-null mice were examined by RNA-sequencing and reduced representation bisulfite sequencing (RRBS). We established primary cell lines from *Tet2*/*Tet3*-null bone marrow in RPMI 1640 with IL-3. Cells were treated with a hypomethylating agent, decitabine and cell viability was measured by WST-8 assay. The *Tet2*/*Tet3*-null cell line was then transduced with human *TET2* catalytic domain (*TET2*^{CD}) using the pGCDN-IRES-GFP retroviral vector. Recipient mice transplanted with bone marrow cells from *Tet2*/*Tet3*-null leukemic mice were treated with 5-azacytidine intraperitoneally.
Results: Human AML showed positive correlation in *TET2* and *TET3* expression. Concurrent *Tet2*/*Tet3* deletion induced fully penetrant, lethal AML with a median survival of 59 days, whereas single deletions of *Tet2* or *Tet3* were healthy within the observation period (Figure 1 A). Gene set enrichment analysis revealed that *Tet2*/*Tet3*-null GMPs were characterized by enrichment of several signatures, including interferon response, Stat3 signaling, TNF-α signaling via NF-κB, and p53 pathway. RRBS showed that *Tet2*/*Tet3*-null GMPs showed marked increase of hyper-differentially methylated regions (hyper-DMRs) compared with *Tet2*-null, *Tet3*-null, or wild-type GMPs. Hyper-DMRs were frequently observed in enhancer regions. Genes hypermethylated (≥20% difference compared with wild-type GMPs) at enhancers in *Tet2*/*Tet3*-null GMPs showed a significant trend toward downregulation in expression. Lethally irradiated syngeneic mice transplanted with bone marrow cells from *Tet2*/*Tet3*-null leukemic mice developed AML within 3 months. *Tet2*/*Tet3*-null leukemic cells were easily propagated in IL-3-containing medium *in vitro*. Decitabine inhibited proliferation of *Tet2/Tet3*-null leukemic cells and upregulated expression of surface Gr-1 and Mac-1, indicating myeloid differentiation of these cells. Recipient mice transplanted with the cell line developed AML. Retroviral overexpression of human *TET2* catalytic domain (*TET2*^{CD}) decreased proliferation of the cell line *in vitro*. Mice transplanted with the *TET2*^{CD} expressing cell line developed AML at longer latencies compared to those transplanted with the mock cell line (Figure 1 B). Progression of AML in recipient mice transplanted with *Tet2*/*Tet3*-null leukemic cells was significantly suppressed by the 5-azacytidine treatment (Figure 1 C).

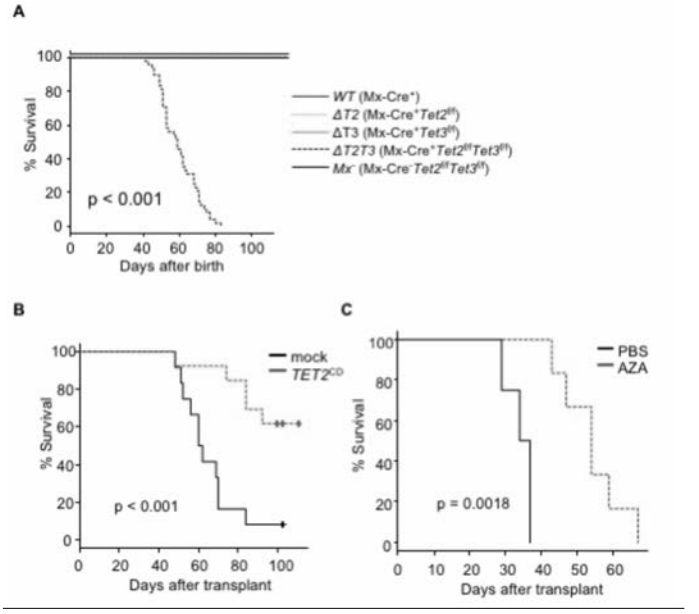

Figure 1.

Summary/Conclusions: Our results suggest that epigenetic dysregulation by concurrent loss of *Tet2* and *Tet3* plays important roles in the pathogenesis of AML. Hypomehtylating drugs may be useful in treating *TET*-deregulated AML

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Acute myeloid leukemia - Clinical 2

P562

NGS IDENTIFIES MUTATIONS PROGNOSTIC OF RELAPSE AFTER ALLO-SCT AND NOVEL CLONE EMERGENCE AT DISEASE RECURRENCE: IMPLICATIONS FOR STRATEGIES TO PREVENT POST ALLO-SCT RELAPSE

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Background: Allogeneic stem cell transplant (allo-SCT) plays the central role in management of Acute Myeloid Leukaemia (AML). Relapse is the major cause of treatment failure after allo-SCT but little is known about either molecular predictors of relapse or the clonal structure of patients with recurrent disease post-transplant.
Aims: 1) Elucidate whether mutations detected at diagnosis are prognostic for disease relapse post-allo-SCT. 2) To characterise clonal structure at relapse following allo-SCT.
Methods: We performed targeted resequencing of 34 AML-associated genes in 113 adults who underwent allo-SCT for AML. In 30 patients we compared mutational profiles at diagnosis and at disease relapse post allo-SCT. Libraries constructed using Fluidigm Access Array were sequenced on a MiSeq platform. FASTQ files were mapped using a BWA aligner (Stampy) and variant calling performed using Varscan, GATK and Pindel. Recurrent somatic variants previously associated with AML were included in the analysis. Novel variants were validated by Sanger sequencing.
Results: Of the 113 patients studied, 91 (80.5%) had intermediate/ favourable and 22 (19.5%) adverse risk karyotype. We detected mutations in 102/113 patients with on average 2 mutations per patient at diagnosis. Our cohort was enriched for samples with mutations in *KIT*, *SRSF2* and *TET2* (p<0.05), but depleted for *NPM1* (p<0.05) compared with data from a previously published cohort of unselected *de novo* AML (CGARN, 2013). 49 patients relapsed post allo-SCT. In multivariate analysis mutations in *IDH1* and *WT1* were significantly associated with an increased relapsed risk (p=0.03 and p=0.013 respectively). *IDH1* and *TP53* mutations were associated with worse relapse-free survival (p=0.047 and p=0.049 respectively). Additionally, p53 mutations were associated with reduced overall survival (p=0.027). Molecular characterisation of paired diagnosis and relapse samples showed novel mutations in 20/30 patients. 11/20 (55%) had changes in mutation number, with or without cytogenetic change. 9/20 (45%) patients had new mutations at relapse not detected at diagnosis (VAF sensitivity of <0.5%). These included: *NRAS* (n=2), *TET2* (n=3) and *DNMT3A*, *IDH2*, *PHF6* and *WT1* in single patients. Multiple distinct mutational events occurred in 5/9 patients. In contrast, 5/30 (16%) had no detectable changes in either mutations or karyotype at relapse and 7/27 (26%) had cytogenetic changes only. Interestingly, in 4 patients changes in clonal structure were identified at relapse by mutational profiling only, with no cytogenetic changes detected. We could deduce clonal evolution from pre-existing clones by mutation profile in 6 patients; or from *de novo* clones with loss of pre-existing clones in 3 patients.
Summary/Conclusions: We identified novel mutations present at diagnosis in adult AML which appear to be associated with an increased risk of relapse after allogeneic SCT. The mixed clonal landscape at relapse suggests multiple genetic mechanisms are likely responsible for relapse. The emergence of novel mutations at relapse suggests that maintenance strategies using targeted therapies tailored to the mutational profile at diagnosis may be ineffective. Rather post-transplant interventions with a broader anti-leukaemic activity, such as donor lymphocyte infusions, or other immunotherapeutic approaches may be preferable. However, the emergence of novel mutations at relapse in NRAS, DNMT3A, TET2 and IDH2 suggests that novel therapies targeting RAS and DNA methylation pathways should be considered. These data, which require validation in a larger cohort, demonstrate that detailed molecular characterisation has the capacity to both predict relapse risk post allo-SCT and inform the design of maintenance strategies.

P563

HIGH PREDICTIVE VALUE OF COMBINED WT1 AND FLOW CYTOMETRY-BASED PRE TRANSPLANT MINIMAL RESIDUAL DISEASE ASSESSMENT IN ACUTE MYELOID LEUKEMIA

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Background: Allogeneic bone marrow transplantation (BMT) offers the greatest chance of cure for patients with high-risk acute myeloid leukemia (AML). Persistence of disease or high levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk after BMT. WT1 expression levels and multicolor flow cytometry (MFC) are the most common tools to evaluate MRD.
Aims: The aim of this study was to analyze the role of pre-BMT combined MRD assessment as predictor for post-transplant relapse risk and to evaluate its the predictive value when comparing different stem cell source and conditioning intensity.
Methods: We retrospectively analyzed the outcome of 224 consecutive AML patients receiving allo-BMT in first or second CR. Pre-BMT marrow samples were analysed for WT1 expression and MFC as MRD evaluation . Median age at transplant was 44 years. Disease phase was CR1 in 161 (72%) and CR2 in 63 patients (28%). One hundred sixty-three (73%) received myeloablative conditioning, whereas 61 patients (27%) received reduced intensity conditioning. Stem cell source was HLA-identical sibling in 79 (35%), haploidentical (HAPLO) in 59 (27%) and alternative donor in 86 (38%). Median follow-up was 59 months (95% CI 46.271.8 months). Relapse-free survival (RFS) was calculated from the time of BMT until last follow-up or documented leukemic relapse. Overall Survival (OS) was calculated from the time of BMT until death by any cause or last follow-up. A positive MFC MRD was defined by the presence of no less than 25 clustered leukemic cells/10^5total events (threshold of $2.5x10^{-4}$residual leukemic cells) at four-color flow-cytometry. WT1 copy number/Abl copy number $500x10^4$ was used as cut-off value for abnormal WT1 expression.
Results: Relapse occurred in 63 patients (28%). Three-year estimate of RFS was 68.8% (median not reached). The cumulative incidence of relapse was significantly affected by occurrence of acute GVHD (lower for grade ≥2, p <0.05), donor source (lower for HAPLO, p<0.005), MRD status before BMT measured with any method (p <0.001 for WT1-based MRD, p<0.03 for MFC based MRD, p<0.0001 for combined MRD). Multivariate RFS analysis revealed that the combined MRD evaluation was the only independent predictor of RFS (p <0.001). Specifically, MFC-MRD was the strongest predictor of longer relapse free survival (p <0.001) since only two relapses occurred in the 24 MFC-MRD negative patients and 3-years RFS was 89.9%. Among MFC-MRD positive patients, WT1 MRD status stratified the risk of relapse as the 3-years RFS was 73.3% and 44.4%, respectively, for patients with normal or increase WT1, p <0.01 (Figure 1). The predictive value of MRD resulted independent from analyzed variables, however, patients with double positive MRD did slightly better when undergoing HAPLO BMT. Moreover, conditioning intensity did not affect RFS duration in MFC MRD negative patients. MRD evaluation was also a strong predictor of long term survival: 3- years OS was 73.5% for MFC negative and 36.7% for double WT1 and MFC MRD positive patients, respectively (p <0.001). Multivariate OS analysis showed that conditioning intensity and combined MRD evaluation significantly influenced OS duration (p <0.005 and <0.001, respectively).

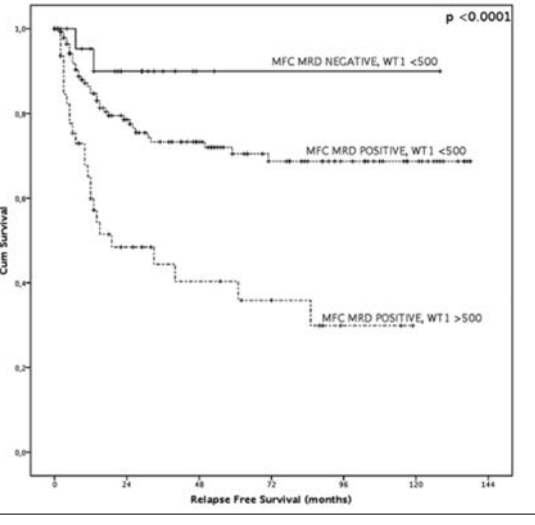

Figure 1.

Summary/Conclusions: Pre transplant MRD evaluation by WT1 and MFC on bone marrow samples is a reliable predictor of relapse risk. Patients with both negative pre-BMT MRD markers have a significantly longer RFS, while patients with both positive MRD markers display an higher risk of relapse. MRD prognostic information may improve the choice of the optimal conditioning intensity and stem cell source. Identifying patients who have an higher risk of relapse could open the way to apply pre-emptive therapeutic strategies to prevent AML relapse, from donor lymphocyte infusion to other innovative approaches.

P564

PROGNOSIS AND TARGETED TREATMENT OF AML WITH DIFFERENT IDH MUTATIONS COULD BE INFLUENCED BY ADDITIONAL MUTATIONS

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Background: Precision medicine aims at specifically targeting different gene mutations, *e.g. FLT3*-ITD (by midostaurin), *DNMT3A* or *TET2* (by hypomethylating agents), *IDH1*R132 (by IDH304 or AG-120), *IDH2* (by AG-221), or *IDH1/2* (by AG-881). However, accompanying gene mutations bearing prognostic information or also being drugable targets and the cytogenetic background may need further attention before treatment decisions.
Aims: To investigate the mutation pattern and cytogenetic background of *de novo* AML with *IDH1*R132, *IDH2*R140, and *IDH2*R172 mutations.
Methods: 1360 patients (pts) with *de novo* AML were evaluated at initial diagnosis by morphology, cytogenetics and analysed for the three mutation hotspots *IDH1*R132, *IDH2*R140 and *IDH2*R172. Furthermore, *ASXL1*, *CEBPA*, *DNMT3A*, *FLT3*-ITD, *FLT3*-TKD, *MLL*-PTD, *NPM1*, *RUNX1*, *TET2*, *TP53,* and *WT1* were investigated. All pts were treated with intensive standard chemotherapy.
Results: In total, 111/1360 (8%) pts showed an *IDH1*R132 mutation (mut), 168 (12%) an *IDH2*R140mut and 35 (3%) an *IDH2*R172mut. Four of these pts were mutated in both *IDH1*R132 and *IDH2*R140. 50% of *IDH*mut occurred in FAB AML M1, 31% were found in AML M2, and 12% in AML M4. In all other FAB classes less than 5% of the *IDH*mut occurred or no *IDH*mut at all (AML M3). Distribution of the three *IDH*mut types within the FAB classes was equal. 78% (238/306) of *IDH*mut carried a normal karyotype. Most frequently *IDH*mut pts were also mutated in *NPM1* (51%), followed by *DNMT3A* (45%), *FLT3*-ITD (23%), *ASXL1* (17%), *RUNX1* (16%), and *MLL*-PTD (13%). *CEBPA*, *FLT3*-TKD, *WT1*, and *TP53* were rarely mutated in *IDH*mut pts. Of note, 50% of the *IDH*mut pts showed an additional targetable mutation. However, accompanying aberrations differed between the *IDH*mut pts: *IDH1*R132mut pts in 103/107 (96%) showed additional mutations, but in 87/107 (81%) a normal karyotype. 63% of these pts were *NPM1*mut, 60% showed a *DNMT3A*mut, 23% a *FLT3*-ITD, 10% an *ASXL1*mut, 10% an *MLL*-PTD, 9% a *RUNX1*mut, and only 3% a *TET2*mut. *IDH2*R140mut pts showed in 91% (150/164) of cases additional mutations, and a normal karyotype in 132/164 (80%) of cases. In comparison to *IDH1*R132mut pts they less frequently showed mutations in *NPM1* (52%) and less often in *DNMT3A* (36%). However, *IDH2*R140mut pts carried more often *ASXL1*mut (24%) and *RUNX1*mut (17%) compared to *IDH1*R132mut pts. 26% showed *FLT3*-ITD, 12% an *MLL*-PTD, and 2% a *TET2*mut. Interestingly, *IDH2*R172mut pts showed in only 27/35 (77%; p=0.005 compared to combined other *IDH*mut) cases mutations and in only 19/35 (54%; p<0.001) a normal karyotype. Additionally, only 3% of pts showed mutations in *NPM1* (p<0.001), only 11% in *FLT3*-ITD, but 36% a mutation in *DNMT3A*, 27% in *RUNX1,* 27% an *MLL*-PTD, 12% in *TET2*, and 10% in *ASXL1*. Therefore, the patterns of accompanying mutations differ significantly between the three *IDH* mutation groups, both regarding potential additional treatment targets and adverse risk markers. Regarding survival *IDH2*R172mut pts showed a more favorable outcome (57% 5-yr-survival) compared to *IDH1*R132 (37%, p=0.049) and *IDH2*R140 (37%, p=0.029) mutated pts. A favorable trend was also seen compared to *IDH* wild type (40%, p=0.067) (Figure 1).

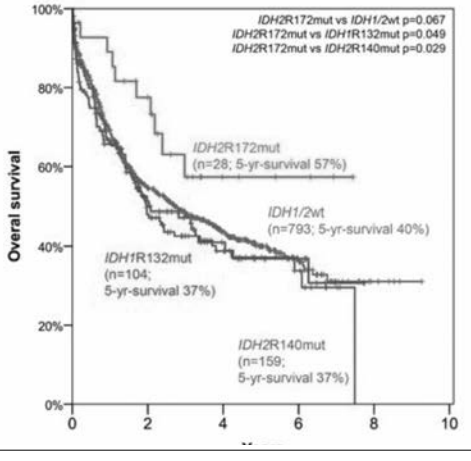

Figure 1. Overall survival according to *IDH* mutations (mut) and *IDH1/2* wildtype (wt) within the intermediate risk group according to the refined MRC criteria.

Summary/Conclusions: 1) Patients with *IDH1* and *IDH2* mutations show different aberration patterns; 2) *IDH1*R132, *IDH2*R140, and *IDH2*R172 show different outcomes; 3) treatment decision is challenging as diverse mutations with distinct targeted treatment options are simultaneously present; 4) *IDH2*R172 mutated patients might benefit from *IDH* targeting treatment, but other drugable targets might be addressed in parallel.

P565

DNMT3A AND IDH1 MUTATIONS WORSEN THE PROGNOSIS OF ELN GOOD RISK (CEBPA OR NPM1 MUTATED/FLT3 UNMUTATED) AML PATIENTS

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Background: Patients with normal karyotype and *NPM1* mutations lacking *FLT3*/ITD (*FLT3*) as well as those having *CEBPA* mutations are regarded to have favorable prognosis according to ELN criteria. However, this classification does not take into account other aberrations frequently associated with *NPM1* and *CEBPA*, such as *DNMT3A* or *IDH1/2* mutations.
Aims: To test possible impact of *DNMT3A*, *IDH1* and *IDH2* mutations on outcome within the ELN good-risk *CEBPA* or *NPM1* mutated patients.
Methods: Presence of *FLT3*, *DNMT3A*, *IDH1* and *IDH2* mutations was detected by Sanger sequencing in 115 *NPM1* or *CEBPA* mutated patients with AML with an intermediate-risk cytogenetics (according to Grimwade *et al.*, 2010) diagnosed between 2001-2014. Median age at diagnosis was 57.5 years (range 23.7-81.9), the initial median WBC count was $33.1x10^9$/L (range 0.4-303.7). The male/female ratio was 59/56 and the median of follow-up was 11.6 months. All patients were treated by remission-inducing regimens and standard post-remission therapy± allogeneic stem cell transplant.
Results: For analyses, patients were divided into 6 groups: Group A: *NPM1*+ only (n=22); Group B: *NPM1+/FLT3*- with *DNMT3A* and/or *IDH1* mutation (n=25); Group C: *NPM1+/FLT3*+ regardless of *DNMT3A/IDH1* mutations (n=45) Group D: *CEBPA*+ only (n=22); Group E: *CEBPA+/FLT3*- with *DNMT3A* and/or *IDH1* mutation (n=5); Group F: *CEBPA+/FLT3*+ regardless of *DNMT3A/IDH1* mutations (n=6). *FLT3*, *DNMT3A* and/or *IDH1* mutations did not significantly influence the probability of achieving CR in *NPM1*+ patients. CR was reached by 18/20 (90.0%) patients within group A, 18/21 (85.7%) in group B and 28/39 (71.8%) in group C. Relapse was detected in only 5/18 (27.8%) group A patients, in 12/19 (63.2%) in group B and in 19/28 (67.9%) in group C. *NPM1* mutated patients with neither *FLT3* nor *DNMT3A/IDH1* mutations (group A) had significantly longer EFS (P=0.009) as well as OS (P=0.017; Figure1A) than group B (*DNMT3A* and/or *IDH1*) mutated ones. *FLT3*, *DNMT3A* and *IDH1* were strongly associated with monoallelic *CEBPA* mutation. 10/19 patients had at least one of these aberrations, compared with only 1/13 cases within a biallelic *CEBPA* mutated ones (P=0.004). Within the *CEBPA*+ cohort, *FLT3* and/or *DNMT3A* and/or *IDH1* mutations significantly decreased the chance of reaching CR on the one hand and increased the risk of relapse on the other. Only 7/11 (63.6%) patients within groups E and F (when analysed together) achieved CR compared with 19/20 (95.0%; P=0.012) cases in group D. 4/7 (57.1%) patients relapsed within groups E+F and only 3/19 (15.8%, P=0.018) patients belonging into group D. Presence of *FLT3* and/or *DNMT3A* and/or *IDH1* aberrations shortened significantly EFS (P=0.009) and OS of *CEBPA* mutated patients (P<0.0001). There was no difference between patients within E and F groups regarding EFS and OS (Figure 1B). *IDH2* mutations did not influence the prognosis of *NPM1*+ or *CEBPA*+ patients whatsoever (not shown).

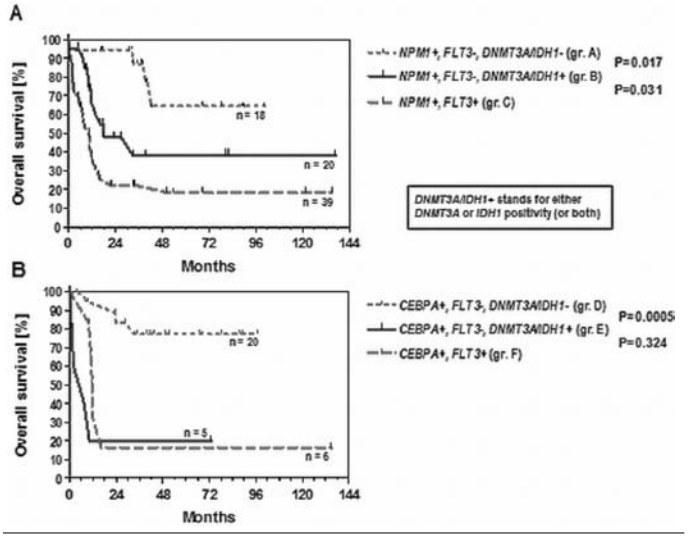

Figure 1. OS in genetic subgroups of *NPM1*+ (A) and *CEBPA*+ (B) patients.

Summary/Conclusions: Patients with *NPM1* or *CEBPA* mutations have favorable outcome only if they lack *FLT3* or *DNMT3A/IDH1* mutations. Not only *FLT3* but also *DNMT3A* and/or *IDH1* aberrations significantly shortened both OS and EFS of these patients. *FLT3*/ITD, *DNMT3A* and *IDH1* positivity coincide with monoallelic *CEBPA* mutation which might be the reason of inferior outcome

of patients with monoallelic *CEBPA* mutation as compared to those with biallelic *CEBPA* (usually lacking the additional mutations). We infer that only patients with *NPM1* or *CEBPA* mutation lacking the additional aberrations should not be transplanted in first CR.

P566

MUTATIONAL SPECTRUM ANALYSIS BY NEXT GENERATION SEQUENCING IN ADULT ACUTE MYELOID LEUKEMIA

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Background: Based on biological function and prognostic roles of these mutations, acute myeloid leukemia (AML) can be segregated into distinct subgroups. However, the spectrum of recurring alterations in larger cohorts needs further exploration and validation. Furthermore, it is not clear what genetic alterations co-operate with those initiating mutations to induce leukemia.
Aims: To investigate genetic alterations in a large cohort of adult AML and to explore potential mutations as therapeutic targets, we performed targeted high throughout NGS of 112 candidates in AML samples from 270 newly diagnosis patients.
Methods: After informed consent was obtained, 270 patients were investigated by a custom targeted NGS gene panel, which covered 112 genes associated with blood diseases. All exons of these genes were sequenced on an Ion Torrent semiconductor plat form and reads were mapped to NCBI hg19 RefSeq. Validation runs showed accurate results, with a mean of >97% coverage of the targeted regions at the average depth of 800X. Five genes mutations including FLT3-ITD, CEBPA, NPM1, CALR, and MPL were also detected by Sanger sequencing, since they are not accurately detected by NGS. Polymorphisms annotated in dbSNP 135 were excluded. All putative mutations were compared against multiple databases (*e.g.*1000genomes,COSMIC, PolyPhen, SIFT). To obtain more detailed genetic information, we also analyzed fusion genes including AML1-ETO, CBF-MYH11, and MLL translocations by RT-PCR, fluorescence *in situ* hybridization(FISH) and karyotype analysis.TP53 gene loss were determined by FISH, in addition to mutations detected by NGS.
Results: On average approximately three (2.84) genes per patient were mutated, and 76 (66.1%) of the 112 genes were revealed to be mutated in at least one patient. The most common gene abnormalities were FLT3 (21.85%), CEBPA (21.48%), NPM1 (20.37%), AML1-ETO (20.00%), N-RAS(19.36%).Mutation frequencies of CEBPA and N-RAS were higher than those reported in the literatures. Whereas, mutation frequencies of *DNMT3a* (10.37%) and *IDH2* (1.48%) were lower. According to the critical initiating step in the pathogenesis of AML, we grouped the adult AML into 9 groups (Figure 1). Then, we analyzed the co-operating mutation in these groups. AML1-ETO and CBFb-MYH11 make up CBF (core binding factor) leukemia. But they had different co-operating mutation constitutions. C-Kit mutations were the most common co-operating mutation for AML1-ETO. Whereas, N-ras mutations were most frequent one for CBFb-MYH11. N-ras mutations were common in all of groups, except NPM1 leukemia. N-ras mutations constituted 15% co-operating mutation in AML1-ETO, 44% in CBFb-MYH11, 30% in MLL translocation, 19% in CEBPA double mutation, 18% in secondary type, and 23% in others. Interestingly, we found 14.81% patients in our cohort had FAT1 mutations, which was not reported before. And FAT1 mutations could be found with high frequency in all of groups. FAT1 mutations could be found in 15% AML1-ETO, 19% CBFb-MYH11, 10% MLL translocation, 11% CEBPA double mutation, 11% NPM1 mutation, 9% in secondary type, and 19% in others.

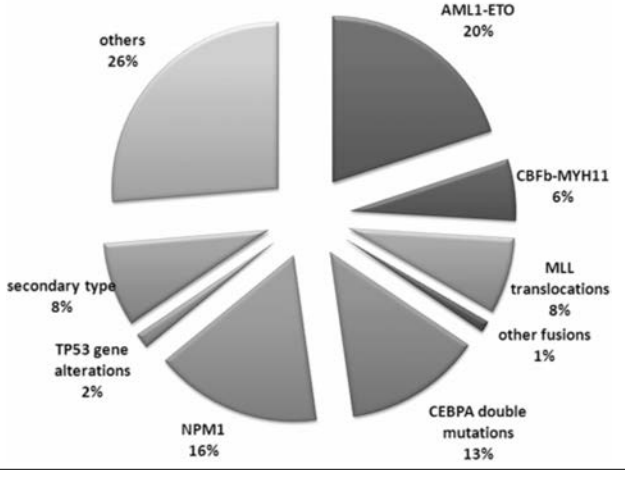

Figure 1. Adult AML were grouped into 9 groups based on the critical initiating step in the pathogenesis of AML.

Summary/Conclusions: Adult AML reveals a highly heterogeneous spectrum of candidate gene mutations. Here we provide an original and comprehensive overview of recurring mutations in AML. N-ras mutations are common in all AML entities, except NPM1 leukemia. These mutations have to be validated in a larger cohort with a focus on clinical implications accompanied by their use as therapeutic targets.

P567

NANOG EXPRESSION AS A RESPONSIVE BIOMARKER DURING REATMENT WITH HEDGEHOG SIGNAL INHIBITOR IN ACUTE MYELOID LEUKEMIA

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Background: Cumulative evidence suggests that dormant self-renewing leukemic stem cells (LSCs) contribute to acute myeloid leukemia (AML) propagation and relapse by evading conventional chemotherapies that target cycling cells. Therefore, there is a great need to find innovative therapies for AML that eliminate LSCs by targeting their specific properties. Aberrant activation of the Hedgehog (Hh) signaling pathway is involved in maintenance of the LSC populations in several experimental systems. PF-0444913 (PF-913) is a novel oral small molecule inhibitor that selectively binds and targets Smoothened (SMO), a membrane protein regulating the Hh pathway. Treatment with PF-913 has shown promising results regarding safety, tolerability, and early signs of efficacy in the early phase study of hematologic malignancies, including AML (Jamieson, *et al. ASH*, 2011). However, the detailed mode of action and biomarkers remain to be elucidated in AML therapy with the Hh pathway inhibitors.
Aims: We examined gene profiling analyses and biomarkers using the pre-clinical model and clinical samples derived from AML patients during PF-913 therapy to clarify the mode of action and biomarkers in AML therapy with the Hh pathway inhibitors.
Methods: We used AML cell lines and patient-derived primary AML cells and bone marrow cells in PF-913 treatment. Using RQ-PCR assays, we examined the change of gene expressions in the canonical Hh pathway molecules. We carried out comprehensive gene set enrichment analysis (GSEA), and pathway analysis during PF-913 treatment both in the pre-clinical experimental systems and the clinical setting (bone marrow blast-rich mononuclear cells derived from AML patients during the PF-913 mono-therapy). We also examined cell cycle analyses using flow-cytometory, immunoblot and immuno-staining assays.
Results: In the immunodeficient NOD/SCID/IL2rg^{null}(NOG) mouse model and the *ex vivo* culturing system of AML cell lines, GSEA revealed that PF-913 treatment induced effects on the self-renewal signatures and the cell-cycling regulations associated with LSC-like properties. Moreover, GSEA revealed that PF-913 treatment in the clinical setting modulated the LSC-like signature, the cell-cycling regulation signature, and the chemokine activity signature in the AML bone marrow cells. Ki-67 immuno-staining of bone marrow derived from AML patients showed that PF-913 treatment temporarily increased cell-cycling status during shorter periods of treatment. We also examined the pluripotency factor, Nanog expression in bone marrow cells derived from AML patients during the PF-913 therapy, based on the previous report that downstream effectors in the Hh pathway, GLI1 and GLI2, directly bind to the Nanog promoter and that the GLI-Nanog axis promotes stemness and growth in several cancers. Change of Nanog transcripts was closely associated with the GLI-target genes in bone marrow blast-rich mononuclear cells derived from AML patients during the PF-913 therapy. Furthermore, by backing to the pre-clinical experimental systems, Nanog transcript level stringently and significantly decreased during PF-913 treatment. In the same pre-clinical model, immunoblot resulted PF-913 treatment decreased the expression of *Nanog* protein level, too.
Summary/Conclusions: Gene profiling analyses revealed that treatment with Hh signaling inhibitor, PF-913 modulates self-renewal and cell-cycling signatures in AML, and Nanog transcript can be a responsive biomarker during the therapy. Our findings imply that PF-913 treatment can improve AML therapy through sensitizing dormant LSCs to chemotherapy and overcoming residual LSC-like diseases in the bone marrow microenvironment.

P568

A NOVEL NEXT-GENERATION SEQUENCING BASED ASSAY FOR FLT3/ITD MUTATIONS IS SENSITIVE AND SPECIFIC AND CAN BE USED TO DETECT MINIMAL RESIDUAL DISEASE IN AML PATIENTS

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Background: FLT3/ITD mutations are common in AML and confer a poor prognosis. A sensitive, specific assay for the detection of minimal residual disease

(MRD) in FLT3/ITD AML could guide decisions on transplant or maintenance therapy. To date, polymerase chain reaction (PCR) based assays for MRD in FLT3/ITD AML have been hampered by competition from the wild type allele, limiting the overall sensitivity to approximately 1 cell in 100. While several groups have reported the development of MRD assays for this disease, none of these diagnostics have been developed in concert with bioinformatics software under a quality system with the intent of being submitted to regulatory authorities as a harmonized assay available to the international community. We have developed a sensitive and specific MRD assay for FLT3/ITD mutations using next-generation sequencing (NGS).

Aims: We report here the development of an assay for FLT3/ITD mutations using a next-generation sequencing (NGS) platform.

Methods: Exons 14 and 15 are amplified by PCR and the products were detected by a refined NGS technique developed at Invivoscribe, Inc. Initial validation was carried out by spiking in fixed amounts of mutant DNA into wild type DNA to establish a sensitivity equivalent to detection of at least one ITD-containing cell out of 10,000 with a minimum input of 100,000 cell equivalents of DNA.

Results: We tested a series of 15 bone marrow aspirate samples from patients previously diagnosed with FLT3/ITD AML. All patients gave informed consent according to the Declaration of Helsinki. The investigator conducting the MRD assay was blind to the clinical information about the sample- no information was provided beforehand regarding the presence or absence of FLT3/ITD mutation, its length, or the mutant-to-wild type allelic ratio. All patients tested were in clinical remission by IWG criteria (J. Clin Oncol 2003; 24:4642) and all samples were derived from the first pull aspirate material used for the clinical confirmation of that remission. In all samples, both the standard CLIA-certified assay for the FLT3/ITD mutation (J Mol Diagn 2003; 5:96) as well as standard multi-parameter flow cytometry (for a leukemia-associated phenotype) were negative for detectable FLT3 mutations or cells with a leukemia phenotype. The first 4 samples were from patients who were newly-diagnosed, had just completed induction therapy, had achieved first CR, and were awaiting consolidation therapy. In all 4 cases, the FLT3/ITD mutation detected at diagnosis was detected in these remission samples, with mutation levels ranging from 1.35E-05 to 1.74E-04. Three samples were from patients who had relapsed and had responded to salvage therapy, achieving a CR2. In these 3 cases, the original FLT3/ITD length mutation was detected, with mutation levels ranging from 1.38E-06 to 1.11E-04 mutant ITD reads/total reads. Six samples were from patients who had undergone allogeneic transplant in remission. The samples were collected during routine post-transplant surveillance, 2-5 years after transplant. No mutation was detected in any of these patients, all 6 of whom are alive and disease free 2.5-5.5 years after transplant. Finally, 2 samples were from patients who had undergone allogeneic transplant for FLT3/ITD AML in first CR. At 2 and 6 months post-transplant, respectively, bone marrow aspirates from these 2 patients confirmed ongoing morphologic remission, with 100% donor chimerism in both the marrow and the T-cell compartment, and a negative standard assay for FLT3/ITD mutations. Using DNA from these same time points, the MRD assay detected FLT3/ITD mutations at levels of 3.67E-03 and 1.04E-04 mutant ITD reads/total reads, respectively. Both of these patients relapsed with AML carrying the detected FLT3/ITD mutation within 6 months.

Summary/Conclusions: This novel MRD assay is specific, and is 2 orders of magnitude more sensitive than current commercially available assays for FLT3/ITD mutations. We anticipate that this assay will be broadly available to the public soon, and will have a significant impact in the clinical management of this disease.

P569

AN *EX VIVO* NATIVE ENVIRONMENT PRECISION MEDICINE TEST SHOWS HIGH CLINICAL CORRELATION WITH RESPONSES TO FIRST LINE ACUTE MYELOID LEUKEMIA TREATMENT

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Background: We have overcome the limitations of 40 years of *ex vivo* testing

Aims: The aim of this study is to determine the ability of Vivia's novel test (based on studying the ex-vivo sensitivity to drugs) to predict the complete remission (CR) rates after induction chemotherapy with cytarabine (Ara-C) and idarubicin (Ida) in 1st line AML.

Methods: This has been an observational clinical trial where bone marrow samples from adult patients diagnosed with *de novo* AML in Spanish centers from the PETHEMA group were included. Whole marrow samples maintaining their Native Environment were incubated for 48h in well plates containing Ara-C, Ida, or their combination. Pharmacological responses are calculated using population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant.

Results: 390 patient samples were used to calculate the dose response (DR) curves for Ara-C alone, Ida alone, and their synergism. For clinical correlation we used 142 patients with median 56 years. The strongest clinical predictors were the Area Under the Curve (AUC) of the DR of Ara-C (P=1.34E-05), and the AUC of IDA (P=3.9E-05). The GAM models revealed a significant relationship (RSquare=0.452 and deviance explained=45%) between these predictors and higher probabilities of post-induction resistance. Figure 1A shows a table illustrating the correlation between clinical outcome (columns) and the test predictions (lines). Using the cut off determined by the GAM models. The test obtain a high Specificity and Positive Protective Value (95% and 80,77%) and a lower sensitivity (50%) with a general prediction of a 81,69%. Interestingly, the 5 cases that the test identify as resistant but were clinically sensitive have high level of minimal residual disease. On the other hand, the test does not properly identify 21/142 that are clinically resistant and the test predicts as sensitive (bottom left quadrant right panel). This mismatched subgroup mimics the problems from molecular markers where a resistant clone present in a minority of leukemic cells cannot be detected yet drives the patient response. Consistent with this analysis, adding the cytogenetic risk factor to the *ex vivo* results, identifying the high risk population by molecular markers that might be present in a minority of the cells, significantly improves the correlation; Figure 1B shows the 90% overall correlation achieved in 117 patient samples adding the cytogenetic risk factor, with a major improvement in the sensitivity from 50% to 72%. Both approaches lead to substantial improvements in estimated overall survival.

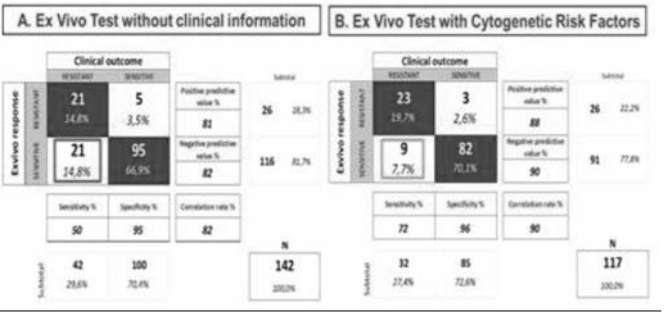

Figure 1. Clinical correlation of the *ex vivo* test alone (A, left) or adding the cytogenetic risk factor (B, right).

Summary/Conclusions: This novel test is able to predict the clinical response to Ida+Ara-C induction with overall correlation and predictive values of 80%, higher than ever achieved. Considering this result and current clinical response rate of 66.7% (70% in this study), clear clinical benefits can be achieved with the use of the test. Adding the cytogenetic risk profile further increase the correlation to 90%. Thus this novel test may be valuable information to guide 1st line patient therapy

P570

RECURSIVE PARTITIONING ANALYSIS FOR GENETIC STRATIFICATION AND PROGNOSTICATION OF ACUTE MYELOID LEUKAEMIA

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Background: The rapid evolution of molecular genetics has resulted in a diverse range of genomic variants that have been variably linked to patient prognosis in Acute Myeloid Leukaemia (AML). Standard regression analysis does not adequately address the complex multigene interactions that may positively or negatively influence individual patient outcome. Recursive partitioning is an alternative approach that empirically computes and ranks in order of importance, all possible prognostic combinations to create a stepwise decision tree resulting in the categorical partitioning of the AML population into subgroups of patients with independent prognosis. Through utilizing Random Forest analysis which repeatedly reforms the decision tree utilizing both subsets of the data and of the variables to avoid the tendency for over-fitting the data to the data set enhancing the generalizability of the decision tree.
Aims: To demonstrate the utility of an algorithmic machine-based learning approach to define independent prognostic subgroups using cytogenetic and genomic variants derived from The Cancer Genome Atlas (CGA) analysis of 200 patients with AML.
Methods: Clinical and mutational data was collected from the CGA AML dataset with cases limited to an age of <70 years. Exome sequencing data from the CGA dataset (http://cancergenome.nih.gov) was analysed utilising Integrated Genomics Viewer (IGV) v2.3 with statistical analysis performed utilising R (R foundation for statistical computing) v3.2.3 and the rpart (v4.1-10), randomForest (v4.6-12) and randomForestSRC (v2.0.7) modules. The analysis was restricted to the 20 most commonly identified mutations within the CGA dataset to exclude uncommon mutations.
Results: The analysis set included 173 patients with complete clinical and genetic information. Of these 143 were aged <70 years. The median overall survival for the entire cohort was 24 months with a median follow-up of 40.5 months. Recursive partitioning analysis was performed with a minimum "bucket" size of 8 patients (5% of the total dataset) and a complexity parameter of 0.001 reflective of the relative complexity acceptable within the model. This resulted in a decision tree that highlighted the importance of favourable risk karyotype and mutations affecting *TP53*, *RUNX1*, *DNMT3A*, *NPM1* and *FLT3-ITD*. (Figure 1). In non-CBF AML a striking adverse prognostic impact was observed for *TP53*, *RUNX1* and *DNMT3A* mutations, particularly when these were in combination with other 'driver' mutations, such as *NPM1* and *FLT3-ITD*. Random Forest Analysis confirmed the prognostic importance of these mutations and predicted an error rate of 37.08% for the predictive model in determining survival for patients within this dataset, thus predicting a greater than 62% probability of correctly predicting survival outcome with these factors. CBF-AML and normal karyotype AML negative for high-risk prognostic molecular markers carried a favourable risk (median OS NR and 27 months, respectively). Patients with high-risk disease included those with *DNMT3A*mut (median OS 11 months), *TP53*mut (median OS 12 months), *RUNX1*mut (median OS 11 months) and adverse-risk karyotype (median OS 12 months). Patients with *FLT3*-ITD (median OS 24 months) had an intermediate prognosis in the absence of other high-risk markers. *CEBPA* double mutants did not appear in the decision tree, likely reflective of the low occurrence rate of *CEBPA* mutations in the dataset.

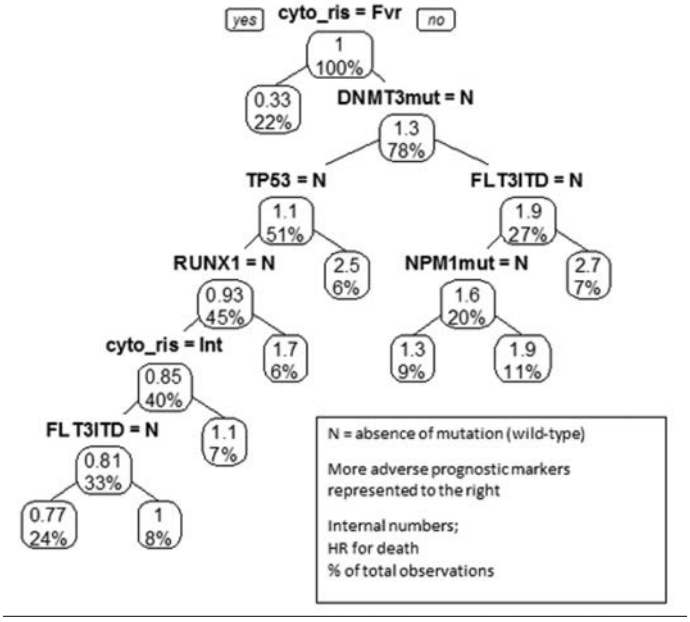

Figure 1.

Summary/Conclusions: Recursive partitioning analysis allows for the formulation of a non-biased analysis tree and highlights the importance of combinations of mutations in determining prognosis for patients with AML. The impact of mutations affecting *TP53, RUNX1* and the combination of mutations affecting *DNMT3A* with other driver mutations was prognostically relevant in patients with intermediate and poor risk karyotype. Confirmation of this decision tree approach using larger datasets will further refine the accuracy and value of this novel algorithmic approach for prognostically sub-classifying AML.

P571

CD7 AND CD34 CO-EXPRESSION IDENTIFY A SUBPOPULATION OF NUCLEOPHOSMIN 1-MUTATED ACUTE MYELOID LEUKEMIA (NPM1+ AML) PATIENTS WITH INCREASED RISK OF RELAPSE

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Background: *NPM1* mutated AML has been considered as a subset of AML patients with relatively good prognosis. However, multiple studies have shown clinical variability in *NPM1* mutated AML.
Aims: Using combined flow cytometry and next generation sequencing (NGS) data we have sought to identify prognostic factors, which would help in individual risk-assessment in *NPM1*+AML.
Methods: Blasts from 83 *NPM1*+ AML patients diagnosed Jan 2012 to Aug 2015 at UHN, Toronto, were analyzed. 76 patients received induction treatment, mostly with daunorubicin 60 mg/m^2 for 3 days and cytarabine 100 mg/m^2 (age >60 years) or 200 mg/m^2(age <60 years) daily for 7 days. At diagnosis, all cases were immunophenotyped using a 4-tube, 10-color flow cytometry panel (1). 30x10^5 cells were analyzed using Navios and Kaluza software (Beckman Coulter). Cases were classified according to 3 immunophenotypic patterns depending on the degree of monocytic differentiation (1: no monocytic differentiation; 2: a monocytic subset, and 3: predominantly monocytic). FLT3-ITD/TKD and NPM1 mutations were identified using fragment analysis on an ABI Genetic Analyzer 3100 /3130 platform. NGS using the TruSight Myeloid Sequencing Panel (Illumina) was performed in 68 patients.
Results: Among 83 NPM1+ AML patients, 69 patients had normal karyotype, 10 had cytogenetic abnormalities and 4 failed. 7 patients were excluded from statistical analysis due to early death. 47 patients achieved complete remission (CR) and 29 relapsed with 11 subsequent deaths. Average time to relapse from diagnosis was 10.5 months. Presence of FLT3 ITD mutation (39 pts) and immunophenotypic marker expression patterns 1-3 were not significantly associated with relapse-risk. However, aberrant expression of CD7+ alone or especially CD34/CD7 co-expression (even in a small subset of leukemic cells) was significantly associated with relapse-risk, independent of FLT3 ITD or TDK mutational status. Figure 1 shows examples of CD7 negative case (left), CD7 in a subset of blasts with minimal CD34/CD7 population (middle) and most blasts CD7/CD34+ (right). CD7+ and CD7- group of patients did not differ significantly in clinical characteristics. However, CD56 was more often expressed in CD7+ patients who relapsed. NGS revealed a heterogeneous mutational profile of known leukemogenic genes. At diagnosis, 15 aberrations (copy number alterations (CNAs), n=10; uniparental disomies (UPDs), n=5) were identified in 25% of patients. None of the studied mutations were significantly associated with increased relapse risk. Interestingly, *PTPN11* mutation showed increased frequency in the group with CD7 expression but was found mostly in patients who remained in CR. Otherwise there was no significant association between NGS results and immunological marker expression patterns. At relapse, 56 genomic alterations (CNAs, n=46; UPDs, n=10) were detected in 55% of patients indicating an increase in genomic complexity.

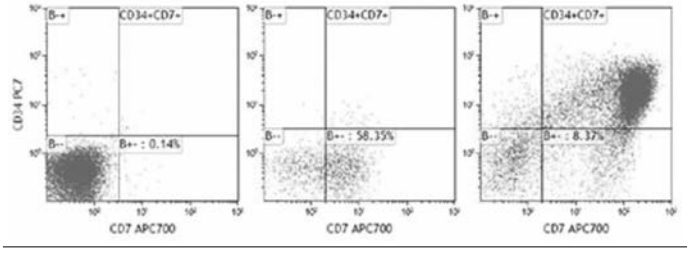

Figure 1.

Summary/Conclusions: Expression of CD7 and/or CD34 were associated with early relapse and/or mortality. The genetic mutational profile of NPM1+ AML is heterogeneous, suggesting that no one specific genetic driver may be responsible for leukemogenesis and affect prognosis. *PTPN11* mutation seems to be associated with better prognosis in NPM1+ AML.

Reference

1. Porwit A, Rajab A. Flow cytometry immunophenotyping in integrated diagnostics of patients with newly diagnosed cytopenia: one tube 10-color 14-antibody screening panel and 3-tube extensive panel for detection of MDS-related features. Int J Lab Hematol. 2015 May;37 Suppl 1:133-43.

Acute myeloid leukemia - Clinical 3

P572

IMPACT OF FIRST LINE ARSENIC TRIOXIDE AND RETINOIC ACID TREATMENT ON OUTCOME IN THERAPY-RELATED ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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Background: Reports of patients (pts) with therapy-related acute promyelocytic leukemia (t-APL) have increased in recent years (yrs) and retrospective studies have suggested that their outcomes were similar to those of pts with *de novo* APL. More recently, arsenic trioxide (ATO)/all-trans retinoic acid (ATRA) has been shown to be the most effective therapy in *de novo* APL with low/intermediate Sanz score (Lo-Coco F. *et al.* NEJM, 2013). So far, this regimen has not been evaluated systematically in t-APL pts.
Aims: To describe clinical outcome in a large series of t-APL pts and to compare outcomes after treatment with ATO/ATRA with that after chemotherapy (CTX) and ATRA.
Methods: We retrospectively studied 76 t-APL pts (median age, 59 yrs; range, 18-80 yrs), treated between 1998 and 2015 within seven study groups/institutions of the US and Europe. Pts had received i) CTX/ATRA (n=34; either daunorubicin/idarubicin and ATRA for induction and different CTXs+ATRA for consolidation), ii) ATO/ATRA (n=19; according to Lo-Coco F. *et al.* NEJM, 2013 (n=16) or Burnett AK, *et al.* Lancet Oncol, 2015 (n=3)), iii) CTX/ATO/ATRA (n=17; according to Gore SD, *et al.* JCO, 2010), and ATRA only (n=6).
Results: In 63 of the 76 t-APL pts, a solid cancer was the primary malignancy, with breast cancer being the most common (n=28), followed by prostate (n=9), head&neck (n=7) and gastrointestinal cancers (n=6). In five of the 76 pts, a hematologic neoplasm was the primary malignancy (non-Hodgkin lymphoma, n=4; Hodgkin lymphoma, n=1). Eight pts had received cytotoxic treatment for autoimmune/rheumatological diseases. The median latency period between diagnosis of primary malignancy/disease and occurrence of t-APL was 4 yrs (range, 1-27 yrs). Sixty pts were of low/intermediate Sanz score. The four therapy groups were comparable in all baseline characteristics except for age (p=0.004) and platelet counts (p=0.003); pts treated with ATRA only were significantly older and had lower platelets. Response data were available for 74 pts. Complete remission after induction therapy was achieved in 82% of the CTX/ATRA pts, in 100% of the ATO/ATRA pts, in 94% of the CTX/ATO/ATRA pts and in 33% of the ATRA pts. Early death rate for the intensively treated group was 6% (CTX/ATRA, n=3; CTX/ATO/ATRA, n=1; ATO/ATRA=0) and 50% in the ATRA group. Only two of the 76 pts relapsed (both of the CTX/ATRA group), both pts were successfully salvaged and went on to allogeneic transplant. Ten pts died in remission (relapse of the prior malignancy, n=5 (CTX/ATRA, n=2, ATRA, n=2, CTX/ATO/ATRA, n=1); infections, n=2 (CTX/ATRA, n=1 and ATO/ATRA, n=1); adverse events during treatment, n=2 (CTX/ATRA, n=1 and ATO/ATRA, n=1); reason unknown, n=1 (CTX/ATRA)). With a median follow-up of 2.4 yrs, the estimated 2-year relapse-free survival was lower in pts receiving CTX/ATRA (80%; 95%>CI: 66-97%) as compared to pts treated either with ATO/ATRA (93%; 95%>CI: 82-100%) or CTX/ATO/ATRA (94%; 95%>CI: 83-100%). The estimated 2-year overall survival (OS) rate for pts receiving CTX/ATRA was again lower (85%, 95%>CI: 73-98%) as compared to pts treated either with ATO/ATRA or CTX/ATO/ATRA (94%, 95%>CI: 84-100%, each). Two-year survival was not evaluable for pts treated with ATRA. Regarding toxicity, there was a trend towards less frequent febrile neutropenia of grade ≥3 in pts treated with ATO/ATRA as compared to CTX/ATRA or CTX/ATO/ATRA (p=0.06).
Summary/Conclusions: The ATO-based regimen for first line treatment of t-APL pts was associated with excellent and sustained response rates. These data demonstrate the important potential of ATO/ATRA in the primary management of t-APL pts.

P573

AZACITIDINE (AZA) *VS* CONVENTIONAL CARE REGIMENS (CCR) IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) WITH MYELODYSPLASIA-RELATED CHANGES (MRC) IN AZA-AML-001 PER CENTRAL REVIEW

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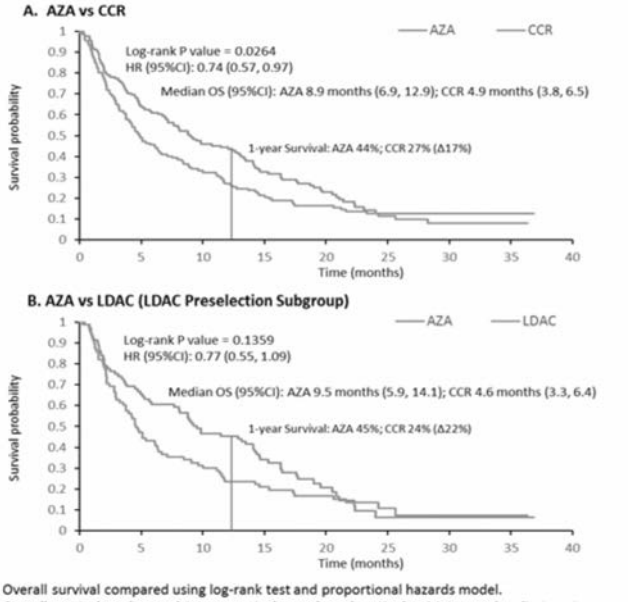
Background: Patients (pts) with AML-MRC are generally older and more likely to have poor-risk cytogenetics, leading to poor prognosis (Weinberg, *Blood*, 2009). A subanalysis of the AZA-AML-001 (AZA-AML) trial evaluated safety and efficacy of AZA *vs* CCR in 158 pts diagnosed with AML-MRC per local assessment (Seymour, ASH 2014). Subsequently, AML-MRC classification was adjudicated centrally according to WHO criteria (Vardiman, *Blood*, 2009).
Aims: Determine the difference in frequency of AML-MRC classification between local and central assessments in AZA-AML, and evaluate the treatment (Tx) effects of AZA *vs* CCR and AZA *vs* low-dose ara-C (LDAC) in the centrally confirmed AML-MRC pt cohort.
Methods: Pts aged ≥65 yrs with newly diagnosed AML confirmed by central review, ECOG PS 0-2, intermediate- or poor-risk cytogenetics, and WBC ≤15x10^9/L, classified as having AML-MRC per WHO criteriaΔΔ are included in these analyses. Pre-randomization, the most appropriate of 3 CCR was preselected for each pt: intensive chemotherapy (IC, standard 7+3), LDAC (20mg SC BID x10d/28d), or best supportive care (BSC) only. Pts were then randomized to preselected CCR or AZA (75mg/m^2/d x7d/28d). Overall survival (OS) was compared for AZA *vs* CCR in all AML-MRC pts, and for AZA *vs* LDAC in the LDAC preselection group. Overall response rate (ORR) included complete remission (CR)+CR with incomplete hematologic recovery (CRi) (IWG 2003).
Results: Upon central review, a much larger pt cohort was identified than based on local reports. In all, 262/488 pts (54%) fulfilled WHO criteria for AML-MRC: AZA n=129, CCR n=133 (IC n=24, LDAC n=79, BSC n=30). In the AZA and CCR arms, respectively, median age was 76 yrs (range 64-90) and 75 yrs (65-87), 51% and 54% had poor-risk cytogenetics, 27% and 22% had ECOG PS=2, and median blasts were 65% (27-99) and 70% (26-100). At baseline (BL) only 79 pts (30%) were reported to have had prior MDS. BL characteristics for LDAC-preselected AZA and LDAC pts were comparable except for ECOG PS=2 (31% *vs* 19%, respectively) and poor-risk cytogenetics (42% *vs* 58%). Median numbers of Tx cycles were: AZA 5 (range 1-27), IC 2 (1-3), LDAC 2 (1-22), and BSC 3 (1-9). OS for all AML-MRC pts was improved with AZA *vs* CCR (HR 0.74 [95%CI 0.57, 0.97], *P*=0.026; Figure 1 A): median OS was 8.9 *vs* 4.9 months, respectively, and 1-yr survival rates were 44% *vs* 27% (Δ17% in favor of AZA). ORR was 25% *vs* 17% with AZA *vs* CCR, respectively (*P*=0.17). In LDAC-preselected pts, median OS with AZA *vs* LDAC was 9.5 *vs* 4.6 months, respectively (HR 0.77 [0.55, 1.1], *P*=0.14; Figure 1 B) and 1-yr survival rates were 45% *vs* 24% (Δ22% in favor of AZA). ORR was higher with AZA *vs* LDAC: 27% *vs* 14%; *P*=0.050. Rates of grade 3-4 cytopenias were similar among AZA, IC, and LDAC, except that febrile neutropenia was less frequent with AZA (23%) than with LDAC (33%) or IC (39%).



Overall survival compared using log-rank test and proportional hazards model.
Overall survival median and 1-year survival rates based on Kaplan-Meier product limit estimates.

Figure 1. Median overall survival for patients with AML-MRC.

Summary/Conclusions: Over one-half of all newly diagnosed AZA-AML pts met the WHO definition of AML-MRC in central review *vs* one-third in local review, suggesting potential under-diagnosis of MRC in the community. This difference may also reflect challenges in diagnosing dysplasia. MDS and AML may reflect a continuum of myeloid disease, particularly in this pt population. Pts with AML with MDS-related changes may respond preferentially to AZA. In both the locally (Seymour, ASH 2014) and centrally identified AML-MRC cohorts, AZA provided a clinically meaningful effect with prolonged OS and improved 1-year survival rates.

P574

IMPACT OF PHYSICIANS' CHARACTERISTICS ON DECISION MAKING IN ELDERLY ACUTE MYELOID LEUKEMIA

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Background: Outside clinical trials, therapeutic options offered to elderly acute myeloid leukemia(AML) patients (pts) are limited. They can be summarized as intensive chemotherapy (ICT), low-intensity therapy or best supportive care (BSC) depending on patient- and disease-related prognosis factors. Although scoring systems have been proposed to rationalize this clinical decision-making, there is a strong heterogeneity in clinical practice which is still poorly understood. Indeed, cancer management study mainly focused on the patients' determinants of care but very few have assessed the influence of physicians' characteristics and none in AML. In behavioral sciences, attitudes towards risk and ambiguity are crucial psychological traits that may explain medical choices and practices. These characteristics are connected with theoretical models of decision under uncertainty which can be divided in Expected Utility (EU) and Non-Expected Utility (Non-EU) models. Choice patterns in decision tasks known as Allais and Ellsberg paradoxes allow classifying individuals in these two classes of models.
Aims: Our study investigated the impact of physician's characteristics on their medical decisions regarding selected clinical vignettes of older AML pts that highlight distinct and difficult representative situations derived from clinical practice.
Methods: Physicians' demographical and occupational characteristics were collected through a national cross-sectional web survey among French onco-haematologists. We also assessed their attitude regarding risks by the self-reported individual willingness to take risks in the daily life on a 0-10 Likert scale (Dohmen & al, 2011) and the response to the binary lottery choice questions of the Allais paradox (Kahneman & Tversky, 1979) for identifying respondents conforming to EU. A last question used two certainty equivalents elicitations (Abdellaoui & al, 2011) in an Ellsberg paradox setting in order to define an index of ambiguity attitudes. The physicians were asked to decide how to treat (ICT, low-intensity therapy or BSC) elderly AML patients presented in clinical vignettes. We present the results for vignette with highest heterogeneity (cf. Figure 1).

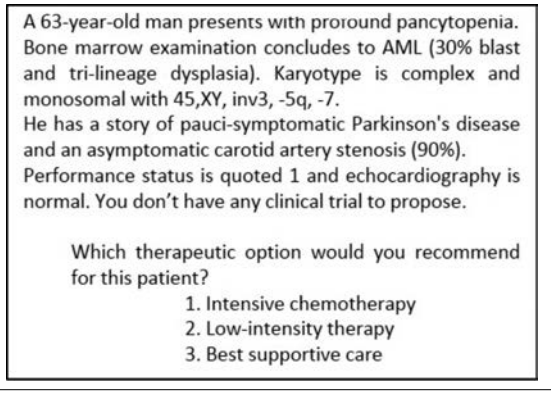

Figure 1. Clinical vignette.

Results: Among the 211 physicians who responded to the survey, the median age was 42 years old [inter-quartile range (IQR): 32-52], 54% were male, 72.5% were consultant or professor, 70.6% worked in academic center, 78% were Hematologists, 61% were involved in AML care in their daily practice. Regarding Likert scale of willingness to take risk, median was 5 (IQR 4-7). Regarding the Allais paradox, EU, non-EU and undefined status represented respectively 42.2%, 44.5% and 13.3%. Regarding attitudes towards ambiguity, averse, neutral, seeking and undefined attitudes represented respectively 43.1%, 16.6%, 15.6% and 24.7%. From the clinical vignette we observed that 51.7% choose ICT, 45% favored low-intensity therapy and 3.3% BSC. Using the elicited treatment recommendation in the vignette as the explained variable, a multivariate logit model (N=159) on variables identified from the bivariate analyses highlighted the following trends: the probability of recommending ICT was 60% lower for women compared to men (p=0,012). Risk averse respondents tended to recommend less ICT (p=0,075) as well as EU respondents (p=0.126). Ambiguity averse respondents tend to recommend more ICT (p=0,073).
Summary/Conclusions: These preliminary results show that physicians' attitudes towards risk and ambiguity, *i.e.* physicians' non professional characteristics, may influence their clinical practice when dealing with older AML pts.

P575

THE ADDITION OF GEMTUZUMAB OZAGAMICIN (GO) TO INTENSIVE CHEMOTHERAPY SIGNIFICANTLY IMPROVES COMPLETE RESPONSES CR RATES AND OVERALL SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Background: Young patients affected by non APL-Acute Myeloid Leukemia (AML) achieve complete remission (CR) using conventional induction chemotherapy with anthracycline plus cytarabine-based regimens in about 55-70%. The addition of Gemtuzumab Ozogamicin (GO) as third or fourth drug demonstrated to improve clinical outcome, in terms of CR rates.
Aims: We retrospectively evaluated and compared the efficacy of different induction schedules, in terms of CR rates and (OS), administered to two groups of AML patients. Group 1 (n=139) was treated with a GO (MyFLAI or MyAIE schedules); group 2 (n=270) received a non-GO based regimen including or not Fludarabine (FLAI, FLAN, FLAG, 3+7 or DAE).
Methods: From 1997 to 2014, 409 patients with newly diagnosed AML were treated in 3 Italian Institutions. According to karyotype (performed in 392/409 patients), FLT3 (available for 244/409 patients), and NPM1 mutational status (available for 157/409 patients), based on the NCCN-2013 risk stratification criteria, 35.2% of the patients were considered at High Risk (HR) (31.6% and 36.4% in the two groups, respectively) and 7.6% at low risk (LR) (7.8% and 7.0%, respectively).
Results: The complete remission (CR) rate after induction was 81.4% and 70.4% for Group 1 and 2, respectively (p=0.008). Deaths during induction (DDI), occurring in the first 50 days from 1st line therapy, were 4/139 (2.9%) in Group 1 and 22/270 (8.1%) in Group 2. Patients treated with GO showed a better OS than patients of Group 2; the 5-years OS in the two groups was 54.01% and 34.9%, respectively, and different according to age (54.0% and 34.9% respectively (p<0.001) in patients <60 years, 30.2% and 13.5% respectively (p=0.001) in patients >=60 years). Notably, the analysis on subgroup of HR patients showed a significantly better OS in Group 1 than in Group 2 (p=0.007, 5-year OS 47.7%; 21.0% respectively) and EFS.
Summary/Conclusions: Our conclusion is that adding GO to any induction regimen is an independent and strong predictor of better OS and higher CR rate. Patients with SR and HR AML could therefore benefit from this new approach to AML front line treatment in terms of OS if compared with other standard regimens.
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P576

HOSPITALIZATION FOR TREATMENT-EMERGENT ADVERSE EVENTS (TEAE) IN OLDER (≥65 YEARS) PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) WITH >30% MARROW BLASTS IN THE PHASE 3 AZA-AML-001 STUDY

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Background: AML treatment (Tx) places an enormous financial burden on both payers and patients (pts). Hospitalization is the largest cost driver in AML care (Zeidan, *Crit Rev Oncol Hematol*, 2015) and AML pts report reduced quality of life when in hospital (Sekeres, *Leukemia*, 2004). The AZA-AML-001 (AZA-AML) study compared azacitidine (AZA) with conventional care regimens (CCR) in older pts with AML. As previously reported, AZA was associated with lower incidence rates (IRs) of hospitalization and total days in hospital for TEAEs per pt-year (pt-yr) of drug exposure *vs* the combined CCR arm (Dombret, *Blood*, 2015). AZA-AML had a preselection study design, allowing for comparisons

between AZA and individual CCR in groups of pts with generally comparable clinical and prognostic features at entry.
Aims: Compare IRs of hospitalization and days in hospital for TEAEs adjusted for pt-yrs of exposure within preselection groups.
Methods: Older pts with newly diagnosed AML (>30% bone marrow blasts), ECOG PS 0-2, intermediate- or poor-risk cytogenetics, and WBC count ≤15x10^9/L were eligible. Pre-randomization, all pts were preselected to receive the most appropriate CCR: low-dose ara-C (LDAC, 20mg SC BID x10 days (d)/28d), intensive chemotherapy (IC, cytarabine 100-200mg/m^2 IV x7d+anthracycline IV x3d induction), or best supportive care (BSC) only. Pts were then randomized to AZA (75mg/m^2x7d/28d) or to CCR and received the preselected Tx. All pts could receive BSC. Safety-evaluable pts had ≥1 dose of study drug and ≥1 post-baseline safety assessment. TEAEs were assessed for up to 28 days after the last AZA or LDAC dose, 70 days after the last IC course, or day of discontinuation for BSC pts. TEAEs were defined as new or worsening AEs during Tx. To address imbalances in duration of exposure between Tx groups, hospitalization and days in hospital for TEAEs are normalized for pt-yrs of exposure using aggregate data.
Results: In all, 471 pts were safety-evaluable (AZA n=236, CCR n=235). Most pts were preselected to LDAC (n=304; 65%). Baseline characteristics of AZA and CCR pts within preselection groups were generally comparable. Duration of exposure (pt-yrs) was longest with AZA in each preselection group (Table 1). AZA was associated with lower IRs of hospitalization and days in hospital for TEAEs *vs* other Tx. IR for number of hospitalizations was not significantly different between AZA and LDAC or AZA and IC, but was ~55% lower with AZA *vs* BSC (relative risk 0.455). Additionally, IRs for days in hospital for a TEAE were lower with AZA by 6.4 to 22.5 fewer days/pt-yr across preselection groups (Table 1).

Table 1.

Incidence rates per patient-year of treatment exposure of hospitalization and days in hospital for TEAEs

	Preselected to BSC only		Preselected to LDAC		Preselected to IC	
	BSC (n=40)	AZA (n=42)	LDAC (n=153)	AZA (n=151)	IC (n=42)	AZA (n=43)
Total exposure, pt-yrs	**9.6**	**28.1**	**82.9**	**111.7**	**14.1**	**35.1**
Patients hospitalized for a TEAE, n (%)	25 (62.5)	29 (69.0)	111 (72.5)	110 (72.8)	21 (50.0)	26 (60.5)
Total number of hospitalizations for TEAEs, n	42	56	186	226	27	60
Hospitalizations per pt-year of exposure, IR	4.38	1.99	2.24	2.02	1.91	1.71
Rate difference AZA:CCR hospitalization	-2.39		-0.22		-0.20	
Relative risk (95%CI) AZA:CCR hospitalization	0.455 (0.305, 0.679)		0.902 (0.743, 1.095)		0.893 (0.567, 1.407)	
Total number of days hospitalized for a TEAE, n	465	798	2905	3202	711	977
Days hospitalized for a TEAE per pt-year of exposure, IR	48.48	28.39	35.03	28.67	50.35	27.82
Rate difference AZA:CCR days hospitalized	-20.09		-6.36		-22.53	
Relative risk (95%CI) AZA:CCR days hospitalized	0.586 (0.522, 0.656)		0.818 (0.778, 0.861)		0.552 (0.502, 0.609)	

AZA, azacitidine; BSC, best supportive care; IC, intensive chemotherapy; IR, incidence rate; LDAC, low-dose ara-C; pt-yrs, patient-years; TEAE, treatment-emergent adverse event

Summary/Conclusions: Accounting for drug exposure when reporting safety outcomes is common in diseases with low mortality rates that require chronic Tx, but may be increasingly relevant for diseases with a high mortality rate, such as AML, particularly for drugs like AZA that are given in repeated Tx cycles. In AZA-AML, incidence rate of hospitalization for a TEAE was >50% lower with AZA *vs* BSC, suggesting that older AML patients should be offered active Tx. AZA was also associated with shorter hospital stays, as evidenced by fewer days in hospital for TEAEs with AZA *vs* LDAC, IC, and BSC.

P577

PHARMACEUTICAL TARGETING OF PYRUVATE DEHYDROGENASE KINASES IN T(8;21) ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) constitutes a heterogeneous clonal hematological malignancy characterized by fast accumulation of immature myeloid cells that are no longer able to differentiate into fully mature blood cells. AML is the most frequent acute leukemia with more than one quarter of a million people diagnosed annually worldwide. Only moderate improvement has been achieved with standard treatment over the last 3-4 decades. Due to genetic heterogeneity and complexity of the disease with considerable variation in therapy response and high relapse rate, AML remains a disease with poor prognosis (median overall survival of 18 months). Hence, there is an urgent need to improve the existing treatment by identification of novel therapeutic targets. By comparative gene expression analysis we have identified aberrantly expressed genes in human AML patient cells as compared to their normal counterparts purified from bone marrow samples of healthy individuals. One of these genes, *pyruvate dehydrogenase kinase 1* (*PDK1*), a regulatory metabolic enzyme, has been shown to be upregulated in several subtypes of AML, and is thought to contribute to the altered energy metabolism exhibited by cancer cells known as the Warburg effect or aerobic glycolysis. Hence inhibiting the activity of PDK1 with small molecule inhibitors provides an interesting novel therapeutic strategy for the treatment of AML.
Aims: The aim of this study was to investigate how targeting of PDK1 by small molecule inhibitors dichloroacetate (DCA) and AZD7545 would affect growth and survival of t(8;21) AML in a series of *in vitro* culture experiments.
Methods: Several different methods were used in order to investigate the role of PDKs including western blot analysis as well as different functional assays to show a switch in the glycolytic acitvity.
Results: The inhibition of PDK1 with DCA was found to reverse the glycolytic phenotype of cancer cells from glycolysis toward mitochondrial respiration leading to cell death in human t(8;21) AML cells. Additionally, the combination of DCA with standard chemotherapy demonstrated a synergistic inhibitory effect. The novel PDK inhibitor AZD7545 was demonstrated to be a more potent inhibitor of PDK1 activity than DCA in t(8;21) AML. However, its ability to switch the Warburg effect is still under investigation. Furthermore in order to establish the use of DCA and AZD7545 in a preclinical setting, a mouse model mimicking human t(8;21) AML was used to determine the expression profile of PDK isoforms. Here PDK2 and PDK3 expression was identified to be predominantly important for the glycolytic phenotype. It was also possible to show that DCA and AZD7545 treatment impaired *in vitro* colony formation ability of clonogenic murine t(8;21) AML cells to a higher extent than wild-type mouse cells.
Summary/Conclusions: Overall, these results indicate that PDKs play an important role in maintaining the Warburg effect in t(8;21) AML, thus implicating that inhibition of PDKs with small molecule inhibitors might represent a novel therapeutic strategy for the treatment of AML patients.

P578

HOPX IS A HEMATOPOIETIC STEM CELL MARKER AND ITS HIGH EXPRESSION PREDICTS CHEMORESISTANCE IN *DE NOVO* ACUTE MYELOID LEUKEMIA PATIENTS

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Background: *Homeodomain-only protein homeobox* (*HOPX*) gene encodes a homeodomain protein and functions as an adaptor protein to mediate transcriptional repression. It was shown to play a role in stemness in intestine, hair follicles, and pulmonary alveolar cells. *HOPX* was also reported as a tumor suppressor gene in lung, colon, esophagus, pancreas, uterus, and stomach cancers.However, the biological effects of HOPX in hematopoietic malignancies have not been explored.
Aims: In this study, we aim to see the clinical and biological relevance of *HOPX* expression in acute myeloid leukemia (AML).
Methods: The *HOPX* gene expression levels were extracted from the mRNA array data derived from 346 newly diagnosed AML patients in the National Taiwan University Hospital, and were analyzed for its clinical and biological relevance.
Results: The 346 patients were divided into two groups based on the median level of *HOPX* expression. Patients with higher *HOPX* expression had significantly higher incidences of mutations in *RUNX1*, *ASXL1*, *IDH2* and *DNMT3A*, but lower incidences of *NPM1* mutation and *CEBPA* double mutations. With a median follow-up of 31.2 months, patients with higher *HOPX* expression had a shorter overall survival (OS) compared with those with lower *HOPX* expression (P=0.003). In multivariate analyses, higher expression of *HOPX* still represented a poor prognostic factor for OS independent of age, WBC counts, karyotype, *FLT3*-ITD, *CEBPA* double mutations and *NPM1*, *MLL*-PTD, and *RUNX1* mutation (P=0.018). The results were recapitulated in the two independent validation AML patient cohorts. Gene set enrichment analysis (GSEA) showed enrichment of both hematopoietic stem cell (HSC) and leukemia stem cell (LSC) signatures in the AML cells with higher *HOPX* levels (P<0.0005 in HSC and P=0.004 in LSC), compatible with a role of HOPX in cell stemness. . Furthermore, the expression of six ATP-binding-cassette (ABC) transporter molecules that were related to poor prognosis and drug resistance, by pumping drugs out of AML cells, were significantly higher in the high *HOPX* group.
Summary/Conclusions: Higher expression of *HOPX* appeared to be an independent unfavorable prognostic factor in our and other AML cohorts. *HOPX* expression is highly correlated with stem cell signatures and ABC-related chemoresistance pathway. These results might at least in part explain the poor survival in AML patients with high *HOPX* expression levels.

P579

KIT MUTATIONS INCREASE THE RELAPSE RISK IN PATIENTS WITH AML WITH T(8;21) OR RUNX1-RUNX1T1 BUT NOT IN PATIENTS WITH INV(16) OR CBFB/MYH11: A SUBGROUP ANALYSIS OF CETLAM PROTOCOLS 2003 & 2012

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Background: Patients with acute myeloid leukemia (AML) and inv(16) or t(8;21) (CBF-AML) are considered of good prognosis. In consequence, general recommendation in these patients is not to perform an allogeneic hematopoietic cell transplantation (alloHCT) in first complete response (CR). However, their relapse incidence after standard frontline chemotherapy may reach 40%. Therefore the identification of potential prognostic markers is considered of great interest in order to improve their current outcome. Among these, *KIT* mutations, identified in approximately one third of patients, have been invoked as a potential relevant prognostic factor in CBF-AML, although their clinical relevance is currently uncertain.
Aims: Analyze the prognostic impact of KIT mutations in CBF-AML, in a series of patients treated following CETLAM protocols (AML-03 and AML-12).
Methods: This study analyzed all consecutive patients with primary CBF-AML enrolled into the AML-03 and AML-12 CETLAM trials for patients up to the age of 70 years. Induction chemotherapy included idarubicin, intermediate-dose cytarabine and etoposide. Consolidation treatment consisted of repeated courses of high-dose cytarabine followed in a small fraction of patients of autologous HCT. Exon 8 KIT mutations were analyzed by PCR amplification and direct sequencing, whereas exon 17 mutations were analyzed by PCR amplification and melting curve analysis in a LightCycler.
Results: The series included 143 CBF-AML patients (73♀, 70♂), 71 with *RUNX1/RUNX1T1* and 72 with *CBFB/MYH11* rearrangements. Median age was 42 years (range: 17-71), and median WBC count 14x10^9/L (range: 1.4-243x10^9/L;). Overall, CR rate was 91%, 5-year overall survival (OS) was 75±4%, and leukemia-free survival (LFS) was 69±5%. No differences in OS or LFS were found between the two CBF-AML groups. *KIT* mutation studies were available in 101 patients with the following results: 1) *RUNX1/RUNX1T1*: 42 patients harboring (76%) wild-type KIT configuration (wt), and 13 (24%) with mutated KIT (mut); 2)*CBFB/MYH11*: 32 (70%) wt, and 14 (30%) mut. Although no differences in OS and LFS were observed between mutant and wt patients when both CBF-AML groups were analyzed together (Figure 1). In contrast, *KIT* mut had impact on outcome in the *RUNX1/RUNX1T1* subgroup: LFS was significantly better in *KIT*wt patients (5 years LFS: 79±7%, *vs* 53±16%; p=0.043) and cumulative incidence of relapse (CIR) was higher in the *KIT*mut group (5 years CIR: 12±4%, *vs* 47±15%; p=0.006). There was a trend for a worse OS in patients with *RUNX1/RUNX1T1* and *KIT*mut (5-year OS in patients with *KIT*wt was 81±6%, *vs* 50±16% in *KIT*mut patients; p=0.068); No differences in CR rate were found depending on presence or absence of *KIT* mutations in any CBF-AML subgroup. Multivariate analysis including age, WBC count, bone marrow blasts and mutational status of *KIT*, confirmed the effect of *KIT* mutations in *RUNX1/RUNX1T1* AML in terms of LFS and CIR (LFS HR=3,5; 95% CI: 1,029-11,961; CIR HR=6,2; 95% CI: 1.534-24.93), but not in OS.

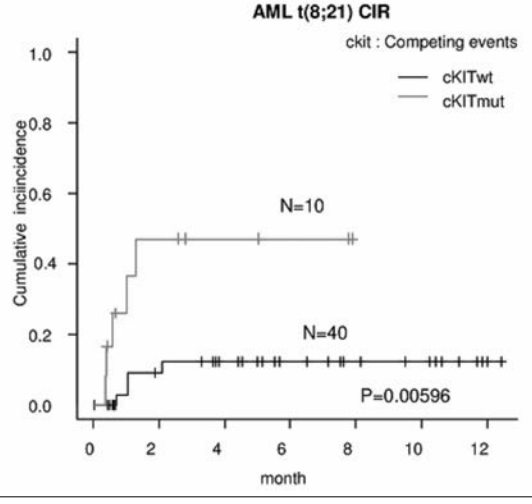

Figure 1.

Summary/Conclusions: In this study, *KIT* mutations exerted an unfavorable prognostic effect in patients within the *RUNX1-RUNXT1* AML subset, although the higher relapse risk observed in this AML subgroup did not translate into a shorter overall survival. The potential benefit of targeted therapies such as *KIT* inhibitors on relapse risk reduction deserves to be investigated in this setting. More data are needed to modify the still valid recommendation of postponing alloHCT for CBF-AML to more advanced phase of the disease.

LB580

RELATIVE BENEFIT FOR INTENSIVE *VERSUS* NON-INTENSIVE INDUCTION THERAPY FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML) USING A COMPOSITE, AGE-COMORBIDITY-CYTOGENETIC, MODEL

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Background: Induction therapy for newly diagnosed AML can be classified as "more" or "less" intensive. Currently less intensive therapies such as azacitidine or decitabine (HMA) appear to be increasing in use among patients >65 years, reflecting concerns about the ability of older patients, who often have various comorbidities, to tolerate intensive therapy. However, the issue of relative efficacy of intense *versus* non- intense therapies arises together with the question about the relative benefit/risk ratios of these options in various populations defined by age, comorbidities, and disease-related characteristics such as cytogenetic and molecular features.
Aims: Our group has designed and validated a new model combining the prognostic effects of age, comorbidity index, and cytogenetic/molecular risks (Blood 2015 126:532). Here, we used this composite model to define distinct prognostic groups and within each, compared two year mortality rates among patients with newly-diagnosed AML according to whether they received intensive or non-intensive therapy at 5 collaborating institutions. Non-intensive therapy principally included HMA or low-dose cytarabine, while intensive therapies primarily consisted of "7+3" or other similar cytotoxic induction regimens.
Methods: We retrospectively collected information regarding comorbidities, laboratory, and survival data from 1079 patients with newly diagnosed AML who received therapy at 5 institutions from 2008–2012. Median follow-up of patients still alive was 30 months.
Results: Patients were ≤49 (29%), 50-59 (25%), 60-69 (26%), and ≥70 (20%) years old. Cytogenetic-molecular risks were favorable (21%), intermediate I and II (36%), or unfavorable (43%) per European Leukemia Net classification. Induction treatments were non-intensive in 18% and intensive in 81% of patients. Using the composite risk model, 17% of patients had scores of 2-4, 37% scores 5-7, 27% scores 8-10, and 19% scores ≥11. As shown before (Blood 2015 126:532), hazard ratios (HR) for mortality increased with increasing scores. Patients with the lowest scores (2-4) almost always received intensive therapies (98%), and were therefore omitted from the comparison analysis. Table 1 shows distribution of regimen intensity per patient age groups: the proportion of patients receiving non-intensive therapy increased with increasing age. Patients with scores 5-7, 8-10, and ≥11 had statistically significantly higher survival rates when given intensive *versus* non-intensive therapies (Table 2). We also looked at outcomes specifically among patients 70-79 years old given intensive (46%) or non-intensive (54%) therapies. Intensive therapies among those older patients resulted in statistically significantly higher survival rates at 2-year (27% *versus* 11%, HR: 0.72, *P*=0.05, Figure 1).

Table 1. Regimen intensity per patient age groups.

Age, years	Total n	Intensive, %	Non-intensive, %
≤49	172	99	1
50-59	215	95	5
60-69	194	80	20
70-74	73	59	41
75-79	53	40	60
80-88	19	5	95

Table 2. Comparisons of hazard ratios (HR) and 2-year rates of survival between intensive and non-intensive first induction therapies.

Pt group	Variable	% of pts		Survival rates		HR	*P*
		Intensive, %	Non-intensive, %	Intensive, %	Non-intensive, %		
Composite scores	5-7	90	10	55	30	0.51	0.005
	8-10	83	17	36	19	0.63	0.02
	≥11	56	34	23	6	0.61	0.008

Summary/Conclusions: Intensive therapy leads to better long term survival even in older patients with significant co-morbidities. Early mortality is not increased in older patients given intensive rather than non-intensive induction therapy, likely due to improvements in supportive care over time. While we cannot exclude the effects of selection bias, our model accounted for the principal covariates associated with outcome. Absent a randomized trial comparing intensive and non- intensive therapies, our results suggest that the former should be offered to all patients up to the age of 80 regardless of their comorbidity burden. Prospective randomized studies are needed to investigate the role of non-intensive therapies in older patients in light of their comorbidity burden. Studies

of physical, cognitive, and social health might further identify groups of patients for whom non-intensive therapies could yield survival benefit.

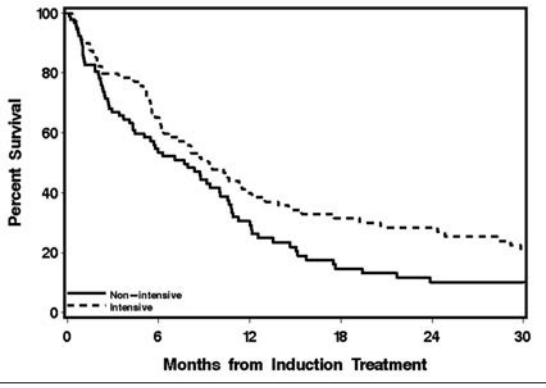

Figure 1.

LB581

NOVEL LEUKEMIA STEM CELL-TARGETED THERAPY FOR ACUTE MYELOID LEUKEMIA BASED ON DUAL INHIBITION OF EZH1/EZH2

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Background: Despite great progress in curing acute leukemia and malignant lymphoma, survival after relapse remains poor. The essential cause of the relapse following conventional chemotherapy is a remaining population of drug-resistant cancer stem cells (CSCs).

Aims: Thus, selective targeting of LSCs is a promising strategy for preventing relapse. The polycomb repressive complex 1 (PRC1) and 2 (PRC2) regulate ubiquitination of histone H2A at lysine 112 (H2AK112) and trimethylation of histone H3 at lysine 27 (H3K27), respectively. We report here that RING1A/B and EZH1/2, which are catalytic subunits of PRC1 and PRC2, are essential for maintenance of CSCs.

Results: We report here that RING1A/B and EZH1/2, which are catalytic subunits of PRC1 and PRC2, are essential for maintenance of CSCs. Using Ezh1-null, Ezh2-conditional and their double knock-out mice, we examined the effects of genetic deletion of EZH1/2 on AML. and found that double deletion of Ezh1/2 induced cell differentiation and apoptosis more severely than single deletions *in vitro* in all subtypes of AML. In AML mice models, deletion of Ezh1/2 induced differentiation of AML cells and complete remission of AML, which was not achieved by single deletion of Ezh1 or Ezh2. Interestingly, the number of CSCs, especially quiescent CSCs, dramatically reduced after deletion of both Ezh1 and Ezh2. However, such strong effects were not observed after deletion of either Ezh1 or Ezh2. We have developed potent and specific inhibitors against both EZH1 and EZH2, and found that the EZH1/2 dual inhibitor induced cell differentiation and apoptosis in most subtypes of AML tested *in vitro*, but a selective EZH2 inhibitor did not affect the growth and survival of AML cells. Oral administration of the EZH1/2 dual inhibitor selectively reduced the number of CSCs and prolonged the survival of the AML mice. Moreover, combination of the EZH1/2 dual inhibitor and Ara-C prolonged survival more dramatically. The dual inhibitor was also effective on treatment of malignant lymphomas in xenograft models.

Summary/Conclusions: Taken together, these results strongly suggest that dual inhibition of EZH1 and EZH2 is a promising therapeutic strategy to eradicate CSCs in a wide range of AMLs. Based on these results we have initiated Phase I clinical trial of the dual inhibitor in malignant lymphoma and we are preparing for AML.

LB582

IMGN632: A NOVEL ANTIBODY-DRUG CONJUGATE (ADC) OF A CD123-TARGETING ANTIBODY WITH A POTENT DNA-ALKYLATOR IS HIGHLY ACTIVE IN PRECLINICAL MODELS OF AML WITH POOR PROGNOSIS

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Background: Targeted delivery of cytotoxic molecules by ADCs recognizing cancer-specific antigens is a promising therapeutic approach. CD123, the IL-3 receptor alpha-subunit, is an attractive cancer target implicated in AML cell survival and proliferation. CD123 is universally expressed on AML blasts, is differentially expressed on AML stem cells relative to normal hematopoietic cells, and is associated with aggressive disease. Here, we report the preclinical evaluation of IMGN632, a novel conjugate of a humanized anti-CD123 antibody with a payload that alkylates DNA without cross-linking.

Aims: Evaluate the activity of IMGN632 in preclinical AML models

Methods: Anti-CD123 antibodies were generated in mice by immunization with a cell line expressing human CD123. The number of CD123 antigens per cell, MDR activity, cell cycle profile and markers of cell death were assessed by flow cytometry on AML cell lines and primary cells from AML patients. Cytotoxicity of the ADC was evaluated on AML cell lines after continuous exposure up to 7 days, and on primary AML patient samples by colony formation after 1-day exposure. Antitumor activity *in vivo* was assessed in immuno-deficient mice bearing human AML xenografts.

Results: We generated a high-affinity anti-CD123 antibody that blocks IL-3-dependent proliferation of AML cells more effectively than previously reported antibodies, and binds to a distinct epitope on the CD123 extracellular domain. The antibody was humanized and engineered so that two cysteine residues were incorporated into its constant region. The payload, an indolino-benzodiazepine dimer containing a mono-imine, was attached to the cysteines via a peptide linker, yielding the conjugate, IMGN632, with two payload molecules per antibody. Following internalization of IMGN632 into CD123 expressing cells the highly cytotoxic payload is released intracellularly. The payload alkylates DNA leading to cell cycle arrest and apoptosis-mediated cell death. IMGN632 was effective at killing twelve human AML cell lines expressing CD123 levels comparable to those of AML patients and carrying one or more known poor prognosis markers for AML (FLT3-ITD, EVI1 overexpression, P53 mutation or high MDR), generating IC_{50} values between 0.5 and 120 pM (median 3 pM). IMGN632 was inactive in CD123-negative cell lines at these concentrations. Anti-leukemic activity of IMGN632 and gemtuzumab ozogamicin (GO), the latter once approved for the treatment of relapsed/refractory AML, were compared *in vitro* in primary AML patient samples (n=15), including four from relapsed/refractory AML patients and seven with strong MDR activity. GO demonstrated variable activity killing 90 percent of AML cells at concentrations between 30 and 30,000 pM (median 1,000 pM). In contrast, IMGN632 was highly active in all samples killing at 100-fold lower concentrations, IC_{90} from 3 to 46 pM (median 14 pM). A non-targeted control ADC was inactive at 1,000 pM. *In vivo*, a single 70 mcg/kg IMGN632 dose resulted in durable complete responses in 5/6 mice bearing MV4-11 subcutaneous xenografts (FLT3-ITD AML model). Likewise, a single 240 mcg/kg IMGN632 dose reduced tumor burden in 6/6 mice bearing Kasumi-3 disseminated xenografts (AML line with P53 mutation and MDR overexpression). Corresponding payload-matched doses of a non-targeted ADC were inactive. IMGN632 was well tolerated at these doses.

Summary/Conclusions: IMGN632 exhibits potent *in vitro* and *in vivo* activity against AML cell lines and patient samples, including those with poor prognostic markers. These findings support advancing IMGN632 into clinical trials.

Genomic complexity in CLL

P580

LONGITUDINAL EVALUATION OF T-CELLS IN CLINICAL MONOCLONAL B-CELL LYMPHOCYTOSIS (MBL)

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Background: Patients with chronic lymphocytic leukemia (CLL) have significant abnormalities in the circulating T-cell compartment – including an increase in the circulating CD8+ T-cells (particularly of the exhausted phenotype), dysregulated cytokine production, and defective immunological synapse formation with antigen-presenting cells. It is not known if these defects are also present in individuals with clinical MBL, a precursor condition to CLL.
Aims: We sought to perform a comprehensive and longitudinal analysis of circulating T-cells in individuals with clinical MBL.
Methods: Using peripheral blood mononuclear cells of individuals with MBL stored in the Mayo Clinic Tissue Bank, we performed 8-color flow cytometry to comprehensively evaluate T-cell subsets (CD4+ and CD8+ naïve, effector, effector memory and central memory cells). We performed these analyses at the time of MBL diagnosis and at a second time point (median of 48 months later) in 18 individuals with MBL. We also evaluated the immunological T-cell synapse at these two time points. We compared these results to age-matched healthy adults (n=5) using the Kruskal-Wallis test. The Mayo Clinic IRB approved this study.
Results: *Analyses at MBL diagnosis:* No difference in the absolute CD4+ count or major CD4+ subsets were observed between controls and individuals with MBL at ascertainment. In contrast, the absolute CD8+ count was significantly higher in individuals with MBL relative to controls (median $0.5x10^9$/L *vs* $0.2x10^9$/L; p=0.03), mostly due to higher CD8+ effector memory cells. No difference in naïve, effector and central memory CD8+ T-cells were observed at ascertainment. *Sequential Studies :* After a median follow-up of 48 months, 9 individuals experienced an expansion of the B-cell clone (Group 1, median absolute lymphocyte count [ALC] increased from $7.5x10^9$/L to $39.0x10^9$/L at time point 2). In the remaining 9 individuals there was no expansion of the B-cell clone (Group 2, median ALC was $7.3x10^9$/L at time-point 1 and $8.7x10^9$/L at time-point 2). Among individuals in Group 1 who had expansion of the B-cell clone, a statistically significant increase in absolute CD4+ central memory, CD4+ effector memory, all CD8+ T-cell subsets (naïve, effector, effector memory and central memory), and exhausted T-cells (CD8+PD1+) was observed at time-point 2. In contrast, among the 9 MBL individuals in Group 2 who had no expansion of the B-cell clone, there were no significant differences in the distribution of CD4+ and CD8+ T-cell subsets at time point 2 compared to MBL diagnosis. We next analyzed differences in T-cell subsets at the time of MBL diagnosis between individuals in Group 1 and 2. The absolute CD8+PD1+ count was significantly higher at MBL ascertainment among individuals in Group 1 compared to those in Group 2 ($0.08x10^9$/L *vs* $0.02x10^9$/L, p=0.01); suggesting that individuals with a higher proportion of exhausted T-cells at diagnosis are more likely to experience expansion of the B-cell clone. *Immunological T-cell Synapse Studies:* As shown in Figure 1a, immunological T-cell synapse function was significantly worse in individuals with MBL compared to age-matched healthy controls. The synapse function also got progressively worse as the B-cell clone expanded among individuals in Group 1 (Figure 1b). In contrast, there was no worsening of synapse function in individuals in Group 2 (Figure 1c), suggesting that the decline in the function of T-cells was directly proportional to an increase in the size of B-cell clone.

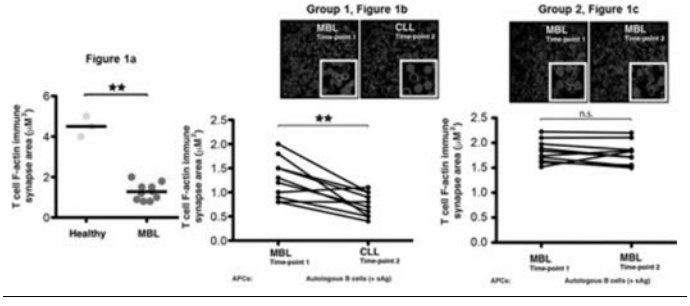

Figure 1.

Summary/Conclusions: Consistent with CLL, there is an expansion of CD8+ T-cells in MBL individuals at the time of ascertainment. MBL individuals who have a higher proportion of exhausted T-cells at diagnosis are more likely to develop an expansion of the B-cell clone over time. Finally, for the first time we demonstrate that T-cells from MBL exhibit impaired immune synapse formation and this functional defect worsens as the size of the B cell clone expands.

P581

COPY NUMBER ANALYSIS OF THE BIRC3 GENE BY DROPLET DIGITAL PCR IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH 11Q DELETION

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Background: Chronic lymphocytic leukemia (CLL) with 11q deletion (11q-) has been associated to a poor prognosis, but the clinical course of patients carrying this lesion is variable. The minimal deleted region (MDR) includes *ATM* and can also include *BIRC3*, a gene often deleted or mutated in advanced/chemorefractory CLL. The prognostic implications of *BIRC3* disruption in addition to *ATM* deletion are not well defined. We have previously conducted a study on *BIRC3* gene in 55 untreated 11q- CLL patients using Cytoscan HD array (Affymetrix) for copy number aberration (CNA) analysis. *BIRC3*, rarely mutated, was deleted in 82% of cases and the biallelic lesion (D+M) was associated with a marked hyperleukocytosis at diagnosis and immediate need of treatment.
Aims: In order to expand these preliminary observations, the study was extended to an additional cohort of 43 11q- CLL patients using the innovative droplet digital PCR (ddPCR) technique for *BIRC3* CNA.
Methods: Genomic DNA was extracted from peripheral blood samples of 43 untreated 11q- CLL. *BIRC3* CNA was performed utilizing the QX200™ Droplet Digital™ PCR System (Bio-Rad) that represents a useful tool to screen known lesions with high accuracy and to provide an absolute quantification of a given target locus relatively to a reference locus. Data were analyzed using the QuantaSoft software and CN value was computed by the ratio between the FAM-target (*BIRC3*) and the HEX-reference (*KBTBD*, centromeric probe on chr 11) molecule concentrations, times to the number of copies of reference species in the genome (*i.e.* [*BIRC3*]/[*KTBD*]*2). By a serial dilution experiment of a CLL case with 95% 11q- by FISH and by preliminary tests on CLL with different types and amounts of FISH lesions, ddPCR proved capable of identifying a deletion when present in at least 10% of CLL cells and to confirm FISH results in 100% of cases. *BIRC3* mutations (exons 6-9) were evaluated by Sanger sequencing. Time-to-first treatment (TFT) was calculated from the date of diagnosis to the date of first therapy or last follow-up.
Results: Given the comparable biological and clinical features of both cohorts, as well as the superimposable TFT (median TFT: 12.3 *vs* 12 months; p=0.36), the 98 11q- CLL patients were pooled together. Their baseline characteristics are reported in Table 1. All patients showed 11q- by FISH (median 70.5%, range 10-99% of nuclei). *BIRC3* was included in the deleted region in 74/98 cases (75.5%) and was mutated in 7/97 (7.2%), being always deleted on the other allele (D+M). After a median follow-up of 70.5 months (range 7.4-235.9), 77/91 evaluable patients have undergone treatment (median TFT 15.6 months, range 0.2-235.9). *BIRC3* deleted cases showed a TFT not significantly different from that of WT cases. Conversely, *BIRC3* D+M lesions was associated with a shorter TFT compared to that of *BIRC3* deleted/WT cases (median TFT 2.3 *vs* 16.3 months, p<0.0001). Notably, *BIRC3* D+M cases had a marked hyperleukocytosis at diagnosis significantly different from *BIRC3* deleted/WT cases (p<0.0001). Finally, 6/7 cases with *BIRC3* D+M required treatment soon after diagnosis.

Table 1. Clinical and biological characteristics of 98 11q- CLL patients.

Gender (M/F)	73/25
Age (range)	61.5 (38-83)
WBCx10^9/L (range)	41.7 (7-302.8)
Stage A/B/C/NA	43/36/9/10
IGHV UM/M	67/31
FISH: 11q- only	42
11q- with 17p-	1
11q- with +12	6
11q- with 13q-	49
BIRC3 mutation*	7
NOTCH1 mutation*	9
SF3B1 mutation*	8
TP53 mutation*	2

*All mutually exclusive with only 1 case harboring both *SF3B1* and *BIRC3* mutations.

Summary/Conclusions: Among untreated CLL with 11q-: 1) *BIRC3* deletion represents a common event (75%), whilst the mutation is rare (7%); 2) *BIRC3* deletion does not seem to influence TFT of 11q- CLL; 3) *BIRC3* D+M is strongly associated with a short TFT; 4) *BIRC3* D+M is mostly associated to an immediate need of treatment and to a marked hyperleukocytosis at diagnosis. ddPCR

represents a handy tool to screen a known CNA with a good sensitivity and accuracy, resulting in a cost-effective approach.

P582

CLONAL EVOLUTION IN CLL IS ASSOCIATED WITH AN UNMUTATED IGHV STATUS, MUTATED TP53 AND SHORTER SURVIVAL

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Background: CLL is a clinically heterogeneous with a course ranging from stable disease for many years to a rather rapid progression requiring treatment. During recent years several genetic parameters have been identified which are associated with progression of the disease. Clonal evolution (CE) on the genetic level is likely to correlate with clinical progression and might be used as a predictor and thus guide treatment strategies.
Aims: The aim of this study was to evaluate the frequency of clonal evolution on the cytogenetic level and its association with the *IGHV* mutation status, *TP53* mutations and clinical outcome.
Methods: 789 CLL cases were selected on the basis that chromosome banding analysis (CBA) had been performed at least at two time points. The median age at first evaluation was 72 years (range: 29-95 years). The first time point of analysis was at primary diagnosis or prior to any treatment in all patients. A total of 1902 CBA were evaluated. The median number of samples per patient was 2 (range: 2-9). The time between samples ranged from 1 months to 118 months (median 17 months). In all patients interphase FISH was performed with probes for 17p13 (*TP53*), 13q14 (D13S25, D13S319, *DLEU*), 11q22 (*ATM*), the centromeric region of chromosome 12 and t(11;14)(q13;q32) (*IGH-CCND1*). In 464 patients both *TP53* mutations and *IGHV* mutation status were evaluated. For 254 of these patients also clinical follow-up data was available. Median follow-up was 64 months and 5-year overall survival (OS) was 88.8%.
Results: At first investigation CBA revealed a normal karyotype in 134 (17%) patients. In cases with an aberrant karyotype the pattern of abnormalities was typical for CLL. CE was observed in 243/789 patients (31%). The median time to CE was 30 months (range 1.5-112 months). The most frequent abnormalities gained during CE observed in >10% of cases were gains of 2p (n=48), 8q (n=53), 13q (n=35), 17q (n=27) and losses of 6q (n=33), 8p (n=52), 11q (n=40), 13q (n=70), and 17p (n=95). In the total cohort an unmutated *IGHV* status was present in 61% of cases and was significantly more frequent in patients with CE (76.5% *vs* 59.4%, p<0.001). *TP53* mutations were detected in 15% of cases in the total cohort and were more frequent in patients in whom CE was observed (25.5% *vs* 10.2%, p<0.001). In the cohort of patients with clinical follow-up data, 70 of 87 (80.5%) patients with CE received therapy during the course of the disease, whereas in patients without CE this was the case in 112/167 (67.1%) (p=0.028). Patients in whom CE was observed had a tendency for shorter OS as compared to those without (5-year OS 84.8% *vs* 90.9%, p=0.07). While no impact of CE was observed in patients with a mutated *IGHV* status, in patients with an unmutated *IGHV* status 5-year OS was 82.8% in cases with CE compared to 92.1% to those without (p=0.05) (Figure 1).

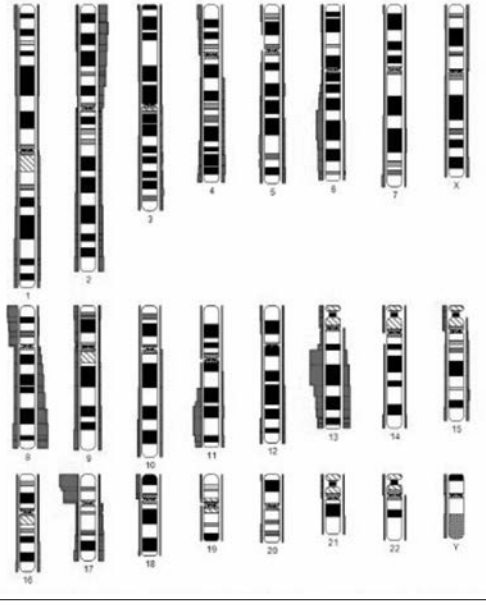

Figure 1. Pattern of chromosome abnormalities acquired during clonal evolution: gained regions are depicted in green to the right and lost regions in red to the left.

Summary/Conclusions: We observed clonal evolution in 31% of CLL patients who were analyzed during the course of their disease. Loss of 17p, 8p and 13q as well as gain of 8q and 2p were the most frequently gained chromosome abnormalities. Clonal evolution was associated with *TP53* mutations and an unmutated *IGHV* status and with inferior OS in particular in patients with an unmutated *IGHV* status. The frequency and impact of clonal evolution needs to be further evaluated in unselected patient cohorts in which chromosome banding analysis is performed on a regular basis.

P583

Abstract withdrawn.

P584

EPIGENETIC REGULATION OF ROR1 IN CHRONIC LYMPHOCYTIC LEUKEMIA DEFINES PREFERABLE TARGET FOR COMBINED EPIGENETIC AND IMMUNE THERAPY

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Background: Definition of leukemia-associated antigens (LAA) expression represents a pivotal step towards inventing new and effective immunotherapy strategies for chronic lymphocytic leukemia (CLL). Expression of LAA could be epigenetically regulated, influencing thereby immune responses to defined LAA. In other tumors, 5-azacytydine effectively increased expression of cancer/testis antigens. Increased expression of LAA under 5'azacytidine exposure might augment immunogenicity of these antigens inducing more effectively anti-LAA cytotoxic immune response. ROR1 might be a specific neo-antigen and may serve as a target for immunotherapy. Moreover spontaneous anti-ROR1 humoral as well as a T cell responses were observed in CLL patients.
Aims: To define effect of 5'azacytidine therapy on expression of LAA and generation of ROR1-specific T-cell responses in CLL patients towards identification of preferable target for future combined epigenetic and immunotherapy approaches in CLL.
Methods: Expressions of *ROR1* and other LAA encoded by *ACTB, FMOD, PLIN2, NFXD1, BAGE, CTAG1B, GAGE1 PRM1, WT1, MAGEA1, MAGEA3* and *RPSAR* in CLL were assessed in 111 CLL patients and 29 H*Vs* using qRT-PCR. Next, the influence of 5μM 5'azacytidine in 24-hours cell cultures of mononuclear cells on the LAA expression was analyzed. Expression of genes encoding LAA were normalized using constitutive gene *GAPDH.* For functional experiments we chose ROR1 since it might represent preferable target for immunotherapy. Mixed lymphocyte peptide cell cultures followed by ELISpot assays for specific interferon gamma release were performed to determine influence of 5'azacytidine on generation of the cytotoxic ROR1-specific T-cell responses in CLL patients and H*Vs* with the expression HLA-A*0201 (as assessed by flow cytometry).
Results: We found expression of the following genes with the median 1/ΔCt of: *ROR1*=0.105, *FMOD*=0.203; *PLIN2*:=0.151; *NFXD1*=0.070; *BAGE*=0.067, *ACTB*=-0.359, *RPSAR*=0.102 and no expression of the *CTAG1B* and *GAGE1* in all CLL cases. We observed low expression of *PRM1* and *WT1* in minority of CLL patients with the median 1/ΔCt of: 0.007 and 0.056 respectively. Higher *RPSAR, FMOD, BAGE* expressions in CLL than HV (median 0.102 *vs* – 0.505, p<0.001; median 0.203 *vs* 0.060, p<0.001; median 0.067 *vs* 0.0001, p<0.001 respectively), and lower expression of *PLIN2* in CLL that HV group (median 0.151 *vs* 0.439) were found. Increased expression of *ROR1, RPSAR, FMOD* after exposure to 5'azacytidine was observed. In functional experiments we found 5'azacytidine led to effective induction of LAA-specific T-cell cytotoxic responses against peptides encoded by *ROR1.*
Summary/Conclusions: This study provides the evidence that LAA are differentially expressed and regulated in CLL. Although *RPSAR*, *FMOD*, *BAGE* showed highest expression in CLL as compared to HV, increased expression of *ROR1, RPSAR, FMOD* was observed after exposure to 5'azacytidine. Moreover, 5'azacytidine effectively augmented anti-*ROR1* cytotoxic T-cell responses. Therefore we could conclude that preferential immunotherapy could target ROR1 and its effectiveness could be augmented by the addition of 5'azacytidine to treatment.
This work was supported by Medical University of Lublin Scientific Grant DS 462.

P585

COMPLEX KARYOTYPE AS IDENTIFIED BY CHROMOSOME BANDING ANALYSIS IS ASSOCIATED WITH SHORTER OVERALL SURVIVAL IN CLL INDEPENDENT OF TP53 ALTERATION AND IGHV MUTATION STATUS

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Background: In standard CLL routine diagnostics interphase-FISH, *TP53* mutation analysis and the evaluation of the *IGHV* mutation status are performed.

Since cultivation of CLL cells was improved by stimulation of CLL cells *in vitro* with DSP30 and IL2 chromosome banding analysis (CBA) is more often performed in CLL. Rigolin *et al.* (Blood 2012) demonstrated that CBA adds prognostic information in CLL patients with "normal" karyotype as determined by interphase-FISH. Recently, Thompson *et al.* (Cancer 2015) reported that a complex karyotype is a stronger predictor than 17p deletion for an inferior outcome in relapsed or refractory CLL patients treated with ibrutinib-based regimens.

Aims: The aim of this study was to evaluate the impact of complex karyotype on overall survival (OS) in CLL patients.

Methods: The basis of this study were 1046 CLL cases evaluated at diagnosis or prior to first therapy. Median age was 67 years (range: 30-89 years). Bone marrow or blood samples were analyzed by CBA and FISH with probes for 17p13 (*TP53*), 13q14 (D13S25, D13S319, *DLEU*), 11q22 (*ATM*), the centromeric region of chromosome 12 and t(11;14)(q13;q32) (*IGH-CCND1*). Further, the *TP53* and *IGHV* mutation status was determined in all patients. Overall survival was evaluated from time of analysis to death or last follow up. For all patients follow up data was available with a median follow up of 68 months.

Results: In order to determine the impact of complex karyotype patients were categorized according to the number of chromosome abnormalities (CA) detected per case. None, 1, 2, 3, 4 and ≥5 abnormalities were observed in 228 (21.8%), 449 (42.9%), 208 (19.9%), 72 (6.9%), 36 (3.4%), and 53 (5.1%) cases, respectively. In 46 (4.4%) cases a *TP53*/17p deletion was identified by FISH and in 76 (7.3%) *TP53* mutations by sequencing. 39 patients carried both a *TP53* deletion and a *TP53* mutation, while in 963 (92.1%) no *TP53* alterations were detected. A significant association was observed between the number of CA and *TP53* alterations: 0-2 CA: 5.5%, 3 CA: 11.1%, 4 CA: 13.9%, and ≥5 CA 39.6% (p<0.001). The number of CA was also associated with an unmutated *IGHV* status (0-2 CA: 35.9%, 3 CA: 52.8%, 4 CA: 50%, and ≥5 CA 66% (p<0.001)). In the total cohort OS at 5 years differed significantly according to the number of CA (0-2 CA: 84.8%, 3 CA: 83.7%, 4 CA: 77.7%, ≥5 CA: 52.6%, p<0.001). Three different definitions for complex karyotypes were evaluated: CK3: ≥3 CA, CK4: ≥4 CA, and CK5: ≥5 CA. We tested the impact of complex karyotype on OS separately in patients with and without *TP53* alterations. In patients without a *TP53* alteration OS at 5 years was significantly lower in cases harboring a complex karyotype than in those without a complex karyotype (CK3: 80.3% *vs* 86.4%, p=0.03; CK4: 73.6% *vs* 86.4%, p=0.004; CK5: 66.4% *vs* 86.3%, p=0.001). Also in patients with *TP53* alterations a complex karyotype was associated with a negative impact on OS (OS at 5 years: CK3: 41.0% *vs* 59.6%, p=0.01; CK4: 35.7% *vs* 59.4%, p=0.01; CK5: 31.3% *vs* 59.1%, p=0.002). In multivariate Cox regression analysis an independent negative impact on OS was identified for *TP53* deletion (relative risk (RR): 3.1, p=0.001), *TP53* mutation (RR: 1.7, p=0.05), number of chromosome abnormalities (RR: 1.1 per CA, p=0.006) and unmutated *IGHV* status (RR: 2.0, p<0.001).

Summary/Conclusions: Although karyotype complexity is associated with *TP53* alterations and an unmutated *IGHV* status, the number of chromosome abnormalities as determined by chromosome banding analysis is an independent negative prognostic parameter in CLL.

P586

IMPROVING THE DIFFERENTIAL DIAGNOSIS OF CD5+ B-LYMPHOPROLIFERATIVE DISORDERS

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Background: The multidisciplinary diagnosis of CD5+ B-lymphoproliferative disorders (B-LPD) can be challenging because there is significant overlap in the clinical, cellular/immunophenotypic and molecular features. There is no pathognomonic molecular abnormality in CLL: deletion of 13q14 is present in approximately half of CLL cases but is also present in more than 10% of MCL as well as other B-LPD. The presence of a CCND1/IGH in a monoclonal CD5+ B-LPD indicates a diagnosis of MCL. The MYD88 L265P mutation is a gain-of-function driver mutation present in the majority of WM/LPL and <5% of CLL cases. An immunophenotypic scoring system is often used to facilitate differential diagnosis but markers such as CD200 & ROR1 may permit a more reproducible diagnosis, particularly when considering recently identified signalling pathway abnormalities.

Aims: To compare immunophenotypic scoring with a diagnostic panel comprising CD200 and ROR1 the differential diagnosis of CD5+ B-LPD

Methods: Analysis was performed on 3082 sequential cases (1041 diagnosis, 2041 follow-up) pre-treatment or relapse). Flow cytometry (5x8-CLR tubes with CD19 & CD20 as a backbone combined with 38 markers including CD5, CD23, κ, λ, CD79b, CD43, CD200 and ROR1) and morphological evaluation was performed in all cases with immunohistochemistry in 1058/3082; molecular analyses were determined by the clinical details and immunophenotype. The final diagnosis was determined by a multidisciplinary team review of all the available data with input from haematopathologists, clinicians, and radiologists. An immunophenotypic score of 0-5 was calculated separately assigning 1 point for expression of CD5 or CD23 and 1 point for weak or no expression of CD79b or sIg or CD20.

Results: A learning set comprising 1172 cases showed ROR1 was expressed in 97.1% of CLL (639/658), 71.7% of MCL (33/46), 23.1% of HCL (6/26), 21.9% of WM/LPL/MZL (75/342), 9.5% of DLBL (4/42), and 3.4% of FCL (2/58). The markers contributing most to the differential diagnosis of CLL *vs* MCL were CD20, CD23, and CD200 (specificity 91.3%, 82.6% and 78.3% respectively). CD23 was expressed in 17.3% (8/46) MCL cases; co-expression of CD200 and CD23 was evident in 8.7% (4/46) MCL cases. The markers contributing most to the differential diagnosis of CLL *vs* WM/LPL/MZL were CD20, ROR1, and CD43 (specificity 83.0%, 78.1% and 70.5% respectively). CD43 and ROR1 were frequently expressed in MCL and so did not help to differentiate CLL *vs* MCL. Similarly CD200 was frequently expressed in WM/LPL/MZL (69.9%, 239/342) and did not contribute to the differential diagnosis of CLL *vs* WM/LPL/MZL. Overall the protein expression profiles that showed specificity for differential diagnosis of CLL *vs* other disorders were CD5, CD23, CD43, ROR1 and CD200 expression with weak CD20, CD81 and surface immunoglobulin/CD79b expression (Figure 1A). This profile was compared with a scoring system in a validation set (n=1469): cases scoring 5 included a small proportion with a CCND1/IGH translocation; cases scoring 4 had a similar incidence of CCND1/IGH and MYD88 abnormalities as cases with a score of 1-3 (Figure 1B). Cases with a fully characteristic immunophenotype had no detectable CCND1/IGH translocations; 2/66 had a MYD88 mutation & both had a CD5-negative MBL in addition to the dominant CLL-phenotype population. Other CD5+CD23+ cases had a high incidence of CCND1 or MYD88 abnormalities.

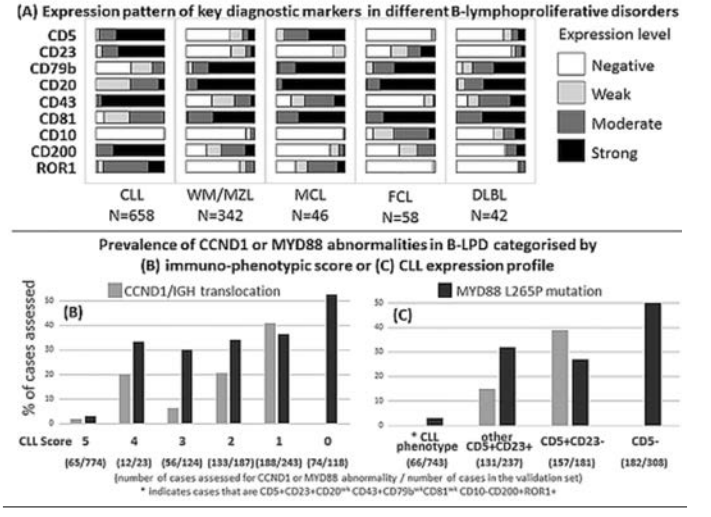

Figure 1.

Summary/Conclusions: A scoring system is suboptimal for diagnosis when additional pathway abnormalities such as MYD88 mutations are taken into account, particularly if cases with a score of 4 or 3 are included. The detection of a MYD88 mutation in a case with a characteristic CLL immunophenotype is frequently associated with the presence of an additional neoplastic non-CLL B-cell population. This study is limited by the fact that molecular testing was guided by immunophenotypic analysis and we are currently assessing sequential cases to determine the incidence CCND1/MYD88 pathway abnormalities in a larger series of cases with a homogeneous phenotype. However the results clearly demonstrate that lack of ROR1 expression in a CD5+CD23+ B-LPD is associated with a high incidence of MYD88 pathway abnormalities; this may represent a group of disorders with biological features intermediate between CLL and WM that should be characterised further.

P587

REFINED KARYOTYPE-BASED PROGNOSTIC STRATIFICATION OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH A VERY LOW RISK GENETIC PROFILE

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Background: Chronic lymphocytic leukemia (CLL) patients with isolated dele-

tion at chromosome 13q (13q-) and devoid of *NOTCH1*, *SF3B1*, *BIRC3, TP53* clonal mutations represent a very low risk subgroup. Cytogenetics with novel mitogens has allowed to reveal chromosomal aberrations in regions uncovered by standard fluorescence *in situ* hybridization (FISH) in CLL and to identify novel genetic subgroups with prognostic relevance beyond known gene mutations (Rigolin *et al.,* GCC 2015).

Aims: To identify in CLL patients with a very low risk genetic profile - *i.e.* 13q- or normal FISH and devoid of *NOTCH1*, *SF3B1*, *BIRC3, TP53* mutations - karyotype (KT)-defined categories using novel mitogens and subclonal *TP53* mutations with the aim of further refining the identification at diagnosis of patients who may never require treatment and with a possible normal life expectancy.

Methods: A cohort of 106 CLLs with a very low risk genetic profile, as defined above, was selected from a series of consecutive CLL patients characterized at diagnosis. FISH and cytogenetics with novel mitogens were performed as described (Bardi *et al.,* J Biomed Biotechnol 2011). The mutational screening of *NOTCH1*, *SF3B1*, *BIRC3* and *TP53* was performed by Sanger sequencing. Subclonal *TP53* mutations were identified by ultra-deep sequencing on Genome Sequencer Junior (Roche-454 Life Sciences) and a dedicated bioinformatic analysis, as described (Rossi *et al.,* Blood 2014).

Results: The characteristics of CLL with 13q- only (n=62; 58.5%) or normal FISH (n=44; 41.5%), with no clonal gene mutations, were the following: 69 males, 37 females; median age 64 years (range 27-85); *IGHV* genes: mutated 72/96 (75%), unmutated 24/96 (25%). KT with novel mitogens was evaluable in 104/106 cases (98%). Overall, a normal KT was present in 39 cases (37.5%), 13q- only in 34 (32.7%), 1 or 2 lesions (other than 13q-) in 25 (24%), ≥3 lesions in 6 (5.8%). Complex KT or additional chromosomic lesions were not associated with the *IGHV* gene mutation status (37.5% of unmutated *vs* 30% of mutated cases). In particular, CLL with 13q- only by FISH and no gene mutations showed additional karyotypic lesions in 25.8% of cases (1-2 additional lesions in 14, ≥3 lesions in 2); CLL with normal FISH and no gene mutations showed karyotypic lesions in 34% of 42 evaluable cases (1-2 lesions in 11, >3 lesions in 4). After a median follow-up of 42.5 months (range 2.0-102.0), according to KT, very low risk CLL patients with normal KT/13q- only were associated with a significantly longer overall survival (OS) (p=0.0004) than cases with additional lesions/complex KT (Figure 1A). The same stratification held true even among 13q- (p=0.005) and normal FISH CLL (p=0.03) subgroups (Figure 1B). Time-to-first treatment (TFT) was significantly longer for very-low risk CLL with normal KT/13q- than for cases with additional lesions/complex KT in the whole series (p<0.0001) and within normal FISH CLL (p<0.0001). *TP53* subclonal mutations were identified only in 3/97 cases (3%), with a minor allele frequency of 0.015-0.03. All showed normal KT/13q-; 2 cases showed mutated *IGHV*. All are alive and 2 are still untreated at the last follow-up.

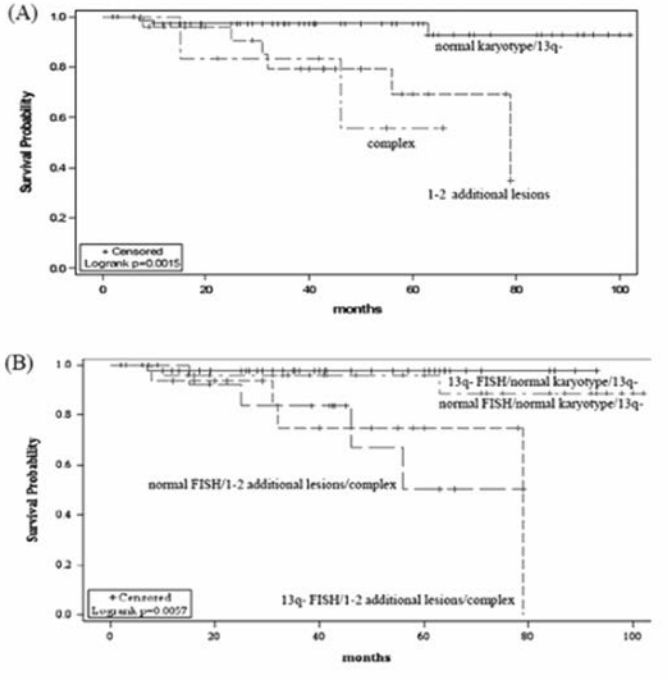

Figure 1.

Summary/Conclusions: In CLL patients belonging to a very low risk category by FISH and gene mutations, whilst *TP53* subclonal mutations are very rare and of uncertain significance, KT using novel mitogens further refines the prognostic stratification allowing to identify a subgroup of patients with excellent long-term prognosis and about 30% of cases with a worse outcome.

P588

PRO-APOPTOTIC EFFECTS OF PRIMA-1MET CORRELATE WITH NOXA GENE INDUCTION IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: *TP53* mutations represent the most adverse predictive and prognostic factor in chronic lymphocytic leukemia (CLL). We have previously shown that missense mutations located in central DNA-binding domain of p53 protein frequently lead to the accumulation of mutated p53 protein with presumably oncogenic properties. A small molecule involved in clinical trials, PRIMA-1^{MET}(Tocris Bioscience), has been declared to be able to change mutated p53 to its wild-type (wt) conformation with rescue of its activity.

Aims: To identify factors correlating with pro-apoptotic activity of PRIMA-1^{MET} in CLL cells.

Methods: Peripheral blood mononuclear cells from patients with CLL (proportion of leukemic cells >85%) were chosen from the *TP53* cohort characterized by the yeast functional analysis FASAY and direct sequencing. A metabolic WST-1 assay determined the viability of cells after PRIMA-1^{MET} treatment. The impact of 48h PRIMA-1^{MET} treatment on p53 protein level was analyzed by western blot (WB). The induction of pro-apoptotic p53 target genes was assessed by qRT-PCR.

Results: Initially, we assessed the effect of PRIMA-1^{MET} on viability of CLL cells. Both p53-mutated and p53-wt samples (n=12) responded by the clear concentration-dependent curve of viability within the concentration range 0.5-4μM; thus indicating that the observed PRIMA-1^{MET} effects on cell viability were p53-independent among the samples. The PARP1 protein cleavage observed in most of the tested samples (7 out of 12) confirmed apoptosis as the principal mechanism of cell death. Using 11 samples and treatment with 2μM and 4μM, we demonstrated that PRIMA-1^{MET} can readily eliminate (n=5) or partially reduce (n=4) high baseline level of mutated p53 protein. Next, we analyzed the expression of p53 target genes and detected a pronounced induction of *CDKN1A* (*p21*), *GADD45* and *PMAIP1* (*Noxa*) in all analyzed types of samples: (i) with strong accumulation of mutated-p53 protein, (ii) with null p53 protein resulting from a truncating *TP53* mutation, and (iii) in p53-wt samples. In addition, we observed a prominent inverse correlation between the final viability of CLL cells and *PMAIP1* induction after 4μM PRIMA-1^{MET} treatment (Spearman´s rho=-0.8462; p=0.0005). A similar effect was also noticeable for the viability of CLL cells and *CDKN1A* induction (Spearman´s rho=-0.6853; p=0.0139). In line with these findings, there was also a significant correlation between *PMAIP1* and *CDKN1A* inductions after 4μM PRIMA-1^{MET} treatment (Spearman´s rho=0.7413; p=0.0058).

Summary/Conclusions: In CLL cells, the PRIMA-1^{MET} is able to markedly diminish or even completely eliminate accumulated mutated p53 protein. However, all accompanying experiments suggest that the observed effects of PRIMA-1^{MET} towards apoptosis induction are in fact p53-independent in CLL cells. Currently, we addressed the question of whether other family members like the p73 protein can account for the observed effects.

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P589

PILOT STUDY TO ASSESS REAGENT AND INSTRUMENT QUALITY FOR REPRODUCIBLE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA: AN ESCCA AND ERIC HARMONISATION PROJECT

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Background: The WHO and iwCLL diagnostic criteria for CLL rely on morphology and immunophenotype based on the co-expression of CD19/CD5/CD23 on B-cells with weak CD20 and monoclonal immunoglobulin expression. The current criteria have some limitations affecting reproducibility, particularly flexibility in marker expression with some centres using a scoring system that permits absence of CD5 or CD23. Through survey of 154 ERIC and ESCCA members involved in the diagnosis CLL, a consensus marker panel was identified, comprising CD19, CD5, CD23, CD20, Kappa and Lambda as a minimum (*i.e.* required for a diagnostic panel) with CD43, CD79b, CD81, CD10, CD200, and ROR1 also recommended for investigation. In addition, definitions for the expression pattern and performance characteristics of the proposed markers were developed that could potentially improve reproducibility of diagnosis across different centres.

Aims: To determine the feasibility of testing whether the proposed diagnostic markers meet the required performance characteristics in individual laboratories.
Methods: A gating strategy to identify the expression levels of component markers on normal peripheral blood lymphocytes was developed and distributed to participating laboratories who tested it on ten historical cases with polyclonal B-cells. The median fluorescence intensity for the relevant markers on defined positive and negative control populations were recorded and returned for central analysis where the relative signal value was calculated.
Results: Data were returned from 9 participating laboratories. The marker combinations used were different in each centre but the results, shown in Table 1, demonstrate that most centres are using appropriate reagents with acceptable instrument settings. The performance characteristics for all 6 markers were met in all 10 cases in 5/9 centres. Suboptimal results were identified for 1 or 2 markers in 3/9 centres. Suboptimal results did not necessarily relate to the reagent used and the same reagent could have different performance characteristics in different laboratories because overall results were also influenced by laboratory procedures and instrument settings.
Summary/Conclusions: Flow-cytometry laboratory performance is influenced by many factors including the clone, fluorochrome, and manufacturer of each reagent, the combination and concentration of reagents used as well as the cytometer instrument settings. Optimising and standardising each component of the process can be labour-intensive. The relatively simple global approach developed by ERIC/ESCCA to assess the CLL diagnostic panel is applicable to a variety of reagent and instrument suppliers and can easily identify potential problems or confirm acceptable performance in individual laboratories. The quality assessment of the diagnostic panel is being extended to other markers including CD43, CD200 and ROR1 with prospective evaluation as part of an ERIC project to improve the reproducibility of differential diagnosis in CLL.

Table 1.

Antigen	CD19	CD20	CD5	Kappa	Lambda	CD23
Relative signal target value	>10	>10	>14	>5	>5	>5
Centre 1	225 (123-479)	127 (51.9-183)	56.3 (16.2-5892)	24.4 (12.6-87.6)	100 (44.8-302)	11 (7.4-17.9)
Centre 2	5462 (4291-6393)	64.8 (36.6-103)	41.1 (17.7-57.2)	* 17.1 (4.9-37.6)	* 2.9 (2.1-4.9)	* 4 (3.1-6.8)
Centre 3	12126 (85.1-14264)	* 5.4 (2.5-7.1)	* 44.2 (2.8-102)	20.2 (7.1-55.5)	35.8 (8.4-116)	* 43.2 (0.8-1670)
Centre 4	* 17.9 (5.6-23.5)	175 (102-306)	237 (52.8-368)	35.6 (12.6-60)	430 (148-612)	* 49 (2.5-223)
Centre 5	16.5 (11.2-18.8)	24.6 (16.7-30.2)	42.9 (15.1-56.7)	22.6 (10.3-65.1)	17.5 (10.3-24.2)	18.7 (8.6-31.7)
Centre 6	56.8 (32.8-81.9)	2812 (398-5030)	37.2 (24.4-105)	19.7 (11.4-65.6)	74.4 (13.6-317)	15.4 (9.7-39.3)
Centre 7	106 (89.9-175)	53.6 (41.2-67.4)	26.2 (17.9-39)	22.1 (6.9-45.1)	149 (72.2-287)	16.9 (8.6-35)
Centre 8	217 (130-234)	82 (58.8-145)	88.6 (51-123)	25.3 (10.7-80.1)	19.6 (7.4-74.8)	10.3 (5.8-14.1)
Centre 9	* 16.3 (5.5-130)	29.9 (18.3-58.7)	* 5.4 (2.4-45.6)	* 12.3 (4.7-29.7)	46.6 (6.5-75.5)	19.1 (9.8-48.4)

The median relative signal, defined as the median fluorescence intensity (MFI) of the positive control population divided by the MFI of the negative control population, for ten cases with polyclonal B-cells from each centre (range in brackets).
A * indicates that the relative signal was below target in 1 or more of the 10 cases evaluated. Grey cell shading indicates that the result was below target in 5 or more cases.

P590

THE SYK/JAK INHIBITOR CERDULATINIB SHOWS PROMISING *IN VITRO* ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: B cell receptor (BCR) kinase inhibitors such as ibrutinib and idelalisib have proved effective for the treatment of chronic lymphocytic leukaemia (CLL). However, these inhibitors appear only to suppress the disease without being curative. A number of patients have developed resistance to ibrutinib following mutation of the BTK or PLCγ2 gene, whilst other patients are unable to tolerate these drugs due to adverse events or progress whilst on therapy for unknown reasons. More recently, our group showed that microenvironmental signals such as IL-4 can partially reverse BCR-kinase inhibition by ibrutinib and idelalisib restoring αIgM induced ERK phosphorylation and calcium flux. Therefore, the development of novel drugs that are still effective once other BCR-kinases inhibitors become ineffective and/or which target signals from the tumour microenvironment is of utmost importance. Cerdulatinib inhibits both Syk (a protein pivotal to BCR signalling) and JAK3 (a protein integral for IL-4 signalling). Importantly, targeting Syk was also shown to induce apoptosis in CLL samples resistance to ibrutinib, identifying Syk inhibition as a promising strategy to treat these patients. Cerdulatinib is currently in a phase I open label dose escalation study and is currently demonstrating clinical activity in patients with relapsed/refractory B cell malignancies including CLL.
Aims: To investigate the dual Syk/Jak inhibitor, cerdulatinib, on CLL cell biology.
Methods: Twenty-four primary CLL samples were treated with cerdulatinib in the presence or absence of IL-4/CD40L or αIgM and apoptosis assessed using propidium iodide/Annexin V staining or PARP cleavage. The effect of cerdulatinib on BCR and IL-4-induced signalling were assessed by immunoblotting and flow cytometry.
Results: At plasma concentrations achievable in patients, cerdulatinib induced apoptosis of CLL cells cultured *in vitro* in a concentration, time and caspase dependent manner, with a mean IC50 of 3μM and 1μM at 48hr and 72hr respectively. Cerdulatinib preferentially induced apoptosis of samples with progressive disease (unmutated IGHV, CD49d+ or ZAP70+) compared to indolent disease (mutated IGHV, CD49d- or ZAP70-). Cerdulatinib significantly inhibited IL-4 induced phosphorylation of STAT6 and αIgM induced calcium flux and downstream signalling via phosphorylation of AKT, ERK and S6 Kinase at concentrations ≤300nM. Cerdulatinib induced similar levels of apoptosis irrespective of pro-survival signalling via IL-4/CD40L or αIgM treatment, suggesting that this drug may be able to overcome microenvironmental signals and thus target tumour cells within the lymph nodes. In addition, cerdulatinib inhibited αIgM and IL-4/CD40L induced expression of pro-survival proteins MCL1 and Bcl-XL. Next we explored the possibility of augmenting cerdulatinib induced apoptosis by simultaneous inhibition with the Bcl-2\Bcl-XL inhibitor ABT-199. *In vitro* in the presence of IL-4/CD40L, ABT-199 synergised with cerdulatinib to induce significantly greater cell death than with either agent alone. Finally we evaluated the effect of cerdulatinib on BCR-induced expression of CCL3 and CCL4 in CLL cells. Cerdulatinib significantly inhibited CCL3 and CCL4 expression at concentrations achievable in patients and to a greater extent than observed with ibrutinib alone.
Summary/Conclusions: Together these data provides the first *in vitro* support for the continued use of cerdulatinib in clinical trials for the treatment of CLL as either a single agent or in combination with other therapies such as ABT-199.

CLL - Efficacy and safety of new treatments 3

P591

SAFETY, EFFICACY AND IMMUNE EFFECTS OF VENETOCLAX 400 MG DAILY IN PATIENTS (PTS) WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Venetoclax (VEN) is an orally bioavailable BCL2 selective inhibitor. Patients (pts) with relapsed or refractory CLL or small lymphocytic lymphoma were treated in a phase 1 trial (M12-175, NCT01328626) with doses ranging from 150-1200 mg/day (Roberts, *et al.*, NEJM 2016).
Aims: Here we report the updated safety and efficacy data in the subset of CLL pts treated at the recommended phase 2 dose (RP2D) of VEN (400 mg daily), with a focus on effects on the host immune system.
Methods: 59 CLL pts were enrolled to receive VEN at the RP2D of 400mg/day. This analysis is restricted to the 57 pts with complete baseline and re-staging evaluations. Immunophenotyping studies were performed by flow cytometry on the peripheral blood at local laboratories.
Results: As of December 1, 2015, the median time on study was 19 months (range 0.5-44). Pts had a median of 3 prior therapies (range 1-11). The overall response rate was 81%; 16% achieved complete remission. The 24-month estimate for progression-free survival was 62% [95% CI=45, 75] and that for duration of response was 77% [95% CI=58, 98]. The most common treatment emergent adverse events (AEs) were upper respiratory tract infection (49%), diarrhea (47%), and neutropenia (44%). Grade ≥3 AEs occurred in 86% of pts, including 40% with neutropenia, 17.5% with infection, and 13.5% with febrile neutropenia. 19/25 pts with neutropenia were treated with GCSF; all of them responded, 2 were also dose-reduced. 8 deaths were reported [Richters transformation (n=4), complications after allogeneic stem cell transplant (n=2), small bowel obstruction, viral pneumonia (n=1 each)]. Significant reductions were seen in blood B-cell counts, however, no appreciable reductions were observed in the mean absolute counts for CD3+ T-cells, CD4+ or CD8+ T-cells, or NK cells. Similarly, no significant changes were observed in immunoglobulin levels. Only 11/57 pts experienced Grade ≥3 infection, at the rates of 14% in the first 6 months and 9% for the subsequent time on study.
Summary/Conclusions: VEN at RP2D of 400 mg/day resulted in a high response rate and durable remissions. While VEN can induce neutropenia, host T-cell numbers and immunoglobulin levels remained unchanged, and the risk of Gr ≥3 infection in this relapsed CLL population was low.

P592

IBRUTINIB FOR RELAPSED CLL PATIENTS OLDER THAN 75 YEARS: PROVEN EFFICACY, TOXICITIES TO KNOW

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Background: Ibrutinib is an option in patient with relapsed/refractory CLL. Nevertheless, the median age in the Phase I-III studies is 67 to 71 years, which does not reflect current practice.
Aims: In France, the data of the ATU (early-access program, n=428 CLL patients) could be used to analyze the safety (adverse events, AE) and efficacy of this treatment in patients older than 75 years old (n=150/428).
Methods: Analysis of efficacy and safety data in 71/150 elderly patients (70.4% male), median age 79 years (75-89 years, 39.4%>80 years) who received the Ibrutinib treatment for their CLL.
Results: At initiation of treatment, the population had frequent vascular comorbidities (hypertension 34%, venous thrombosis 10%, arteritis 7%), heart comorbidities (myocardial infarction, 10.3%, arrhythmias 13.4%, valvular disease 4%), or kidney dysfunction (31.3% with reduced creatinine clearance 30-60ml/min). As such, they required antiplatelet therapy and/or anticoagulant in 30% and 12% of cases, respectively. Baseline CLL characteristics included: advanced Binet stage (stage C 68%), deletion 17p and/or TP53 mutation 53.3%, deletion 11q 21.6%, median of 3 lines of previous treatments (range 1-9). Patients started ibrutinib at 420mg/d in all but one patient, still 55.7% of patients had a dose reduction to 280mg/d due to toxicity during the first year of follow-up, after a median of 8 weeks (range 2-52)). Standard dose was reintroduced in 10/39 pts after resolution of AE, among which 5/10 had to decrease dose from one level again. Ibrutinib was definitively stopped in 34% of pts after a median of 5 months (range 1-16), mainly for AEs (infections 5.6%, cardiac complications 7%, bleedings 11.3%, hematological toxicity), rarely for PD (5.6%). After a median follow-up of 15.1 months, overall response rate at 12 months (45 evaluable patients) was 93.4%, including 75.5% partial remission (35,6% with or 40% without lymphocytosis). Overall survival (OS) and progression-free survival (PFS) rate at 12 months were 92% and 83%, respectively. At last follow-up (median: 15.1mo), 63.4% of patients were alive on treatment, 15.5% alive without ibrutinib, and 21.1% (15/71) died from: infection (7/15), CLL progression (6/15, 3/6 had proven Richter transformation), heart disease (2/15). In statistical analysis, the only factor influencing PFS (HR=6,369; p=0,004) and duration of response (HR=7,052, P=0,017) but not OS was a previous medical history of arteritis/myocardial ischemia. As reported from clinical trials, a drug hold>7 days tended to be associated with a reduction of the duration of response, but not a dose reduction from 420 to 280mg/d. In this series, estimated median OS was 21 months. Safety profile was fairly known and manageable: grade 1-2 bleeding (19%), cardiac toxicity (7%), diarrhea (24%), myalgia/arthralgia (20%), the frequency of which decreasing over the first 6 months. On the other hand, ibrutinib was associated with a risk of hematologic AE and infection (20% of patients experienced infection of at least grade 2), irrespectively from date of assessment (3, 6 or 12 months).
Summary/Conclusions: In very elderly patients with CLL, Ibrutinib was an effective strategy requiring nevertheless an adequate management of toxicities, dose reduction being often efficient.

P593

MUTATION ANALYSIS BY TARGETED NEXT GENERATION SEQUENCING IN ULTRA HIGH RISK CLL PATIENTS TREATED WITHIN THE CLL2O STUDY

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Background: In chronic lymphocytic leukemia (CLL), *TP53* mutation (*TP53mut*), 17p deletion (17p-) or fludarabine-refractoriness are strongly associated with resistance to chemotherapy-based treatment and a poor prognosis. The German and French CLL study groups initiated the CLL2O trial, which aimed to achieve sufficient response rates and remission duration by combining the CD52-antibody alemtuzumab with dexamethasone followed either by alemtuzumab-maintenance or allogeneic stem cell transplantation. The trial enrolled only CLL patients refractory to a fludarabine-based regimen (with or without 17p-) or with 17p- (front line or relapsed).
Aims: While incidence and prognostic value of gene mutations have been extensively evaluated in first-line CLL treatment situations, only scarce data is available from clinical trials focusing on high risk patients.
Methods: We performed targeted next generation sequencing (tNGS) of eight recurrently mutated genes (*TP53, NOTCH1, SF3B1, ATM, BIRC3, POT1, FBXW7, MYD88*) on baseline samples of 111 CLL2O patients. Statistical analyses were performed by Fisher's exact test, Kaplan-Meier estimates, and Cox proportional hazards regression with significance as $p<.05$ (two-sided).
Results: The full CLL2O cohort (n=131) comprised 17p- frontline (n=42), 17p- relapsed (n=28) and refractory (with 17p- (n=30) and without 17p- (n=31)) patients. Analyses are reported for the full cohort and for those predefined subsets of the trial. Among all 111 patients with mutation data available, *TP53* mutations were detected in 79 (71.2%), *NOTCH1* in 32 (28.8%), *SF3B1* in 27 (24.3%), *ATM* in 24 (21.6%), *BIRC3* in 7 (6.3%), *POT1* in 4 (3.6%), *FBXW7* in 3 (2.7%) patients and *MYD88* in a single (0.9%) patient. There were associations of muta-

tions with genomic aberrations, *e.g. TP53* with 17p- (p<.001), *ATM* with 11q- (p=.037), but there was no correlation between *TP53mut* and other recurrent mutations. Remarkably, 11 pretreated patients showed 2 to 4 *TP53mut*, while only a single untreated patient had two *TP53mut*. In the full trial cohort, none of the mutations had a significant impact on response, progression free survival (PFS) or overall survival (OS). Notably, in patients with 17p-, the incidence of *TP53, SF3B1 and NOTCH1* mutations was similar in treatment naïve (n=37) and pretreated (n=74) patients and the overall genomic landscape appeared not markedly different between those cohorts (Figure 1). These findings indicate that most genomic variants preexist and are not induced by fludarabine-based chemotherapy in 17p- CLL. Of note, in the 17p- frontline cohort, there was a significant association of *TP53* wild-type (hazard ratio 0.14; p=.02) and *ATMmut* status (hazard ratio 0.16; p=.04) with prolonged PFS. In contrast, within the refractory and 17p- relapsed subgroup, there was no significant association of any mutation with outcome. In the F-refractory cohort, when comparing the groups with (n=26) and without (n=21) 17p-, there was an association of *ATMmut* with absence of 17p- (p=.01), but no significant correlation to outcome.

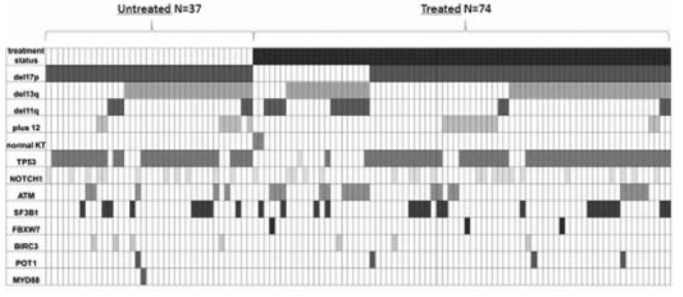

Figure 1.

Summary/Conclusions: In conclusion, within the full trial cohort none of the recurrent mutations had an impact on outcome in the CLL2O trial. Fludarabine-based treatment does not appear to increase the overall complexity of the genomic landscape of 17p- CLL, but it might increase the number of intraindividual *TP53mut* in a given tumor. *TP53* is a prognostic factor in previously untreated, but not in pretreated and refractory patients enrolled within the CLL2O trial. Of note, in the F-refractory cohort 20/21 (95,2%) patients without 17p- and with *TP53* wild-type had at least one other alteration such as 11q-, *NOTCH1mut*, *ATMmut* or *SF3B1mut.*

P594

IDELALISIB IN COMBINATION WITH RITUXIMAB IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)/SMALL LYMPHOCYTIC LYMPHOMA (SLL): REAL-WORLD EXPERIENCE THROUGH AN EARLY ACCESS PROGRAM IN EUROPE AND AUSTRALIA

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Background: Idelalisib (IDELA) is a first-in-class selective PI3Kδ inhibitor approved in EU for use in combination with rituximab (R) for patients (pts) with relapsed CLL; or as first-line CLL treatment in the presence of del(17p) or TP53 mutation (TP53m) in pts unsuitable for chemo-immunotherapy. A post-authorization, pre-national reimbursement early access program (EAP) was initiated for Australia, Belgium, Greece, Ireland, Spain and UK, for pts without other therapeutic options and who could not be included in an IDELA clinical trial. Pts with relapsed/refractory (R/R) CLL/SLL and treatment naïve (TN) CLL/SLL with del(17p)/TP53m, were eligible for the CLL/SLL cohort. IDELA data outside clinical trials and in real-world settings is limited.
Aims: This analysis was to characterize baseline demography of pts treated with IDELA+R in both TN CLL/SLL pts with del(17p)/TP53m and R/R CLL/SLL, outside of a clinical trial setting. Serious adverse events (SAE) reported are also presented.
Methods: The EAP enrolled pts from March 2015, analysis data-cut off was 15 January 2016. Available data were collected from de-identified pt registration data and SAE reports. This analysis summarizes baseline pt characteristics which were either mandatory or optional on the enrolment form. SAEs were reported whilst pts were on treatment. The proportion of missing data (unreported or unrecoverable) is also shown in the Table 1.
Results: Median follow up was 133 days (2-315). 170 pts with R/R CLL/SLL had documented prior treatment regimens (166 with specific treatment and 4 with number of lines only). Most commonly used prior therapies included anti-CD20 (85.6%), cyclophosphamide (69.3%), fludarabine (56.6%), bendamustine (43.3%) and prednisolone (40.0%). SAE data collected were consistent with previous clinical study SAE reporting with SAEs in 37/263 (14.1%) pts. SAEs included 8 (3.0%) pneumonia, 7 (2.7%) liver test elevations, 5 (1.9%) neutropenia, 5 (1.9%) pneumonitis, 4 (1.5%) skin reactions, 3 (1.1%) diarrhea and 3 (1.1%) febrile neutropenia.

Table 1.

Baseline Demography	TN n (%)	R/R n (%)	Total, n (%)
Histology, CLL/SLL*,*	45 (17.1)	218 (82.9)	263 (100)
Age, year			
Median (range)*	70 (40-87)	70.5 (30-90)	70 (30-90)
Male*	28 (62.2)	156 (71.6)	184 (70.0)
Binet stage			
A/B/C	2/3/1 (33.3/50/16.7)	10/60/93 (6.1/36.8/57.1)	12/63/94 (7.1/37.3/55.6)
Documented	6	163	169
Missing data	39	55	94
ECOG			
0/1/2/3	4/3/0/0	57/92/15/3	61/95/15/3 (35.1/54.6/8.6/1.7)
Documented	7	(34.1/55.1/9.0/1.8)	174
Missing data	38	167	89
		51	
Del(17p)/TP53m			
Either	45 (100)	69 (49.3)	114 (61.6)
Neither	0	71 (50.7)	71 (38.4)
Missing	0	78	78
Prior therapies			
Median (range)	0	2 (1-11)	2 (0-11)
Documented		170	215
Missing data		48	48

*Indicates mandatory information collected upon enrolment
*SLL enrolled in Australia only

Summary/Conclusions: This is the largest cohort reported to date for pts with CLL/SLL treated with IDELA+R, outside of clinical trials. Pts included in the EAP had similar demographic characteristics to those previously reported in clinical trials.[1] To date, available results indicate an acceptable tolerability profile of IDELA+R in the real-world setting for pts withTN CLL/SLL and 17p deletion/ TP53m and for pts with R/R CLL/SLL.

Reference

1. Eichhorst B, *et al*. Ann Oncol 2011; 22(Suppl 6):vi50-vi54.

P595

CHROMOSOME 8 ABNORMALITIES ARE ASSOCIATED WITH AN EVEN WORSE OUTCOME AND KARYOTYPE COMPLEXITY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND TP53 ABERRATIONS

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Background: *TP53* aberrations (deletions and/or mutations, *TP53*ab) in chronic lymphocytic leukemia (CLL) are associated with dismal clinical outcome and reduced life expectancy. However, mounting evidence suggests that within cases carrying *TP53*ab other cytogenetic features may also influence the clinical outcome. Alterations in chromosome 8, in particular 8p losses (affecting several regions, from 8p12 to 8p23) and 8q gains (usually involving 8q24, where *MYC* is located), are enriched in *TP53*ab patients and have been proposed as features with potential prognostic value within this aggressive group.
Aims: To assess the prevalence of 8p losses (8p-) and 8q gains (8q+) in CLL patients with *TP53ab*, characterize their clinicobiological profile and evaluate their prognostic value.

Methods: A total of 101 patients with *TP53*ab from 17 Spanish and Greek institutions were included in the study. 8p- and 8q+ were analyzed in peripheral blood samples by FISH using *LPL* (8p22) and *MYC* (8q24) probes (AbbottMolecular) in 75 and 101 cases, respectively. Clinical and cytogenetic data from patients with 8p- *vs* normal 8p (N-8p) and 8q+ *vs* normal 8q (N-8q) were compared.
Results: A total of 11/75 patients (14.7%) showed 8p- and 18/101 cases (17.8%) carried 8q+. In 6/75 patients (8%) both abnormalities were concomitant. As for the clinicobiological profile at diagnosis, 8p- and 8q+ cases displayed no differences regarding age, Hb/platelet/leucocyte values, as well as FISH detected abnormalities del(11q), del(13q) and trisomy 12 compared to cases with normal 8p (N-8p) and normal 8q (N-8q), respectively. However, cases with 8p- exhibited a higher incidence of B symptoms compared to N-8p cases (P=0.039). Interestingly, 8p- cases carried a higher number of 17p-deleted cells as well as a higher median number of chromosomal alterations detected by chromosome banding analysis (CBA) (7 *vs* 3) and, therefore, a higher frequency of complex karyotypes (CK, defined as ≥3 structural/numerical aberrations) compared to N-8p cases (P=0.001, P=0.041 and P=0.006, respectively). Similarly, cases with 8q+ also showed a higher median number of chromosomal abnormalities (6.5 *vs* 2) and higher frequency of CK compared to N-8q cases (P=0.001 and P=0.002, respectively). Ten-year survival analysis revealed a significant shorter overall survival (OS) for both 8p- and 8q+ (P=0.002 for 8p- *vs* N-8p, and P=0.007 for 8q+ *vs* N-8q). CK also had a negative impact on OS (P=0.018) (Figure 1). In multivariate analysis only CK retained independent significance (P=0.032, HR: 2.5).

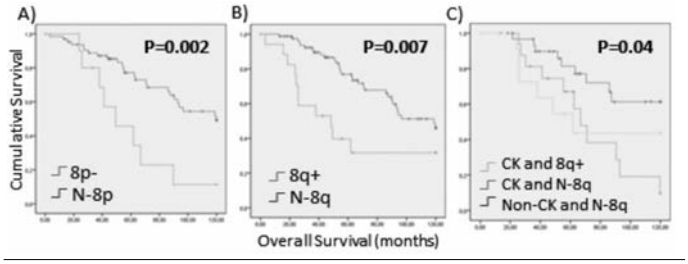

Figure 1. Kaplan Meier plots for ten-year OS in patients carrying (A) 8p-, (B) 8q+ and (C) complex karyotype with 8q+ or N-8q.

Summary/Conclusions: 1. The prevalence of 8p- and 8q+ in this cohort of CLL patients with *TP53ab* was 14.7% and 17.8% respectively. 2. The detection of chromosome 8 abnormalities (8p- and/or 8q+) by FISH in patients with CLL and *TP53*ab is associated with karyotype complexity and further deteriorates outcome. 3. Genomic complexity assessed by CBA negatively impacts on survival even amongst patients with *TP*53ab. 4. Validation in large cohorts is required to elucidate if the observed dismal OS in CLL patients with 8p- and/or 8q+ is due to chromosome 8 alterations *per se* or by the genomic instability associated with complex karyotypes.
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P596

OUTCOMES WITH SINGLE-AGENT IBRUTINIB BY PRIOR LINE OF THERAPY AND FOLLOWING IBRUTINIB DISCONTINUATION IN PATIENTS WITH CLL: ANALYSES FROM PHASE 3 STUDIES

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Background: Ibrutinib (ibr) is the first-in-class, oral, once daily Bruton's tyrosine kinase inhibitor EMA-approved for adult patients with CLL with ≥1 prior therapy. Difference in outcomes with ibr may exist by prior lines of therapy (LoT). Variable overall survival (OS) after ibr discontinuation (DC) have also been reported.
Aims: To evaluate outcomes with ibr based on prior LoT, and following ibr DC in patients with CLL.
Methods: We analyzed data from two randomized phase 3 trials of ibr: PCYC-1115/16 (RESONATE-2) in patients ≥65 years with treatment naïve (TN) CLL; PCYC-1112 (RESONATE) in previously treated (PT) CLL, excluding patients with del17p for a more homogenous dataset for analysis. All patients provided informed consent. Progression-free survival (PFS) and overall response rate (ORR) were assessed by investigator.
Results: Data from 271 patients were included in this analysis. In TN *vs* PT patients, median age was 73 *vs* 66 years. PT patients had a median of 3 prior therapies including CD20 antibody (93%), purine analog (87%), or alkylating agents (93%; bendamustine 41%). Median PFS and OS were not reached (NR) for TN or PT patients, with 89-92% progression free at 2 years for patients treated with ibr in 1st- or 2nd-line (Table 1). ORR was high regardless of LoT (91% in TN, 92% in PT patients). 85% of TN patients and 61% of PT patients continue ibr treatment. Adverse event (AE) profile was similar for both groups. Patients receiving ibr in earlier LoT were less likely to DC ibr due to progressive disease (PD) (Table 1). Median OS post ibr DC is NR for patients who received ibr in 1st- or 2nd-line (n=23) *vs* 7-9 months (mo) in 3rd-line and beyond (n=34).

Table 1. Outcomes with Ibrutinib based on prior LoT including outcomes following discontinuation.

	Subgroups by Prior LoT (N=271)			
	0 (n=136)	1 (n=27)	2 (n=41)	≥3 (n=67)
Median age, years (range)	73 (65-89)	64 (30-85)	66 (46-86)	67 (44-83)
Median follow up, mo (max)	22 (32+)	30 (36+)	31 (34+)	30 (37+)
Continuing study ibr, n (%)*	116 (85)	19 (70)	28 (68)	36 (54)
24-mo PFS, %	92	89	80	69
30-mo OS, %	97	93	83	82
ORR (w/PR-L), %	91	100	93	88
CR/CRi	17	7	15	6
PR-L	3	0	2	9
DC due to, n (%)*				
PD	4 (3)	2 (7)	5 (12)	11 (16)
AEs	13 (10)	1 (4)	6 (15)	7 (10)
Deaths	2 (2)	2 (7)	1 (2)	5 (7)
Other	1 (1)	2 (7)	1 (2)	4 (6)
Median time post DC*, mo				
Follow up	9	5	9	10
OS	NR	NR	9	7
OS: DC due to AE	NR	NR	16	NR
OS: DC due to PD	NR	NR	8	6
Patients with subsequent therapy, n	4	1	6	11
Common subsequent therapy	2 FCR, 1 BR, 1 clb	1 EPOCH-R	3 R-CHOP	4 idela±R, 3 ofa

*5 patients who DCed study ibr to receive commercial ibr not included.

Summary/Conclusions: Treatment with once daily ibr led to favorable PFS and OS, and high ORR regardless of LoT in patients with CLL. Patients who received ibr in earlier LOT as 1st- or 2nd-line therapy were less likely to progress and experienced better post-ibr survival outcomes.

P597

SINGLE-AGENT IBRUTINIB *VS* STANDARD OF CARE FOR PATIENTS WITH RELAPSED/REFRACTORY (R/R) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): AN ADJUSTED COMPARISON OF RESONATE™ WITH THE CLLEAR DATABASE

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Background: The phase 3 RESONATE™ trial (NCT01578707) compared ibrutinib (ibr) with ofatumumab (ofa) in patients (pts) with previously treated CLL and not eligible for purine analogue treatment. Ibr showed significantly improved progression-free survival (PFS; hazard ratio [HR]=0.22; p<0.001) and 18-month overall survival (OS; adjusted for crossover) (HR=0.35 [0.16-0.75]) (Diels, ISPOR-EU 2015).
Aims: In the absence of a head-to-head comparison of single-agent ibr with other frequently used treatments, we investigated the relative efficacy of ibr *vs* physician's choice in R/R CLL pts by comparing pt-level data from RESONATE™ with R/R pts in a real-world setting (the CLLEAR database). The CLLEAR database holds data on CLL pts from the Czech Republic, collected

from academic centers. Electronic Medical Records for 450 pts from 3 centers, collected between 2012 and 2015 were available from this database.
Methods: Pt-level data from the RESONATE™ trial (ibr, n=195; ofa, n=196) were compared with data from the CLLEAR database, adjusting for confounding factors using multivariate statistical modelling. From this database, pts who received second-line or later line treatment were identified. Longitudinal follow-up in subsequent treatment lines was available for pts in second (n=87), third (n=33), fourth (n=15), and subsequent (n=13) lines. In order to account for non-comparability of ibr pts with the CLLEAR cohort due to lack of randomization, the statistical approach of a multivariate Cox proportional hazards model was used to compare PFS and OS between treatments, including line of therapy, age, gender, disease stage (based on Binet/Rai), and ECOG performance status as covariates.
Results: Across all treatment lines, the most frequent treatment regimens used were FCR or FCR-based treatment (20.9%), alemtuzumab monotherapy (15.5%), chemotherapy+monoclonal antibodies (14.2%), rituximab monotherapy (9.5%), corticosteroid only (9%), R-CHOP or R-CHOP–based (6.1%), and BR (4.7%); the remaining percentages comprised a multitude of various other therapies, each used in less than 4% of pts. Line of therapy, age, gender, disease stage, and ECOG performance status were all independent risk factors for worse outcome of PFS and OS. Median age at treatment initiation was 67 years for both pt cohorts. Median number of prior therapies in RESONATE™ was 3, compared with 1 for CLLEAR. The observed HRs for PFS and OS of ibr *vs* physicians' choice from the CLLEAR database were 0.19 (0.13-0.27) and 0.26 (0.17-0.42). After adjustment for differences in baseline characteristics, HRs improved to 0.10 (0.06-0.16) for PFS and 0.15 (0.08-0.28) for OS. The impact of adjustment on PFS and OS was mainly driven by differences in number of prior lines of therapy. Adjusted HRs for PFS and OS of ibr *vs* FCR-based treatment in the second line were 0.07 (0.02-0.25) and 0.11 (0.02-0.57), respectively.
Summary/Conclusions: Investigation of clinical outcomes suggests that ibr administered to pts in the RESONATE™ trial was more effective than physician's choice amongst pts from a real-world cohort of Czech pts. PFS and OS for ibr is improved compared with standard therapies currently available for pts with R/R CLL. These results are consistent with a previous report from a Swedish observational study showing superiority of the ibr cohort from RESONATE™ over physicians' choice (Österborg, ASH 2015) and further support the growing evidence that ibrutinib significantly improves both PFS and OS *vs* current and prior commonly used regimens.

P598

NO IMPROVEMENT IN LONG-TERM OVERALL SURVIVAL AFTER THE INTRODUCTION OF CHEMO(IMMUNO)THERAPY FOR CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS BELONGING TO STEREOTYPED SUBSET #2

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Background: Several population-based studies support the notion that overall survival (OS) in CLL has improved in recent years. Relevant to this, a turning point in the management of CLL was the introduction of chemoimmunotherapy, in particular the fludarabine-cyclophosphamide -rituximab (FCR) regimen, the first to lead to prolonged OS compared to previous chemotherapy approaches. However, certain CLL subgroups have proven refractory to chemo(immuno) therapy, the paradigmatic example being patients harboring aberrations within the *TP53* gene who display resistance to all such approaches. Aside from this, relatively little is known about the trends in OS over time among other CLL prognostic subgroups.
Aims: To investigate trends in long-term survival of CLL patients with particular biomarker profiles treated with chemo(immuno)therapy.
Methods: We evaluated parameters affecting OS within a cohort of 3503 patients treated for CLL with chemo(immuno)therapy in 15 institutions in Europe and the US. The patient distribution in the present study was as follows: males: n=2410 (69%), median age at treatment: 63.5 years; CD38 positivity: n=707/1853 (39%); del(17p): 111/1059 (11%); del(11q): 199/937, 21%; trisomy 12: 133/706, 19%; del(13q): 323/570, (57%); unmutated IGHV genes (U-CLL): n=2216 (63%); membership in stereotyped subset #2 (IGHV3-21/IGLV3-21): n=166 (5%). The latter was assessed separately due to representing an aggressive CLL subgroup, independently of IGHV gene somatic hypermutation status. Patients included in the analysis received primary treatment between May 1980 and July 2013 and were subdivided into two groups: (A) treated before 2006 (n=2092) and; (B) treated in 2006 and after (n=1411). This time-point was chosen due the following implementation of chemoimmunotherapy in clinical practice after 2005.
Results: The two groups did not differ at diagnosis with regards to gender, IGHV mutational status, stereotyped subset #2 membership or cytogenetic profile. In contrast, significant differences ($p<0.05$) were identified concerning: patient age at time of first treatment (median: 63 for group A *vs* 64.5 for group B); CD38 positivity (42% in group A *vs* 33% in group B, $p<0.0001$); OS (measured from the time of diagnosis): 9.5 years in group A *vs* 17.5 years in group B ($p<0.0001$). The superior outcome of patients treated after 2005 (group B) was evident across subgroups defined by age, gender, IGHV mutational status, CD38 expression, del(11q), trisomy 12 and del(13q) ($p<0.05$ for all comparisons). In contrast, the OS was not improved after 2005 for cases carrying del(17p) (median OS: 7 and 5.2 years in groups A and B respectively, p=0.61), which is not unexpected given the documented low efficacy of chemo(immuno)therapy amongst patients with such aberrations. Notably, a similar lack of improvement in OS (median OS: 7 and 9 years in groups A and B respectively, p=0.14) was identified for cases belonging to stereotyped subset #2, further highlighting this subset as a particularly aggressive CLL subgroup.
Summary/Conclusions: Advances in chemo(immuno)therapeutic approaches in CLL during the last decade have translated into prolonged OS in all prognostic subgroups of the present study except those carrying *TP53* abnormalities as expected, but also to those assigned to stereotyped subset #2, that are generally devoid of such gene aberrations. This latter finding, reported here for the first time, supports the clinical relevance of B cell receptor IG stereotypy in CLL and indicates the need for alternative treatment options for subset #2 patients.

P599

VENETOCLAX IS ACTIVE IN CLL PATIENTS WHO HAVE RELAPSED AFTER OR ARE REFRACTORY TO IBRUTINIB OR IDELALISIB

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Background: Patients (pts) with chronic lymphocytic leukemia (CLL) who relapse after or become refractory (R/R) to BCR pathway inhibitors have poor outcomes. Venetoclax (VEN) is a selective, oral BCL-2 inhibitor with significant activity in R/R CLL.
Aims: Primary endpoints are ORR by iwCLL criteria [week (w) 8, 24, then every 12w] and safety.
Methods: In this ongoing Ph 2 study, pts with CLL R/R to ibrutinib (IBR, Arm A) or idelalisib (IDE, Arm B) receive VEN 20 mg daily followed by 5-week ramp up to 400 mg daily.
Results: 54 pts were enrolled (41 A, 13 B), including 25 refractory to IBR and 6 to IDE; 12 and 6 were intolerant with CLL progression after stopping IBR and IDE, respectively; 3 in each arm had both IBR and IDE. 54% had >5 prior therapies, 83% had unmutated *IGHV*, 20% had ALC >100x10^9, 35% had del(17p),

and 24% had ≥1 node ≥10 cm. Median time on VEN was 31.9 w (0.6– 52.9) for Arm A and 23.7 w (5.4–52.9) for Arm B. 8 in Arm A discontinued VEN (4 PD; 1 each respiratory failure, multi-organ failure, death of unknown cause, consent withdrawal); 2 in Arm B (1 PD, 1 non-response). Efficacy in 48 evaluable pts is shown in the Table 1. In the refractory subsets, 14/22 Arm A pts and 3/5 Arm B pts achieved response. 8/27 pts who reached w24 were MRD-negative (10^{-4}) by flow cytometry in blood (all Arm A: 2 CR, 1 nPR, 4 PR, 1 SD).

Table 1.

Best response in evaluable* pts, n (%)	Arm An=38	Arm Bn=10
ORR	23 (61)	5 (50)
CR	3 (8)	0
PR/nPR	19 (50)/1 (3)	5 (50)/0
SD	10 (26)	4 (40)
PD	1 (3)	1 (10)
DC before assessment	4 (11)	0

**3 in each arm are not yet evaluable*

Safety was consistent with prior reports. AEs in >20% pts: neutropenia (48%), diarrhea (37%), nausea (35%), anemia (32%), fatigue (24%), hyperphosphatemia (20%). Grade 3/4 AEs >10%: neutropenia (39%), thrombocytopenia (22%), anemia (20%), leukopenia (13%), pneumonia (13%). SAEs in ≥2 pts: pneumonia (9%), febrile neutropenia (7%), increased potassium, multi-organ failure, septic shock (4% each). 2 pts had laboratory TLS without clinical sequelae.

Summary/Conclusions: In CLL pts R/R to IBR or IDE, VEN monotherapy showed promising activity, including MRD negativity at 24w, with acceptable safety. This is the first prospective study to demonstrate efficacy in this poor prognosis population. Follow-up will assess depth and duration of response.

Chronic myeloid leukemia - Biology

P600

SOMATIC MUTATIONS IN NEWLY-DIAGNOSED CHRONIC MYELOID LEUKEMIA DETECTED BY WHOLE-EXOME SEQUENCING

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Background: Although tyrosine kinase inhibitors (TKIs) have significantly improved the prognosis of chronic myeloid leukemia (CML), the ability of TKIs to eradicate CML remains uncertain and patients must continue TKI therapy for indefinite periods. The *BCR-ABL1* gene is a strong driver mutation in CML pathogenesis, and there have been few reports of somatic mutational analysis in CML.
Aims: Our study objective is to identify somatic mutations in newly-diagnosed chronic-phase of CML (CML-CP) patients who were registered in Japan adult leukemia study group (JALSG) CML212 study by whole-exome sequensing (WES).
Methods: The JALSG CML212 study is a multicenter prospective randomized study to compare the cumulative achievement of CMR for adult *de novo* CML-CP (UMIN Clinical Trials Registry, UMIN000007909). Patients were randomly assigned to receive either dasatinib or nilotinib. Samples from the initial 24 patients enrolled in the study between May 2013 and Jan 2014 were analyzed. Genomic DNA was extracted from PBMCs at the time of diagnosis. As a germline control, DNA was obtained from buccal mucosal cells. Whole exome capture was accomplished by liquid phase hybridization of sonicated genomic DNA with a mean length of 150–200 bp for the bait cRNA library, which was synthesized on magnetic beads. The captured targets were subjected to massive sequencing using HiSeq 2000 sequencing system with the pair end 100 bp read option. Copy number analysis was performed using DNAcopy, in which the total number of reads covering each bait region and the allele frequency of heterozygous SNPs detected by WES were used as input data. The mean coverage of more than 95% of the target sequences was analyzed at an average depth of more than ×20. The mutations identified by WES were confirmed by Sanger sequencing and deep sequencing. Single nucleotide variants (SNVs) were extracted from WES as somatic mutations. All mutations were compared with published SNP data. Known synonymous SNPs with p values ≥0.001 compared with the valiant allele frequency (VAF) of peripheral blood leukocytes and oral mucosa by the Fisher's exact test were excluded from further analysis. Correlations between the number of mutations and clinical factors were identified by the Pearson product-moment correlation coefficient using EZR software. Gene ontology (GO) analysis was used to evaluate functional enrichment in GO terms among mutated genes detected by WES.
Results: The median age of the patients was 55 years, 75% were male, and the mean WBC count at diagnosis was 96000/μL. The median IS-*BCR-ABL1* was 57%. 8.3% of patients had additional mutations besides translocation of (9;22) that were detected by the G-band staining method. We identified 190 somatic mutations on 184 genes other than the *BCR-ABL1* fusion gene (median 8, range 1-17). Summary of mutations were shown in Figure 1. *TET2* or *TET3*, *AKT1*, and *RUNX1* were mutated in one patient each. Especially mutations in epigenetic regulators, *ASXL1*, *TET2*, *TET3*, *KDM1A* and *MSH6* were mutated in 5 patients (21%). Two patients had mutations on *ASXL1*, both were loss of function mutations and existed within exon 12. Other recurrent mutations were identified in *CLSTN2*, *COL7A1*, *CSMD2*, and *DYSF*. By GO analysis, mutated genes were mostly enriched with cell signaling and cell division pathways. DNA copy number alterations revealed that 2 out of 24 patients had uniparental disomy (UPD) in chromosomes 1p and 3q, respectively.
Summary/Conclusions: We identified mutations of *TET2*, *TET*3, *ASXL1*, *AKT*1, *RUNX*1, and *PRDM9*, besides multiple novel recurrent mutations previously reported in association with hematological malignancies. Further analyses such as the transition of these mutations by deep sequencing are required

to elucidate associations with therapeutic responses or prognosis, and functional analysis of these candidate genes in CML cell lines is required to confirm these findings.

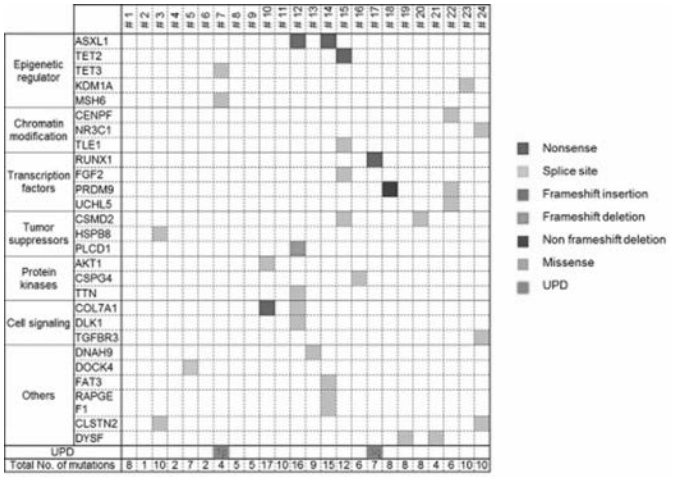

Figure 1.

P601

ROCK AS THERAPEUTICAL TARGET FOR MORGANA LOW CML

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Background: Atypical chronic myeloid leukemia (aCML) is an haematological neoplasm characterized by a median overall survival of 12.4 months. The standard treatments for aCML patients are chemotherapeutic drugs. However, these treatments result inefficient in inducing remission from the pathology. Recently we demonstrated that the haploinsufficiency of Morgana, an Hsp90 co-chaperone, *in vivo* is sufficient to induce a lethal and transplantable CML-like myeloid neoplasm characterized by non recurrent cytogenetic abnormalities in the bone marrow. Morgana is able to bind to and inhibit Rho-kinases, which are emerging as key oncogenic players in haematological disorders
Aims: Identify fundamental altered pathways in aCML to uncover the biological basis of the disease and find new therapeutical targets.
Methods: The bone marrow of morgana heterozygous mice and chronic myeloid leukemia patients has been analyzed extensively by flow cytometry and immunoistochemestry. Murine and human CML bone marrow cells and *in vitro* cellular models (K562 and THP-1 cells) have been tested for sensitivity to the ROCK inhibitor Fasudil.
Results: We demonstrated that diseased morgana heterozygous mice show ROCK hyperactivation in the bone marrow and that inhibition of these kinases results in apoptosis of morgana^{low} bone marrow leukemic cells without affecting normal cells survival. Moreover, in THP-1 cells Morgana downregulation enhances ROCK activity promoting cell proliferation while ROCK inhibition significantly reduces the proliferation of these cells. Interestingly, we found that the Morgana-ROCK pathway is altered in the 16% of Philadelphia-positive CML patients where ROCK hyperactivation, cooperating with BCR-ABL signalling, leads to imatinib resistance. In this context, treatment with a ROCK inhibitor restores the efficacy of imatinib to induce apoptosis. In addition, we found Morgana downregulation and ROCK hyperactivation in the bone marrow of all aCML patients we tested.
Summary/Conclusions: Taken together these results point out Morgana as an oncosuppressor and ROCK as potential therapeutical target for Morgana^{low} CML patients.

P602

A NOVEL C-TERMINAL HSP90 INHIBITOR WITH THERAPEUTIC EFFECT IN IMATINIB RESISTANT CML

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Background: The introduction of specific BCR-ABL tyrosine kinase (TK) inhibitors, especially imatinib mesylate (Gleevec), revolutionized the clinical treatment of Chronic Myeloid Leukemia (CML). However, in many cases stable remission cannot be sustained through several escaping mechanisms, such as mutations in the ABL-kinase (*e.g.* T315I & M351T). These insights raise an urgent need to develop alternative treatment strategies. An attractive approach is targeting Heat Shock Protein 90 (Hsp90), which acts as a molecular chaperone and facilitates the folding of several oncogenic proteins including, BCR-ABL.FDA approved Hsp90 inhibitors are available and show anti tumor activity but to the best of our knowledge they all target the N terminal domain of Hsp90 and initiate heat shock response (HSR) with severe side effects (Wang & McAlpine, 2015). Thus C-terminal Hsp90 inhibition can be an attractive approach in targeting Imatinib resistant CML with low toxicity. We have identified hotspots in the C-terminal domain of Hsp90 (Ciglia *et al.*, 2014) and designed several non-peptidic inhibitors, which target Hsp90's C-terminal dimerization.
Aims: Generation and characterization of novel compounds targeting C terminal dimerization of Hsp90 *in vitro* and *in vivo*.
Methods: The specificity of selected inhibitor (DDK88) to Hsp90 was determined by Hsp90 dependent luciferase refolding assay followed by efficacy experiments using imatinib sensitive and resistant myeloid leukemic cell lines *in vitro* as well as in *in vivo* Xenograft model.
Results: In the present study, we have extensively characterized a novel and promising therapeutic compound (DDK88) for patients with Imatinib resistant CML *in vitro* and *in vivo*. DDK88 exhibits anti-proliferative and cytotoxic activity in several human myeloid leukemic cell line models (*e.g.* K562 - 5.72±0.31μM, KCL-22 - 2.74±0.52μM) and induces cell cycle arrest, early differentiation and inhibits colony formation. DDK88 disrupts Hsp90's chaperone activity to BCR-ABL protein. Hence *in vitro* application of DDK88 revealed down regulation of BCR-ABL protein expression and its downstream signaling network including, STAT5a, CRKL, AKT and mTOR proteins. Moreover, imatinib resistant CML cell lines are equally sensitive to DDK88 (*e.g.* K-562r - 6.24±0.52 μM, KCL-22r - 2.86±0.63μM) as compared to imatinib sensitive cells. In the same way, DDK88 inhibits proliferation and BCR-ABL kinase activity of 3 clinically relevant imatinib-resistant (~10 μM) BCR-ABL mutant (T315I, M351T & E255K - ~3μM) cell lines. Notably, unlike clinical inhibitors targeting N-terminal (*e.g.* AUY922), DDK88 does not induce HSR, evaluated by protein expression of HSF-1, Hsp70, Hsp40 and Hsp27. DDK88 has a therapeutic window as its inhibitory effects on healthy cord blood (CB) derived mononuclear cells (MNCs) and CD34+ cells were significantly less potent as compared to the human leukemic cell lines. Furthermore, *in-vivo* proof of concept studies demonstrate the efficacy of DDK88 at 0.5 mg/kg in a K-562-Luciferase Xenograft tumor model. DDK88 reduced tumor burden with respect to tumor weight (DDK88 0.2±0.01g *vs* vehicle 1.26±0.44g (p=0.04)). Immunoblot analysis of tumor samples derived from DDK88 treated mice revealed the absence of HSR as well as downregulation of BCR-ABL kinase activity and its associated downstream signaling pathways.
Summary/Conclusions: This study provides *in vitro* and *in vivo* characterization of a novel anti-Hsp90 compound, which is specific against its C-terminus. Hence further improvement and testing of DDK88 in pre-clinical studies can be a promising strategy to target imatinib resistant CML and to avoid HSR.

References

1. Ciglia E, Vergin J, Reimann S, Smits SHJ, Schmit L, Groth G, and Gohlke H. (2014) Revealing hot spots in the C-terminal dimerization domain that determine the stability of the molecular chaperone Hsp90. *PLoS ONE* 9, e96031.
2. Wang Y, McAlpine SR. Heat-shock protein 90 inhibitors: will they ever succeed as chemotherapeutics? Future Med Chem. 2015;7(2):87-90. Epub 2015/02/17. doi: 10.4155/fmc.14.154 pmid:25685998.

P603

RESISTANCE IN CHRONIC MYELOID LEUKEMIA: THERAPEUTIC TARGETING OF ESCAPE VIA CSF2RB

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Background: Treatment of chronic myelogenous leukemia (CML) with BCR-ABL tyrosine kinase inhibitors (TKI) achieves high rates of molecular response. However, BCR-ABL-positive leukemic stem and progenitor cells persist implying the need for lifelong treatment. Bone marrow stroma plays an important role in inhibiting apoptosis. Cytokines such as interleukin 3 (IL-3) and granulocyte/macrophage-colony stimulating factor (GM-CSF) mediate BCR-ABL-independent survival of progenitor cells via common receptor subunit CSF2RB. Disruption of the CSF2RB axis by the Janus kinase1/2-inhibitor ruxolitinib overcomes cytokine-mediated resistance *in vitro*. We previously demonstrated upregulation of CSF2RB in BCR-ABL-transformed cells as potential resistance mechanism, and now provide an indepth molecular and functional analysis.

Aims: The aim of this study was to examine the functional and biological relevance of CSF2RB upregulation in BCR-ABL positive leukemia cells in response to TKI treatment as mechanism by which IL-3 and GM-CSF induce TKI resistance.
Methods: Human M07p210 cells were used to study the functional relevance of CSF2RB-mediated rescue of TKI-treated leukemia cells by siRNA-mediated knockdown of CSF2RB. Cell proliferation and viability of BCR-ABL positive CML CD34+ progenitor cells under the influence of nilotinib/ dasatinib and ruxolitinib were analyzed *in vitro*. Expression of CSF2RB in M07p210 cells and BCR-ABL positive CML CD34+ progenitor cells in response to nilotinib and dasatinib was examined by western blot and RT-PCR. Bone marrow samples harvested at 3 months on nilotinib therapy were used to study CSF2RB regulation in CML-patients with defined levels of molecular response.
Results: In M07p210 cells and CML CD34+ progenitor cells, BCR-ABL inhibition with TKIs induces significant upregulation of CSF2RB on protein and mRNA-level. Moreover, cytokines exert distinct survival signals including STAT5 activation, despite effective BCR-ABL-inhibition in the presence of nilotinib. Furthermore, selective knockdown of CSF2RB prevents cytokine-mediated cell survival in nilotinib-treated M07p210 cells. Combination treatment of M07p210 and treatment naïve CML CD34+ progenitor cells with nilotinib/ dasatinib and ruxolitinib overcomes cytokine-mediated tyrosine kinase inhibitor resistance as demonstrated by additive growth inhibition in the presence of GM-CSF. Normal progenitors remain unaffected indicating differential activity. In colony assays with treatment naïve CML progenitors, combined application of nilotinib/ dasatinib and ruxolitinib significantly diminished granulocyte-monocyte and erythroid colony formation in the presence of rescuing cytokines. In picked progenitor colonies, mRNA-upregulation of CSF2RB was observed in 2 of 7 nilotinib-treated patient samples. Interestingly, marked upregulation of CSF2RB was also seen in 2/7 samples exposed to combined treatment. In contrast, total leukocytes from bone marrow harvested at 3 months after start of nilotinib treatment in 8 patients shows unidirectional upregulation of CSF2RB mRNA independently of the molecular response.
Summary/Conclusions: CSF2RB is upregulated in response to nilotinib and dasatinib *in vitro*, and in clinical samples derived from nilotinib treated patients. Marked individual variations of CSF2RB expression in correlation to clinical outcome can be observed in bone marrow mononuclear progenitors indicating patient specific cell intrinsic regulation of CSF2RB. In contrast, consistent CSF2RB upregulation in total bone marrow lysates confirms the bone marrow cytokine-response counteracting nilotinib treatment most likely governed by the bone marrow stroma. Efficacy and tolerability of combined BCR-ABL/ Jak1/2-inhibtion with ruxolitinib and nilotinib to target persistent stem cells is currently investigated in clinical trial NCT02253277.

P604

MITOCHONDRIAL DNA MUTATIONS IDENTIFY CLONAL HETEROGENEITY IN CHRONIC MYELOID LEUKAEMIA

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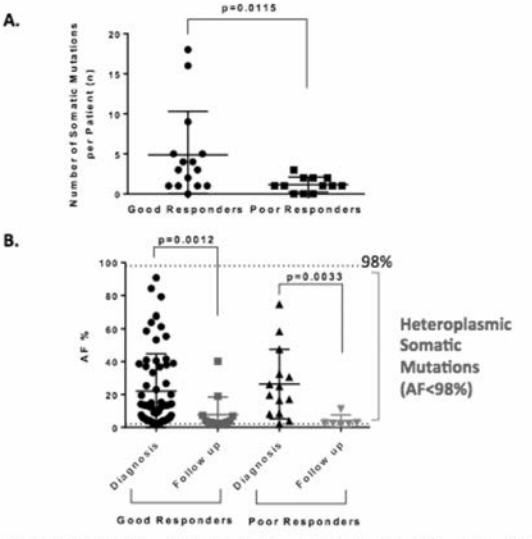
Background: Genomic instability in chronic myeloid leukaemia (CML) is reported to be associated with increased reactive oxygen species (ROS). The mitochondrial (mt) genome is susceptible to ROS-induced mutations due to oxidative stress in the mitochondrion and limited DNA repair mechanisms. MtDNA is present in multiple copies per cell: homoplasmy indicates that all mtDNA copies in a cell are identical, whereas coexistence of variant and wild type is referred to as heteroplasmy. Significant variations in treatment outcome occur despite all CML patients having the BCR-ABL1 fusion gene. We hypothesized that mtDNA mutations might reflect high mutational stress and an adverse prognosis.
Aims: To determine the landscape of somatic mtDNA mutations in CML, and to determine whether mt mutations are due to oxidative stress.
Methods: The whole mt genome was amplified in two overlapping fragments, pooled in equimolar concentrations and sent for next generation sequencing. We studied 27 CML patients at diagnosis (Dx) and follow-up (FU) after 12 months of therapy in the ALLG CML9 trial using up-front imatinib, with selective switching to nilotinib for sub-optimal molecular response. Patients were selected as good (n=15) or poor (n=12) responders based on achievement of major molecular response (BCR-ABL1^{IS} ≤0.1%) at 12 months. Mesenchymal stem cells or hair follicles were used to exclude germline polymorphisms. Comparison of technical duplicates was used to establish a 2% cut-off to distinguish mutations from background sequencing errors.
Results: No indels were found. We identified 73 somatic point mutations in 14/15 (93%) good responder patients, and 14 somatic mutations in 9/12 (75%) poor responder patients at Dx. Mutations were distributed across the mt genome with no evident hotspots. The distribution did not differ between good and poor responders, and did not show the excess of G>T transversions that is considered characteristic of ROS-induced damage. The number of somatic mutations per patient was higher in good responders (1-18 per patient; mean 4.87) than in poor responders (mean 4.87 *vs* 1.17 per patient; p=0.0115) (Figure 1A). There was no correlation between Sokal or EUTOS score and the number of mutations. All somatic mutations at Dx were heteroplasmic, with the exception of one patient carrying 10/18 homoplasmic mutations. No difference in the allele frequency (AF) of mutations was found between good (2.1% -90.8%; mean 22.1%) and poor responders (3.7%>74.6%; mean 26.4%) (Figure 1B). Most (85/87) mutations found at diagnosis reverted to homoplasmy in FU samples. Only two patients had mutations showing persistent heteroplasmy with a change in the AF. New heteroplasmic mutations were identified in FU samples in 7/15 (47%) good responder patients, and in 5/12 (42%) poor responders. The AF of mutations at FU was lower than at Dx (2.1%>40.3%, mean 6.5%;) (Fig 1B), but was higher than the estimated level of the residual CML clone (BCR-ABL^{IS} 0%>2.7%; mean 0.46%).



Mitochondrial DNA mutations are common in CML and identify clonal heterogeneity. (A) Number of somatic mutations per patient at diagnosis. Good Responders tended to have more mutations than did poor responders. **(B) Comparison of the Allele Frequency (AF) between good and poor responder patients at diagnosis and follow up.** The AF of mutations at follow-up is lower than at diagnosis.

Figure 1.

Summary/Conclusions: MtDNA mutations are common in CML, and identify clonal diversity both at diagnosis and after imatinib treatment. The pattern of mtDNA mutations was not consistent with ROS-mediated damage. The presence of mt mutations was not associated with clinical risk score and had no prognostic effect. Most mutations found at diagnosis were undetectable at follow-up, consistent with molecular response of the CML clone, but the emergence of heteroplasmy in follow-up might indicate the presence of a non-CML haematopoietic clone in some patients.

P605

CLONAL STRUCTURES OF LEUKAEMIA STEM CELL POPULATIONS IN MYELOID BLAST PHASE CHRONIC MYELOID LEUKAEMIA

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Background: Chronic myeloid leukaemia (CML) is a model of the multistep processes in cancer. In myeloid malignancies initiating mutation originates in a haemopoietic stem cell (HSC) to give rise to pre-leukaemic stem cell populations. After blast transformation, multiple leukemic stem cell (LSC) populations can be identified. We have shown that CD34+LSC populations were most closely related to normal progenitor populations but had shared elements of a normal stem cell expression signature[1]. In chronic phase (CP)-CML, the leukaemia-propagating population is the HSC, and the progenitor subpopulations do not have stem cell characteristics. Studies to isolate LSC populations in BP-CML have been limited, identifying the granulocyte-macrophage progenitor subpopulation as a possible LSC source[2]. Moreover while CP-CML is dependent on BCR-ABL, the mutations and additional cytogenetic abnormalities underlying disease progression are poorly annotated.

Aims: In this study we aim to: i)Identify changes in size of immunophenotypic (IP) compartments in the progression from CP to BP-CML, ii)characterize which haemopoietic stem/progenitor cell (HSPC) subpopulations of BP-CML patients contain LSCs iii)understand the clonal structures associated with disease progression.
Methods: We have performed extensive IP analysis of 14 AP-CML, 4 CP-CML and 11 BP-CML patients and compared the various HSPCs compartments to normal bone marrow (BM) (N=3). Furthermore we purified HSPC-like populations from 5 BP-CML patient samples and performed *in vivo* xenograft studies using NSG mice with serial transplantation to identify populations with LSC potential. Finally multicolor FISH analysis was performed in 3 patient samples and in engrafted human cells from primary and secondary murine recipients.
Results: Our data conclusively demonstrate that functional LSCs are present in multiple stem/progenitors populations in myeloid BP-CML,. We have described the clonal structures in the samples of 3 patients. Sequential acquisition of 17p13 loss or of 17p13loss and isochromosome 17 was seen in patient samples. More complex clonal structures, with the presence of subclonal atypical BCR-ABL and 17p13loss were detected after transplantation in NSG mice. The monitoring of clonal structures in a patient with 2 sequential samples suggested that the clonal architecture is dynamic and can change with progression of the disease.
Summary/Conclusions: Our results demonstrate that myeloid BP-CML is a heterogeneous disorder with variable LSC populations. Further interrogation of these populations and of clonal hierarchy will identify novel therapeutic targets of LSC populations.
DK and HM are joint first authors, MC and PV are joint senior authors

References

1. Goardon N, *et al.* Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer cell. 2011
2. Jamieson CH, *et al.* Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. NEJM. 2004

P606

ROLE OF THE AURORA KINASE A AXIS IN IMATINIB RESISTANCE OF CHRONIC MYELOID LEUKEMIA CD34+ PROGENITORS

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Background: Aurora Kinase (AK) A has a pivotal role in chronic myeloid leukemia (CML) genomic instability. Its constitutive activation associated with the *BCR-ABL*1 TK activity promotes the progression of mitosis irrespective of the integrity of replicated DNA. Published studies have proven the therapeutic advantage of AK inhibitors in CML patients either responsive or resistant to imatinib (IM). Such AK inhibitors potential has been ascribed to their inhibitory activity on p210 TK activity.
Aims: In this study, we have focused on the specificity and mechanisms of action of AKs and Plk inhibitors in the putative LSC compartment (CD34+).
Methods: The CD34+ hematopoietic cell fraction was investigated for the phosphorylation levels of AKA and other pro-survival components of its signalling pathways: - FoxM1, a proliferation-associated transcription factor implicated in the advantage of clonal hematopoiesis over the normal counterpart, particularly in the leukemic stem cell (LSC) compartment, which is not dependent on BCR-ABL1 tyrosine kinase (TK) for proliferation and survival; - Polo-like kinase 1 (Plk1), a ser-thr kinase involved in M/G1 progression. CD34+ cells were isolated from bone marrow samples of 10 CML patients at clinical diagnosis by means of immuno-magnetic selection (miniMACS from Miltenyi Biotec). CD34+ cells from peripheral blood of healthy donors, pooled to avoid individual differences were used as normal controls. Informed consent was obtained from all the patients. RT-PCR (reverse transcriptase-polymerase chain reaction), WB (western blotting), IP/IB (immunoprecipitation and immunoblotting) were used to investigate gene expression and protein interactions.
Results: Our results proved a FoxM1 increment associated with IM resistance. An IM-resistant K562 cell line (LD50 0.37 microM *vs* 0.026 microM of parental cells) generated in our lab exhibited FoxM1 over-expression and hyper-phosphorylation contingent upon the upstream activation of AKA and Plk1. In fact, in IM-resistant K562 cells, both Plk1 inhibition by volasertib (1microM) and AKs inhibition by danusertib (1microM), activated a significant increment of apoptotic cell death compared to parental cell line. AKA, FoxM1 and Plk1 involvement in IM resistance was confirmed in mononuclear cell fraction from bone marrow samples of 3 CML patients who developed IM resistance independent from BCR-ABL1 point mutations. Interestingly, the putative BCR-ABL1+/CD34+ LSC compartment, which is neither dependent on BCR-ABL1 TK for proliferation and survival nor killed by IM and second generation inhibitors, showed a hyperphosphorylation of AKA and a consequent overexpression and hyper-activation of FoxM1 and Plk1. Moreover, clonogenic assays performed by using CD34+ progenitors from 3 CML patients at diagnosis showed that volasertib and danusertib are capable to reduce the clonogenic potential of the CD34+ compartment to a much greater extent compared to 1st and 2nd generation TKIs (see Table 1).

Table 1.

LD50	PT1	PT2	PT3
IMATINIB	0.255 M	0.374 M	0.472 M
NILOTINIB	0.197 M	0.277 M	0.324 M
DASATINIB	0.269 M	0.311 M	0.295 M
VOLASERTIB	0.112 M	0.093 M	0.162 M
DANUSERTIB	0.098 M	0.084 M	0.073 M

Summary/Conclusions: The BCR-ABL1+/CD34+ compartment provides a sanctuary for disease relapse upon drug withdrawal as well as a putative source of drug-resistance. We have identified a new signaling pathway involved both in drug resistance and in CD34+ cell survival. Our data open the route to novel therapeutic approaches worth exploring in order to overcome drug resistance. Danusertib and volasertib are in clinical trials in hematologic malignancies.

P607

PRESENCE OF SOMATIC AND GERMLINE MUTATIONS IN EPIGENETIC MODIFIERS IN CML-CP

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Background: Chronic myeloid leukaemia (CML) originates from a single genetic aberration (BCR-ABL1); however the clinical disease is remarkably heterogeneous and the genetic mechanisms of resistance to tyrosine kinase inhibitors (TKI) are still poorly understood. Recently, we have identified consistent differences in genome-wide DNA methylation patterns in chronic phase (CP) CML patients compared to healthy controls, whireas epigenetic modifying enzymes have been found frequently mutated in other haematological neoplasms.
Aims: The aim of this study is to analyse a panel of mutations in epigenetic modifiers in CML-CP using Ion Torrent PGM next-generation sequencing. The panel design was based on gene expression analysis we generated and literature search. Potential mutations found at diagnosis may be used as novel prognostic biomarkers for TKI response.
Methods: 52 samples from untreated patients with newly diagnosed CML-CP ($CD34^+$) who started on imatinib were included in the study, classified as responders (n=26) /non-responders (n=26) based on BCR-ABL1/ABL ratio at 3 months. As constitutional non-leukaemic DNA, for non-responders we used DNA from T cells, cultured *in vitro* for 7 days, and for responders DNA from the patients in deep molecular remission (whole blood). 14 samples from healthy donors ($CD34^+$) and 5 samples from CML-BC (blast crisis) were also used. A custom panel covering the coding region of 71 epigenetic enzymes was designed.
Results: A mean depth of 273/amplicon was obtained, detecting mutations as low as 4%. After excluding "bad" variants of low quality, common SNPs with minor allele frequency (maf) >1%, variants found in healthy controls and intronic variants, we kept the non-synonymous variants predicted disease causing, deleterious and damaging by Mutation Taster, PolyPhen-2 and SIFT respectively, and found 104 variants in 46/71 of the genes. However, when constitutional DNA was used as non leukaemic control, we found that only 35 were CML-related somatic mutations, including missense, nonsense, frameshift/non-frameshift insertions and splice site variants, present in 25 genes. 26 mutations were found in non-responders (NR), 13 in responders (R). Interestingly all nonsense variants (in *ASXL1, IKZF1, DNMT3A, EP300*) and most insertions (in *ASXL1, WT1),* were found only in non-responders. Similarly, nonsense mutations in *ASXL1 and IKZF1* were found in 2 CML-BC patients. The frequency of the mutated allele for most mutations was <10%. Mutations were detected in the R and NR group in equal proportion (11/26 patients), however, the presence of ≥2 mutations was more common in NR**.** In addition, we examined the correlation of presence of mutations with gene expression, detecting a correlation in some cases, and their influence on overall survival (OS), finding an influence, especially in the non-responders, more prominent when there were ≥2 mutations. Moreover, we found 69 missense variants that were also present in the constitutional DNA, with frequency of the mutated allele ~50%, considered as germline mutations. These variants were present in 32 genes with 39 and 34 variants in NR and R respectively**,** while there was no difference in the number of R, NR that carried at least one mutation (both groups 20/26 patients)**.** Specific variants in *RUNX1, TET1, TET2* were found in ≥3 patients.
Summary/Conclusions: The mutation analysis of epigenetic modifiers in CML-CP identified the presence of somatic mutations, some of which (found only in NR) can be considered as predictive biomarkers for IM failure, and germline mutations that may predispose to CML.

Chronic myeloid leukemia - Clinical 2

P608

ASSESSMENT OF BCR-ABL1 TRANSCRIPT LEVELS BY DIGITAL PCR (DPCR) IN 116 PH+ CML PATIENTS TREATED WITH TIROSIN KINASE INHIBITORS (TKIS): A COMPARATIVE ANALYSIS BETWEEN DPCR AND QPCR

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Background: Assessment and monitoring of BCR-ABL1 levels by quantitative PCR (qPCR) is essential for the management of CML patients treated with TKIs. Currently, up to 30-40% of CML patients treated with TKIs can achieve a deep molecular response (DMR: BCR-ABL1 ≤0,01% IS), but only 50% maintain a stable Treatment Free Remission (TFR) after discontinuation of TKIs therapy. qPCR has some intrinsic limitations and it does not appear to be an optimal assay to select the best candidates to TKIs discontinuation. Digital PCR (dPCR) can give an absolute quantification of target nucleic acids by partitioning the PCR reaction mix over a large number of wells, each containing a single copy or no copies of the target region. The number of target copies originally present in the sample can be calculated from the number of partitions in which amplification has successfully occurred. The dPCR is expected to give a better sensitivity and accuracy than qPCR in the assessment of molecular Minimal Residual Disease (MRD) in CML patients treated with TKIs.
Aims: The aim was to quantify the BCR-ABL1 transcript levels by dPCR in 116 CML patients treated with TKIs (imatinib, nilotinib or dasatinib) and achieving a major molecular response (MMR or $MR^{3.0}$) or DMR ($MR^{4.0}$, $MR^{4.5}$and $MR^{5.0}$). The results obtained by dPCR were compared with the ones obtained by qPCR.
Methods: The dPCR was performed on a QuantStudio 3D Digital PCR System (Life Technologies) by using a TaqMan-MGB probes targeting the BCR-ABL1 transcript. The absolute quantities of BCR-ABL1 transcript were expressed as number of copies/ul. Samples for dPCR testing were obtained from CML patients treated with TKIs, on time-checks planned for MRD monitoring by standard qPCR performed according to the last International Guidelines. The analysis by dPCR and qPCR were performed on 33 cases with stable $MR^{3.0}$, 37 cases with stable $MR^{4.0}$, 34 cases with stable $MR^{4.5}$, 12 cases with stable $MR^{5.0}$. Blank samples served as negative controls, while 10 healthy donors served as normal controls. Patients enrolled in the study gave informed consent.
Results: In patients with $MR^{3.0}$ the number of BCR-ABL1 copies/ul assessed by dPCR were significantly higher than those of patients with $MR^{4.0}$ (0.0002), $MR^{4.5}$ ($p<0.0001$) or $MR^{5.0}$ ($p<0.0001$). BCR-ABL1 transcript levels were detectable by dPCR also in cases (n°=43) resulted undetectable by qPCR. No linear correlation was found between the BCR-ABL1 copies/ul assessed by dPCR and the values of BCR-ABL1/ABL1% IS (R=0.196) or the absolute copy number of BCR-ABL1 transcripts assessed by qPCR (R=0.184). However, a case by case analysis revealed a linear correlation (R=0.729) only for the cases with an absolute copy number of BCR-ABL1 transcript >15 assessed by qPCR. One-way ANOVA test revealed a correlation between dPCR BCR-ABL1 levels and the $MR^{3.0}$class only. The 84% of deep responders fell under the value of 0.468 BCR-ABL1 copies/ul indicated by the ROC analysis as the value below which the patients with lower levels of MRD might be dissected (specificity=63.64%, sensitivity=84.34%; AUC=0,847).
Summary/Conclusions: The study suggests that dPCR is more accurate and sensitive than qPCR especially in the patients with DMR and currently we are conducting a systematic monitoring of MR by dPCR to evaluate its reliability. Larger and prospective studies are warranted to confirm the higher sensitivity and accuracy of dPCR and its usefulness to better select the candidates for TFR.
Acknowledgments: This work was supported in part by EuropeanLeukemiaNet (ELN) - European Treatment and Outcome Study (EUTOS) and by Cofin 2009.

P609

MOLECULAR RESPONSES OF B2A2 BCR-ABL1 TRANSCRIPT ARE INFERIOR TO B3A2 TYPE IN NEWLY DIAGNOSED PATIENTS WITH CHRONIC MYELOID LEUKEMIA AT CHRONIC PHASE TREATED WITH FRONTLINE IMATINIB -TAIWAN CML STUDY

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Background: b3a2 (e14a2) and b2a2 (e13a2) are the two major *BCR-ABL1* transcripts in CML. The prognostic relevance of the two transcript subtypes after frontline therapy with tyrosine kinase inhibitor is unclear.
Aims: We aimed to determine the impact of the two *BCR-ABL1*subtypes on outcome of CML patients following frontline imatinib therapy.
Methods: *BCR-ABL1* transcript subtype was determined in 655 newly diagnosed CML-CP patients enrolled in Taiwan CML Study from Jun-2004 to March-2014. The frequencies of transcript subtypes were 62.4% (n=409) for b3a2, 34.5% (n=226) for b2a2, 1.8% (n=12) for both b3a2 and b2a2, and 1.3% (n=8) for other rare types. The *BCR-ABL1* levels expressed as International scale (IS) were measured in a central laboratory every 3 months following frontline imatinib 400mg/d. A comparison of the molecular responses and outcome of patients between b3a2 and b2a2 transcript subtypes was made.
Results: Of the 635 patients with b3a2 and b2a2 transcripts, there was no difference in age, gender, WBC count, circulating blast percentage, platelet count, or Sokal risk score between the two subtypes. At 3 months, *BCR-ABL1* IS ≤10% was achieved in 49% of b3a2 patients compared with 42% of b2a2 type (*P*=0.114). At 6 months, 19% of b3a2 patients had *BCR-ABL1* IS >10% compared with 37% of b2a2 (*P* <0.0001). At 12 months, 35% of b3a2 type had *BCR-ABL1* IS >1% compared with 46% of b2a2 patients (*P*=0.027). Patients with b2a2 transcripts had a slower rate of decline as compared with that of b3a2 transcripts over the time from 3 months to 7 years after imatinib treatment. The cumulative incidence of MMR at 2 years in b3a2 and b2a2 patients were 55.6% and 38.2%, respectively (*P* <0.0001); at 4 years was 71.2% for b3a2 and 59.2% for b2a2 (*P* <0.0001). The cumulative incidence of $MR^{4.5}$at 4 years was 28% and 26.7% for b3a2 and b2a2, respectively (*P*=0.323); at 6 years was 42.2% for b3a2 and 41.5% for b2a2 (*P*=0.323). The median time to MMR was not different between patients carrying the two transcript types, 14.4 months for b3a2 and 15.5 months for b2a2 (*P*=0.659). The median time to $MR^{4.5}$for b3a2 was 32.6 months and 34.1 months for b2a2 patients (*P*=0.994). With a median follow-up of 49.3 months, 30 (4.7%) patients had disease progression, accelerated phase (n=13) and blast crisis (n=17; 10 myeloid and 7 lymphoid). There was no difference in the occurrence of disease progression between the two transcript subtypes (3.7% for b3a2 and 6.6% for b2a2, *P*=0.117). Lymphoid-blast phase was highly associated with b2a2 transcript (6/7) compared to b3a2 type (1/7) (*P*=0.010). The overall survival at 10 years for b3a2 and b2a2 transcripts were 94.0% and 93.9%, respectively (*P*=0.635). The progression-free survival at 10 years was 92.7% for b3a2 and 87.9% for b2a2 patients (*P*=0.089).
Summary/Conclusions: Taiwan CML Study demonstrated that patients with b2a2 transcripts had significant higher failure rates at 6 and 12 months, a lower cumulative incidence of MMR by 2 and 4 years, a higher frequency of lymphoid crisis and a trend of inferior progression-free survival as compared to those with b3a2 patients.
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P610

THE INCIDENCE AND NATURAL HISTORY OF DASATINIB COMPLICATIONS IN THE TREATMENT OF CHRONIC MYELOID LEUKAEMIA

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Background: Dasatinib (DAS) has shown superiority over imatinib in achieving cytogenetic and molecular responses in chronic phase (CP) CML but with a different toxicity profile, which may impact on its overall benefit. Reported tox-

icities from the DASISION trial at 5 years include pleural effusions (PE) (29%), pulmonary hypertension (PHTN) (5%), and low rates of arterial ischemic events. While the literature well describes the incidence of these events, the response to therapy and the impact of subsequent dose modifications on the outcome of the toxicity have generally not been comprehensively characterised.
Aims: To review the incidence of these side-effects in a snapshot survey of Australian patients (pts) receiving DAS, either as first or subsequent line of therapy for CML-CP, with a focus on risk factors and the response of the toxicity to therapeutic changes.
Methods: Retrospective study of all eligible pts (received DAS for CML-CP and access to sufficient data) at 17 Australian institutions. Each pt's history was tracked in detail to identify any complications potentially attributable to DAS, and the treatment and outcome of these complications in response to cessation or alterations in DAS dose or schedule.
Results: 221 pts were evaluable, with a median age at DAS commencement of 53 years (range 20 – 86) and median starting dose of 100mg (20-140*)*. 51 (23%) received DAS as 1st line therapy, 133 (60%) 2nd line and 37 (17%) 3rd line. The median follow up from DAS commencement was 27 months (4 –116 months). No side effects were reported in 105 (48%) pts. Of the 116 (52%) pts with side-effects, the most common side effect was PE, observed in 53 (24%) pts with a median age of 65 years (41-86 years) and with median time to onset of 11 months (0.5-59 months). Most (40 pts, 76%) were receiving DAS as 2nd line therapy*.* DAS dose at PE onset was 100mg in 37 pts (70%), 140mg in 9 pts (17% PEs; notably 38% of pts on this dose developed PE), 70mg in 4 pts, 80mg in 2 pts and 50mg in 2 pts. The Figure 1 documents outcomes in pts with PE at 100mg dose with the key findings being a very high risk of recurrence in the absence of dose reduction and a substantial risk despite dose modifications. Evaluation of the CML status in these patients 6 months after changes in DAS dose and schedule is currently underway. Additional management of PE included diuretics (79%), thoracocentesis (43%), and steroids (47%). Only 1 of 7 pts given maintenance low dose steroids developed recurrence. Other side-effects included Grade 3-4 haemorrhage in 8 pts (4%), 3 of whom had platelets <50 $x10^9$/L and fatal intracerebral haemorrhage in a pt with a normal count. Ten (5%) pts had elevated pulmonary artery pressures meeting criteria for PHTN (RVSP >36mmHg) [median DAS dose 100mg (100-140)]; all described dyspnoea, although notably 8 had either concurrent PE or developed PE within 6 months. Of 5 PHTN pts with available follow up data, RVSP normalised in 4 post DAS cessation. The two episodes of peripheral vascular disease requiring intervention occurred in pts previously treated with nilotininb. There were no atypical infections apart from one pt with an empyema. Two pts had benign cervical lymphadenopathy. There were no deaths clearly attributable to DAS. Side-effects led to permanent DAS cessation in 51 pts (23%).

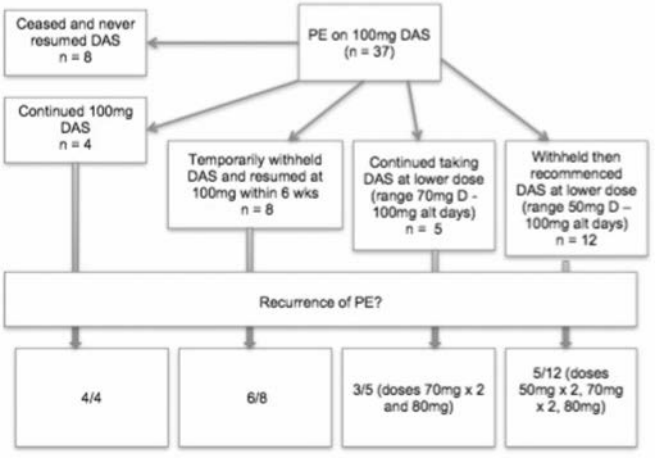

Figure 1.

Summary/Conclusions: The incidence of common DAS side effects (PE, PHTN) were similar to those seen in the DASISION study. Other side-effects were uncommon. Age and dose were risk factors for PE. In pts with PE on 100mg, recurrence was very high in the absence of dose reduction, and was still high despite reduction but appeared to be reduced by maintenance low dose steroids. These findings support the results of Porrini (ASH 2011) who found DAS at lower doses is effective in the elderly. With dosing appropriate for age, possibly supported by titration according to DAS levels and molecular response, severe side effects of DAS requiring permanent drug cessation are likely to be rare.

P611

THE HOCT1/ABCB1 DIPLOTYPE IS ASSOCIATED WITH COMPLETE CYTOGENETIC RESPONSE AND TOLERABILITY TO IMATINIB IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA

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Background: Drug transporters, such as hOCT1 (SLC22A1) and ABC members (ABCB1, ABCG2), have been already shown to play a role in both intracellular and systemic plasma concentrations of imatinib. In particular, our group showed that the hOCT1 c.480C>G polymorphism conditions higher rates of adverse events and shorter EFS. Analogously, the ABCB1 polymorphisms have been related to a worse prognosis, but the item is still debated.
Aims: Here we address imatinib pharmacodynamics through a mathematical analysis based on patient's pharmacogenetic data and gender. All analyses were conducted in the context of the TIKlet protocol (ClinicalTrials.gov code: NCT01860456).
Methods: Fifty-three CML patients receiving imatinib, 30 males and 23 females, were enrolled at the Hematology Unit of Pisa and at the Department of Internal Medicine of Orbassano (TO), Italy. The following polymorphisms were assessed: rs72552763 [MI420I], rs12208357 [c.181C>T] and rs683369 [c.480C>G] (hOCT1), rs1128503 [c.1236C>T], rs2032582 [c.2677G>T/A] and rs1045642 [c.3435C>T] (ABCB1), rs4149117 [c.334G>T] (SLCO1B3). Patients were grouped according to the absence (wild-type) or the presence (heterozygous or polymorphic homozygous) of at least one polymorphic allele.
Results: We found that the complete cytogenetic response was in strong relationship with combinations of hOCT1 [c.480C>G] and ABCB1 [c.3435C>T] polymorphisms. Indeed, genotypes identical for both loci (*i.e.*, wild/wild or polymorphic/polymorphic) exhibited a longer time to CCyR than those with mixed types (wild/polymorphic or polymorphic/wild), p-value=0.013. Since the drug tolerability has a strong incidence on the discontinuation of the therapy, we studied also whether the onset of toxicities was related to any of the considered variables. Interestingly, the same combinations of hOCT1 [c.480C>G] and ABCB1 [c.3435C>T] polymorphisms found related to efficacy were found associated with the maximum grade of toxicity as well, p-value=0.022. In particular, patients with mixed hOCT1 c.480C>G and ABCB1 c.3435C>T genotypes displayed higher levels of toxicity. As ancillary investigation we studied also the time to the onset of the main toxic effects and we found that the time of manifestation of the cramp toxicity was shorter in females than in males (5.3 *vs* 22.6 months) and the time to manifestation of the edema toxicity was associated with a combination between gender and ABCB1 c.3435C>T (2.5 *vs* 16.4 months).
Summary/Conclusions: To the best of our knowledge this study sheds a first light on the possible role of the combination between hOCT1 [c.480C>G] and [ABCB1 c.3435C>T] polymorphisms as predictor of both efficacy and toxicity of the imatinib treatment.

P612

REAL-LIFE COMPARISON OF DASATINIB AND NILOTINIB AS SECOND-LINE THERAPY AFTER IMATINIB FAILURE FOR CHRONIC PHASE CML

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Background: Use of 2nd generation tyrosine kinase inhibitors (2G-TKIs) dasatinib (DAS) and nilotinib (NIL) in chronic phase (CP) chronic myeloid leukemia (CML) patients failing imatinib (IM) result in around 50% of sustained cytogenetic response. However, it's unclear if there's a significant difference in efficacy of the two 2G-TKIs, especially in the long-term.
Aims: To compare efficacy of DAS and NIL in CP-CML patients after IM resistance or intolerance.
Methods: We retrospectively analysed 163 CP-CML patients resistant or intolerant to IM that received either DAS (n=95) or NIL (n=68) as second-line therapy.

We compared the characteristics of the two groups at the time of CML diagnosis and at the time of IM failure, including the cause of switch to 2G-TKI, duration of IM therapy, IM dose escalation and Hammersmith score to predict the probability of response to 2G-TKIs. Cytogenetic and molecular responses were evaluated according to the ELN recommendations. Time to treatment failure (TTF) was calculated from the start of 2G-TKI to any of the followings: progression to accelerated or blastic phase (ABP), death for any cause at any time, treatment discontinuation for primary or secondary resistance or intolerance. Progression free survival (PFS) was calculated from the start of 2G-TKI to ABP or death. Overall survival (OS) was calculated from the start of 2G-TKI to death.

Results: Considering CML characteristic at diagnosis, the DAS and NIL cohorts were comparable for age, sex and risk score (Sokal and EUTOS). Median duration of IM therapy was similar (DAS 19 months, NIL 14 months), but 27/95 patients (28%) had IM dose escalation before DAS compared to only 9/68 (13%) before NIL (p=0.03). There was a higher rate of switch to DAS than to NIL for secondary resistance (26/95, 27% *vs* 7/68, 10%; p=0.01) while more patients changed from IM to NIL due to intolerance (31/68, 46%, *vs* 21/95, 22% for DAS; p=0.002). Rates of primary resistance did not differ (47/95, 49% for DAS *vs* 28/68, 41% for NIL; p=0.37), as other causes of switch (1/95, 1% for DAS *vs* 2/68, 3% for NIL; p=0.77). Hammersmith score was almost identical in the two groups. Complete cytogenetic response (CCyR) was attained in 53/73 (73%) patients not in CCyR at the time of DAS start, compared to 31/48 (65%) patients not in CCyR at the time of NIL start (p=0.46). Mean time to attain CCyR was similar (7.1 months for DAS and 5.3 months for NIL; p=0.30). Major molecular response (MMR) was achieved in 55/89 (65%) patients not in MMR at the time of DAS start and in 39/61 (65%) patients not in MMR at the time of NIL start (p=0.82). Again, mean time to MMR was not different in the DAS e NIL cohorts (12.4 *vs* 8.5 months; p=0.14). With a median follow-up of 44 months (range 1–124), 5-year TTF was similar for DAS (65%, 95%CI 52-75%) and NIL (61%, 95%CI 43-74%; p=0.40) [Figure 1a]. Thirty-two of 95 patients (34%) stopped DAS due to toxicity (19/32, 59%), resistance (11/32, 31%) or other causes (3/32, 10%); 22/68 patients (32%) interrupted NIL for toxicity (11/22, 50%), resistance (8/22, 36%) or other causes (3/22, 14%), Probability of survival and progression were almost identical, with a 5-year PFS of 84% (95%CI 68-89%) for DAS and 92% (95%CI 79-97%) for NIL (p=0.27) [Figure 1b] and a 5-year OS of 89% (95%CI 78-95%) and 96% (95%CI 85-99%) (p=0.31), respectively.

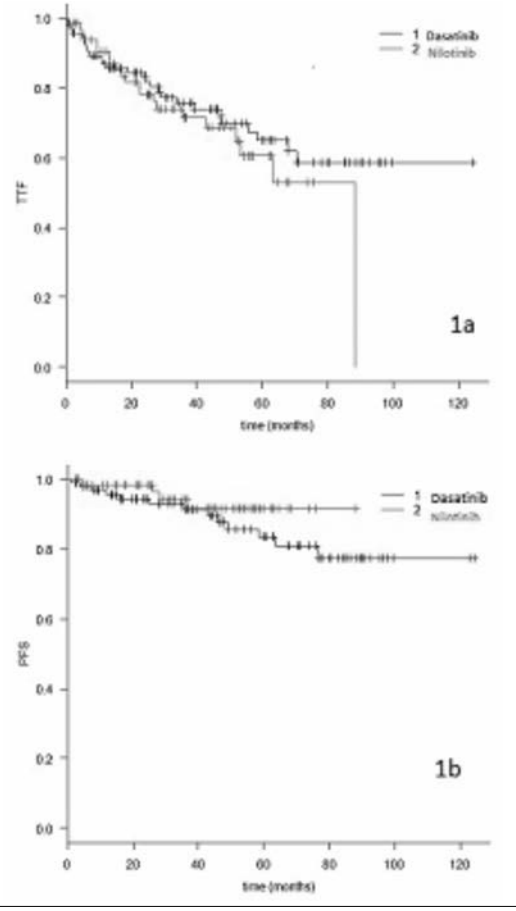

Figure 1.

Summary/Conclusions: With the limits of a retrospective analysis, our data suggest similar efficacy of DAS and NIL after IM failure in CP-CML, with high rates of cytogenetic and molecular responses and excellent long-term survival.

P613

FACTORS THAT INFLUENCE PATIENT WILLINGNESS TO ATTEMPT TREATMENT-FREE REMISSION IN CHRONIC MYELOID LEUKAEMIA

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Background: Most patients (pts) with chronic myeloid leukaemia (CML) on long-term tyrosine kinase inhibitor (TKI) treatment will achieve a deep molecular response (commonly defined as MR4.0; BCR-ABL ≤0.01%). Around half of these pts would remain in treatment-free remission (TFR) if their TKI were stopped after several years of stable MR4.0 or better. TFR is increasingly becoming a goal of treatment, yet little has been reported about pt perceptions of TFR.

Aims: To examine factors that influence expressed willingness to attempt TFR.

Methods: We developed a survey incorporating CML-specific factors (disease history, treatment toxicity, adherence) and questions relating to perceived susceptibility to relapse and consequences of relapse, benefits of and barriers to attempting TFR. Verbatim responses to an open-ended question regarding willingness to attempt TFR were grouped into themes. The survey incorporated two validated inventories: the Morisky Medication Adherence scale and the MDAnderson Symptom Inventory for CML TKI toxicity. CML pts in MR4.0 were eligible if they were taking any approved TKI. Pts were recruited from around Australia after receiving an invitation letter explaining the survey.

Results: There were 87 eligible respondents (Table 1). 82% of pts (95% CI 73-90%) indicated that they would be willing to cease TKI under close medical supervision if their doctor (Dr) thought it appropriate. On a scale of 0-100 (where 100 is extreme willingness) the median score was 69. No demographic or CML-related (disease duration, TKI) variable in the survey was significantly associated with the outcome variable. On average, respondents rated their TKI toxicity severity as 2.3 (SD: 1.7; Range: 0-7) on a 0-10 scale, (where 10 is very severe). This was positively correlated (r=0.76; p<0.01) with interference of TKI toxicity in daily life (mean 2.66). Increasing age was positively correlated with TKI toxicity severity (r=0.34; p<0.05), but not with interference of toxicity. Neither measure of TKI toxicity was significantly associated with willingness to stop TKI treatment. Stated treatment adherence was moderate (mean score 6.9/10 [SD: 1.1; Range: 3.5-8]). Adherence was positively correlated with age (r=0.27; p<0.05), but not with willingness to attempt TFR. Qualitative responses were related to willingness (59%); reluctance (34%); and ambivalence or conditional willingness (38%) to attempt TFR. The most frequently cited reasons to stop TKI included: TKI toxicity, actual (n=21) or potential (n=5); and confidence in the Dr (n=11) or molecular response (n=8). The leading reason not to stop was fear of disease progression or TKI resistance (n=7). Some patients believed that age or comorbidities made them unsuitable to attempt TFR; that TKI treatment was essential to prevent relapse or death; or cited negative experiences of friends stopping chemotherapy for other cancers. Conditional willingness was most often related to availability of medical supervision and testing (n=14) and probability of maintaining TFR (n=7).

Table 1. Patient characteristics.

Mean age (years)	56
Male:Female	38:49
Median BCR-ABL%	0
Current undetectable BCR-ABL	34
Current TKI	
Imatinib	56%
Nilotinib	26%
Dasatinib	17%
Ponatinib	1%
Median years since CML diagnosis	6
Median years on current TKI	3
Median MDASI-CML symptom score*	29

*0=no symptoms; 200=worst possible

Summary/Conclusions: Most respondents (82%) would be willing to stop their TKI with the support and supervision of their Dr. Although TKI toxicity was the commonest reason to attempt TFR, there was no correlation between symptom severity and willingness to stop TKI. This might indicate that even mild TKI toxicity is sufficient motivation for pts to attempt TFR. Potential future toxicity was also relevant to some patients. Reluctance to stop TKI was often associated with needs for additional information or misunderstanding of the current data. Understanding pt motivations and concerns is important if TFR is to be incorporated into the routine treatment pathway for CML.

P614

GENETIC VARIABILITY OF OXIDATIVE STRESS AND DNA REPAIR GENES POTENTIAL PROGNOSTIC AND THERAPEUTIC BIOMARKERS IN CHRONIC MYELOID LEUKEMIA

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Background: Oxidative stress (OS), resulting from an imbalance between Reactive Oxygen Species (ROS) production and antioxidant defenses, contributes to cell damage, apoptosis and ineffective hematopoiesis. Chronic myeloid leukemia (CML) is a clonal neoplastic disease associated with the reciprocal translocation t(9;22), encoding the *BCR-ABL1* oncogene. BCR-ABL protein induces, among other mechanisms, production of reactive oxygen species (ROS) by activation of the PI3K pathway, increase glucose metabolism and mitochondrial dysfunction. The antioxidant enzymes superoxide dismutases (SOD) and catalase (CAT), as well as DNA repair enzymes, such as OGG1, are important cell defense components against OS. Polymorphisms in genes that codify these enzymes may contribute to differences in susceptibility of individuals to oxidative damage, since it can lead to reduced protection against OS, influencing CML development and therapeutic response.
Aims: In the present study we investigate the influence of polymorphisms in genes related with oxidative stress (*CAT, GPX1, MPO, SOD1*, and *SOD2*) and DNA repair (*OGG1, NEIL1*, and *XRCC1*), and in the transcription factor *NFE2L2*, as a prognostic risk marker in CML patients [namely the impact in overall survival and tyrosine kinase inhibitors (TKIs) response]. Moreover, we also analyzed their participation in the development of mutations in *BCR-ABL1* gene.
Methods: This study enrolled 75 patients diagnosed with CML. The genetic polymorphisms of *CAT* (rs1001179), *GPX1* (rs1050450), *MPO* (rs2333227), *SOD1* (rs2070424), *SOD2* (rs4880), *OGG1* (rs1052133), *NEIL1* (rs5745920), *XRCC1* (rs1799782), and *NFE2L2* (rs13001694), were assessed by RFLP-PCR and Tetra-primer-ARMS-PCR. The oxidative stress (reactive oxygen species/total antioxidant status ratio) and DNA damage (8-hydroxy-2'-deoxyguanosine) were measured using commercial kits. The statistical analysis was carried out by variance analysis, $\chi 2$ test and Fisher exact test ($p<0.05$).
Results: Our results show that *SOD2* CC genotype influence the mutation status of *BCR-ABL1* gene (odds ratio 9.25x, I.C.95% 1.24-18.82; $p=0,007$), being the CC genotype also associated with higher oxidative stress levels. On the other hand, patients with *MPO* GG and AG genotypes have a high rate of suboptimal response to TKIs (odds ratio 4.92x, I.C.95% 1.24-9.10; $p=0,043$), and these genotypes were also associated with higher oxidative stress levels. Moreover, the overall survival of CML patients can be influenced by *NEIL1* [CML patients with CT genotype had lower survival (166±5 months) than patients with CC and TT genotypes (204±6 months; $p=0.041$)] and *NFE2L2* [CML patients with TT genotype had lower survival (88±7 months) than patients with CT and TT genotypes (216±8 months; $p=0.003$)].
Summary/Conclusions: Our results show that genetic polymorphisms in oxidative stress and DNA repair related genes modulate oxidative stress and DNA damage levels in CML patients. Moreover, these polymorphisms influence the prognosis of CML patients, their response to TKI treatment and the development of *BCR-ABL1* mutations, which suggest their potential as new prognostic and therapeutic biomarkers.
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P615

LANDMARK ANALYSES IN THE PIVOTAL PONATINIB PACE TRIAL: IMPACT OF EARLY RESPONSES ON 3-YEAR OUTCOMES IN HEAVILY PRETREATED CP-CML PATIENTS

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Background: Ponatinib is a potent oral tyrosine kinase inhibitor (TKI) active against native and mutant forms of BCR-ABL1. It is approved for patients with refractory CML or Ph+ALL, or with the T315I mutation. Responses at early landmark time points have been correlated with positive long-term outcomes in patients treated with other TKIs in the first- and second-line settings.
Aims: Landmark analyses are limited for populations treated with multiple prior TKIs. Here, we evaluate the impact of achieving early landmark responses with ponatinib on 3-year outcomes in heavily pretreated patients in the ongoing Phase 2 PACE trial (NCT01207440).
Methods: Molecular responses (International Scale; BCR-ABL ≤0.1% [major molecular response (MMR)], >0.1–1%, >1%–10% and >10%) and cytogenetic responses (Ph+ metaphases ≤35% [major cytogenetic response (MCyR)]) at 3, 6 and 12 months and their association with outcomes at 3 years past landmark were examined in a post hoc analysis of heavily pretreated patients with chronic-phase (CP)-CML (n=267). *P* values were calculated using the log-rank test. Data cutoff date was 3 August, 2015. All patients gave informed consent.
Results: Median time from diagnosis was 7 years (range 0.5-27) and median age was 60 years (range 18-94); 61% of patients had received ≥3 prior TKIs. At 3, 6, and 12 months, 48%, 62% and 71% of patients, respectively, achieved MCyR, and 14%, 29% and 39%, respectively, achieved MMR, among patients evaluable at each landmark. Greater reductions in BCR-ABL1 transcript levels at most landmark time points were associated with improved PFS and OS at 3 years (Table 1), as was MCyR. Deeper responses at all landmark time points were also associated with the achievement of MR4.5 over time.

Table 1. Estimated PFS and OS at 3 years past landmark by BCR-ABL1 level.

Landmark/Response		n	PFS	*p-value	n	OS	*p-value
3 mo	BCR-ABL1 ≤0.1%	32	97%	—	33	97%	—
	BCR-ABL1 >0.1–1%	47	70%	.39	48	85%	.60
	BCR-ABL1 >1%–10%	51	61%	.0080	55	81%	.13
	BCR-ABL1 >10%	82	54%	.0003	94	78%	.16
			overall:	0.0013		overall:	0.34
6 mo	BCR-ABL1 ≤0.1%	58	91%	—	61	93%	—
	BCR-ABL1 >0.1–1%	42	60%	.0023	44	83%	.036
	BCR-ABL1 >1%–10%	30	61%	<.0001	32	90%	.33
	BCR-ABL1 >10%	57	53%	<.0001	74	78%	.0076
			overall:	0.0001		overall:	0.029
12 mo	BCR-ABL1 ≤0.1%	63	91%	—	63	97%	—
	BCR-ABL1 >0.1–1%	26	74%	.011	27	85%	.039
	BCR-ABL1 >1%–10%	19	70%	.0041	22	96%	.18
	BCR-ABL1 >10%	41	56%	<.0001	50	80%	.0020
			overall:	0.0001		overall:	0.012

*Calculated across the entire post-landmark timespan and unadjusted for multiple comparisons

Summary/Conclusions: Deep, early reductions in BCR-ABL1 transcripts were positively associated with long-term survival in this refractory population. These data support the prognostic value of achieving early landmark cytogenetic and molecular responses with ponatinib in heavily pretreated patients.

P616

BCR-ABL1 TRANSCRIPT LEVEL ON DAY+28 CAN PREDICT EARLY MOLECULAR RESPONSE AT 3 MONTHS AND MAJOR MOLECULAR RESPONSE AT 12 MONTHS IN CHRONIC MYELOID LEUKEMIA PATIENTS WHO TREATED WITH FRONTLINE DASATINIB

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Background: In BCR-ABL1 tyrosine kinase inhibitor (TKI) treated chronic phase chronic myeloid leukemia (CP-CML), early molecular response (EMR) at 3 month is currently identified as being one of the most important prognostic factors. Sokal risk score and dose intensityduring first 3 months were strongly associated with achievement of EMR. As dasatinib is a novel, oral tyrosine kinase inhibitor with improved potency, identification of very early molecular response (VEMR) would be beneficial.

Aims: We evaluated the possibility of the VEMR at I month predicting outcomes in newly diagnosed CP-CML patients treated with dasatinib.
Methods: In this multi-center, observational, open-label study, 102 patients with CP-CML were enrolled to receive dasatinib at a dose of 100 mg once daily. The primary end point was complete molecular response (CMR) by 18 months. Secondary end points including molecular response (MR) by 1, 3, 6, 12, 18, 24, 36 month, time to and duration of MMR and CMR, bcr-abl kinetics by digital PCR, and safety were tested. A receiver operating characteristic (ROC) curve from molecular data on Day+28 was calculated to predict EMR and MMR at specific timepoints.
Results: Median age was 49 years (19-81 years) and 61 patients were male. With median follow-up duration of 17 months (0.9-27.2 months), 92 (90.1%) out of 102 patients were still on dasatinib treatment and 10 patients discontinued due to disease progression or treatment failure in 3 patients or adverse events in 4 patients or other causes. The cumulative MMR at 12 months was 65.2%. The cut-off value of BCR-ABL1 transcript on Day+28 calculated by ROC curve was 40%. Among 92 patients who have available molecular data of both D+28 and 12 months, fifty nine (64.1%) patients had less than 40% of BCR-ABL1 transcript (VEMR) on Day+28. In 59 patients who had VEMR, 50 (84.7%) patients achieved MMR at 12 months. However, only 30.3 (10 out of 33 patients) % of patients with no VEMR achieved MMR at 12 month. More detailed data will be updated at presentation.
Summary/Conclusions: Our study shows that VEMR at I month can be an important predictor for further molecular responses as well as long-term outcome. Therefore it would be helpful to monitor BCR-ABL1 transcript level at 1 month in patients who treated with more potent TKIs.

P617

THE IMPACT OF EARLY MOLECULAR RESPONSE ON LONG-TERM OUTCOMES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) TREATED WITH DASATINIB OR IMATINIB FROM THE DASISION TRIAL

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Background: An exploratory, landmark analysis of the randomized phase 3 DASISION trial (NCT NCT00481247) demonstrated improved long-term outcomes (progression-free survival [PFS] and overall survival [OS]) in newly diagnosed CML-CP patients treated with dasatinib or imatinib who achieved *BCR-ABL1* ≤10% *vs* >10% at 3 months. The early molecular milestone of *BCR-ABL1* ≤10% at 3 months was achieved by a higher proportion of patients on dasatinib (84%) compared with imatinib (64%).
Aims: To evaluate the effect of EURO (Hasford) risk score on the achievement of *BCR-ABL1* ≤10% at 3 months and to evaluate long-term efficacy (PFS, OS, major molecular response [MMR], and $MR^{4.5}$) in treatment-naïve CML-CP patients from DASISION. A multivariate analysis was performed to explore the relationship between the survival of a patient and several explanatory variables including treatment, gender, ECOG performance status, race, age, smoking status, and geographical region.
Methods: In DASISION, patients received dasatinib 100 mg once daily (n=259) or imatinib 400 mg once daily (n=260). *BCR-ABL1* transcript levels were measured on the International Scale. Patients with a EURO score ≤780 were considered low risk, >780 to ≤1480 intermediate risk, and >1480 high risk.
Results: Most patients in DASISION were at intermediate risk according to EURO score (124 [48%] on dasatinib and 123 [47%] on imatinib); 86 patients on dasatinib and 87 patients on imatinib (33% each arm) were at low risk, and 49 patients on dasatinib and 50 patients on imatinib (19% each arm) were at high risk. A higher percentage of patients in the dasatinib arm achieved *BCR-ABL1* ≤10% at 3 months *vs* the imatinib arm in all risk groups (low: 91% *vs* 73%; intermediate: 80% *vs* 66%; high: 83% *vs* 44%). The largest difference was observed between those at high risk: 17% of high-risk patients on dasatinib did not achieve *BCR-ABL1* ≤10% at 3 months *vs* 56% on imatinib. Within each EURO risk score group, a higher proportion of patients on dasatinib *vs* imatinib had *BCR-ABL1* ≤1% at 3 months (low: 56%, 95% confidence interval [45-68%] *vs* 20% [12-30%]; intermediate: 48% [38-57%] *vs* 12% [7-20%]; high: 33% [20-48%] *vs* 5% [1-16%]). Five-year PFS, OS, MMR, and $MR^{4.5}$ rates were higher for patients with *BCR-ABL1* ≤10% *vs* >10% at 3 months in all risk groups (Table 1). Based on multivariate analysis of OS at 5 years, women were found to be at a lower risk of death than men (hazard ratio: 0.38, 95% confidence interval: 0.19, 0.75). No association was found between survival and other explanatory variables.
Summary/Conclusions: In all EURO risk score groups, a higher percentage of patients on dasatinib achieved *BCR-ABL1* ≤10% at 3 months than patients on imatinib, with the most striking difference in high-risk patients. The proportion of patients achieving *BCR-ABL1* ≤1% at 3 months was higher with dasatinib *vs* imatinib, and the 95% confidence intervals for the two treatment arms did not overlap. Long-term outcomes were superior for patients with *BCR-ABL1* ≤10% at 3 months across all risk groups. These analyses suggest that dasatinib should continue to be considered as a first-line therapy for CML-CP patients, irrespective of EURO risk score.

Table 1.

		DASATINIB		IMATINIB	
		BCR-ABL1 at 3 months			
Risk category	5-year rates, % (95% confidence interval)	≤ 10%	> 10%	≤ 10%	> 10%
Low risk	PFS	94 (86-98)	86 (42-100)	95 (86-99)	91 (71-99)
Low risk	OS	97 (90-100)	86 (42-100)	97 (89-100)	100 (85-100)
Low risk	MMR	94 (86-98)	71 (29-96)	82 (70-91)	41 (21-64)
Low risk	$MR^{4.5}$	61 (48-72)	14 (<1-58)	53 (40-66)	23 (8-45)
Intermediate/ high risk	PFS	91 (85-96)	80 (61-92)	93 (85-97)	79 (67-89)
Intermediate/ high risk	OS	95 (90-98)	87 (69-96)	96 (90-99)	86 (75-93)
Intermediate/ high risk	MMR	84 (76-90)	30 (15-49)	80 (70-87)	41 (29-54)
Intermediate/ high risk	$MR^{4.5}$	50 (41-59)	3 (<1-17)	45 (34-55)	8 (3-18)

LB618

RESULTS FROM ENESTFREEDOM: TREATMENT-FREE REMISSION (TFR) FOLLOWING FRONTLINE NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP)

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Background: Proof of concept for TFR in CML-CP was demonstrated in the STIM trial, which enrolled pts with sustained deep molecular response (MR) on long-term imatinib (IM; median duration of 59 mo); ≈ 40% of pts enrolled in STIM maintained TFR (no loss of deep MR) after stopping IM. Similar results were observed in subsequent tyrosine kinase inhibitor (TKI) TFR studies, although differences in design (eg, pt population, monitoring requirements, criteria for reinitiation of therapy) preclude direct comparison of results across studies. Several studies have shown an association between maintaining TFR and longer TKI treatment duration.
Aims: To present results from the single-arm phase 2 ENESTfreedom study (NCT01784068) evaluating the ability to stop NIL and maintain TFR in pts who achieved a sustained deep MR on frontline NIL.
Methods: Eligible pts had CML-CP, typical b2a2 and/or b3a2 *BCR-ABL1* transcripts, and ≥ 2 y frontline NIL, and had achieved $MR^{4.5}$ (*BCR-ABL1* ≤ 0.0032% on the International Scale [IS]) on treatment. All pts provided informed consent. On study, pts continued NIL for 1 y (consolidation phase; RQ-PCR assessments every 12 wk); pts meeting response criteria during the consolidation phase (no assessment worse than MR^4 [*BCR-ABL1*^{IS}≤ 0.01%], ≤ 2 assessments between MR^4 and $MR^{4.5}$, and $MR^{4.5}$ in the last assessment) were eligible to stop NIL (TFR phase; RQ-PCR assessments every 4 wk for the first 48 wk). NIL reinitiation (ReRx phase) was triggered by loss of major MR (MMR [*BCR-ABL1*^{IS} ≤ 0.1%]). This analysis was conducted when all pts who entered the TFR phase had completed 48 wk of TFR, entered the ReRx phase, or discontinued from the study (data cutoff, 30 Nov 2015).
Results: A total of 215 pts were enrolled and entered the consolidation phase; 190 of these pts stopped NIL and entered the TFR phase. Median age at enrollment was 55 y, and median NIL duration prior to TFR was 43 mo (range, 33-89 mo). At wk 48 of the TFR phase, 51.6% (95% CI, 44.2-58.9%) of 190 pts remained in MMR without reinitiation of treatment (primary endpoint), and 47.4% (95% CI, 40.1-54.7%) were in $MR^{4.5}$ without reinitiation of treatment. A total of 86 pts lost MMR and reinitiated NIL. Of these 86 pts, 85 pts regained MMR, and 76 pts regained $MR^{4.5}$(1 pt discontinued from the study without regaining MMR [pt decision] 7.1 wk after entering the ReRx phase). The median time to regain MMR was 7.9 wk. No new safety signals were observed with NIL treatment. Fewer pts developed adverse events (AEs) in the TFR phase (65.8%) *vs* the consolidation phase (83.2%). Myalgia has been reported as part of an IM withdrawal syndrome in prior studies. Here, in the first 48 wk of the TFR phase, 47 pts (24.7%; grade 3/4 in 1.1%) stopping NIL reported AEs

in the musculoskeletal pain grouping (defined as musculoskeletal pain, myalgia, pain in extremity, arthralgia, bone pain, and/or spinal pain), of whom 32 had no previous such events. Most of these events occurred early in the TFR phase (39 pts had an event within the first 24 wk; median duration, 24.0 wk).
Summary/Conclusions: In ENESTfreedom, over 50% of pts remained in TFR 48 wk after stopping NIL, a clinically meaningful rate for pts with a relatively short duration of NIL therapy (≈ 3.6 y). Among pts who did not remain in TFR, all who remained on study regained MMR, and most regained $MR^{4.5}$ with NIL reinitiation.

Myelodysplastic syndromes - Clinical 2

P618

IMPACT OF AZACITIDINE THERAPY ON OVERALL SURVIVAL OF NEWLY DIAGNOSED PATIENTS WITH HIGH-RISK MYELODYSPLASTIC SYNDROMES: A POST HOC ANALYSIS OF THE ERASME STUDY

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Background: Treatment of myelodysplastic syndromes (MDS) has undergone a sweeping change in recent years following the emergence of demethylating agents such as azacitidine (AZA). However, recently published data establish a clear need for population-based studies to confirm the findings of clinical trials in the real-life setting.
Aims: To evaluate the use and outcomes of AZA in higher-risk MDS (HR MDS; International Prognostic Scoring System [IPSS] Int-2/High-risk) patients (pts) in a real-life setting.
Methods: The ERASME study is an observational, prospective study of pts with MDS (WHO 2008 classification). This sub-analysis included only HR MDS pts who had been chosen for one of the following management therapies at time of diagnosis: active therapy with AZA or allogeneic hematopoietic cell transplant preceded by AZA (AZA-HCT). The presence of therapy-related MDS and cytogenetic abnormalities was taken into consideration. Overall survival (OS) of these pts was compared against best supportive care using the Kaplan-Meier and time-dependent methods.
Results: A total of 150 pts with HR MDS were recruited between January 2013 and August 2015. 113 pts (median age 73.0 years [yrs; interquartile range 66.0-77.0]; 52% male) were treated with AZA and 37 pts (median age 81.0 yrs [75.0-85.0]; 57% male) with best supportive care (considered only for survival analysis). Therapy-related MDS was detected in 19% of cases. IPSS score was Int-2 in 51% of pts and High-risk in 49%. Prognostic cytogenetics was classified as good in 35% of pts, intermediate in 4%, poor in 22%, and very poor in 27% (14% were unclassified due to missing metaphase data). The first therapy administered was AZA for 84 (74%) pts and AZA-HCT for 29 (26%) pts. The main reasons for treatment strategy selection were risk classification (99%), age (80%), symptomatology (58%), and comorbidities (52%). The median number of AZA cycles was 4.0 (1.0-7.0), with 41 pts (36%) receiving ≥6 cycles. Most pts were treated on a 7-day regimen (n=91 [81%]; missing=14%) and a dose of 75 mg/m^2 (n=71 [63%]; missing=2%). The most common adverse events (AEs) were asthenia in 20 pts, pyrexia in 18, constipation in 14, febrile neutropenia in 12, and neutropenia in 10. AEs led to dose reductions in only 8% of cases. Overall, median OS for pts treated with AZA on the 7-day regimen at the 75 mg dose *vs* pts treated with other regimens was 19.4 months (mos; 12.8-23.2) *vs* 14.9 mos (11.8-18.6) (*P*=not significant [ns]), respectively. Observed OS in pts treated for ≥6 cycles *vs* those treated for <6 cycles was 20.3 mos (14.2-23.2) *vs* 14.9 mos (6.6-not reached [NR]) (*P*=0.05). OS in pts who did not have comorbidities *vs* those who did was 20.3 (7.4-NR) *vs* 16.6 (12.8-22.2) (*P*=ns). At last study follow-up (36 months), 53 pts (47%) had died after a median of 7.4 mos (4.2-13.7): 39 (46%) and 14 (48%) pts on AZA and AZA-HCT, respectively. Median OS in HR MDS pts treated with AZA was higher than in HR MDS pts receiving supportive care: 16.6 mos (13.1–21.7) *vs* 8.4 mos (5.7-13.0) (*P* <0.01). Median time-dependent OS was also higher with AZA than with supportive care: 14.8 mos (12.8-18.7) *vs* 5.0 mos (2.5-11.6) (*P* <0.01).
Summary/Conclusions: The findings from this post hoc subanalysis of the prospective population-based study show that treatment with AZA prolongs OS in pts with HR MDS in a real-life setting when compared with supportive care. The results support the hypothesis that OS could be compromised with significantly more baseline comorbidities or reduced total dose recommended of AZA.

P619

IMPACT OF BONE MARROW FIBROSIS IN RESPONSE TO AZACITIDINE IN 94 PATIENTS WITH MYELODISPLASTIC SYNDROMES (MDS), CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML) AND ACUTE MYELOID LEUKEMIA

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Background: Azacitidine (AZA) is effective in high risk MDS, CMML-2 and low blast count AML patients not suitable for more intensive treatment. Factors that may influence response to AZA are still under investigation. Bone marrow fibrosis is a potentially negative prognostic marker on overall survival (OS), but its clinical significance in this setting of patients remains to be clarified.
Aims: To evaluate efficacy and clinical predictors of OS and overall response rate (ORR) to AZA in a real life patient cohort.
Methods: We retrospectively evaluated 94 consecutive patients treated at two Institutions from June 2009 till February 2016 with AZA subcutaneously (5+2+2 schedule) every 28 days, outside clinical trials. We analyzed data from routine laboratory analysis, bone marrow histology, morphology and cytogenetics at diagnosis. Statistical analysis was performed using Student' t test for continuous variables, and chi-square test for categorical ones. OS was measured from the starting of AZA treatment. Median age at diagnosis was 72.5 years (range 39-90); male to female ratio was 1.7 (58/34). The initial diagnosis according to WHO 2008 classification were low blast count AML in 15 cases (15.9%), AREB2 in 40 (42.6%), AREB1 in 25 (26.6%) CMML in 5 (5%) and RCUD/RCMD/MDS NOS in 9 (10%). The International Prognostic Scoring System (IPSS) risk for all MDS patients was int-2 (N=60) or high (N=20) at the onset of AZA therapy. Median number of cycles administered was 7 (range 1-44).
Results: Seventy-nine patients (84%) receiving ≥4 cycles of therapy were available for response evaluation according to International Working Group 2006 criteria. After a median of 6 cycles (4-13), ORR was 47.8% (CR 23.1%, PR 14.1% and SD with hematologic improvement 10.3%), SD was 23.1%, and PD 29.5%. Median OS from the beginning of therapy was 19.9 months (range 4-75 months). AREB-1 and AREB-2 presented a lower OS (11.4 and 19 months) than AML (22 months) and CMML/RCUD/RCMD/MDS NAS (42 months). As expected, a statistically significant difference in median OS was observed between int-2 and high risk (31 *versus* 15 months respectively, p<0.005). In univariate analysis monocytosis, severe neutropenia, peripheral and marrow blasts percentage, and bone marrow cellularity did not influenced ORR. IPSS cytogenetic risk category (43 favourable, 10 intermediate, 41 poor) did not influence ORR, but it had an impact on OS that is significantly lower in poor than in intermediate risk (median OS 12 *vs* 26 months, p=0.05). OS in low risk cytogenetic category was 31 months. As regards the grade of marrow fibrosis, patients with MF-0 had significantly lower PD rate than those with any grade of fibrosis (25% *vs* 52%, p=0.01). Moreover, ORR was higher in patients with MF-0 *versus* the others (48% *vs* 40%), although not significantly (Figure 1). In our study, patients with any grade of fibrosis had worse median OS then patients with no fibrosis (15 months *vs* 26 months).

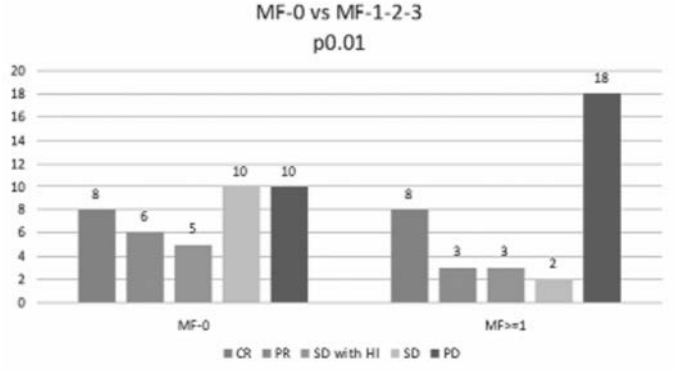

Figure 1.

Summary/Conclusions: Our results confirm effectiveness of AZA in patients with high risk MDS and AML with low marrow blast counts. To the best of our knowledge, this is the first report showing that bone marrow fibrosis negatively impacts response to AZA therapy. Further studies are needed to clarify the clinical significance of marrow fibrosis and its impact on OS and ORR among patients treated with hypomethylating agents.

P620

AGE DISTRIBUTION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) IN GERMANY AND THEIR PROGNOSIS ACCORDING TO THE INTERNATIONAL PROGNOSTIC SCORING SYSTEM (IPSS): DATA FROM THE REGULAR CARE MDS REGISTRY

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Background: The IPSS and the revised-IPSS were generated with data from 816 and 7,019 patients (pts) of academic centers with a median age of 69 and 71 years (y), respectively. The IPSS should be applied to all MDS-pts to assess prognosis and define the optimal therapeutic strategy.
Aims: The aim of the current evaluation of the regular care MDS-registry was to describe the age distribution of MDS in regular care and the meaning of the IPSS in various classes of age.
Methods: Pts were eligible if a bone marrow (BM) biopsy was performed and pts have given written informed consent. Basic data and the quarterly course of the disease were documented in an online registry. Statistical analysis: Depending on the distributions of each variable frequency tables, means (SD), medians were calculated overall and stratified by age classes. Corresponding overall tests were performed (Chisquare, Kruskal-Wallis). Furthermore, survival was estimated by IPSS and age stratified Kaplan-Meier curves and compared using a bivariate Cox regression model.
Results: Between July 2009 and March 2015 (69 months) 1689 pts from 85 institutions mainly outpatient practices, were recruited and eligible. Median age of 681 (40.3%) women and 1008 (59.7%) men were 74.8y (min-max: 26.5-94.2). The duration of observation, frequencies of IPSS, need for transfusions (Tx) prior to diagnosis (d), comorbidities and the Charlson comorbidity index (CCI) are given in the Table 1. Higher age and IPSS risk were significantly associated with the risk of death (Figure 1).

Table 1. Age distribution and MDS parameter.

Item	All	- 69y	70-74y	75-79y	80y +	p value
N(%)	1689(100)	451(26.7)	410(24.3)	420(24.9)	408(24.2)	
Mean months ofobservation (mean (SD))	24.55(18.86)	29.24(20.45)	24.86(18.71)	23.82(18.15)	19.78(16.58)	p<0.0001
Low risk N (%)[a]	387(33.1)	103(31.9)	93(30.9)	98(34.3)	93(35.9)	p=0.214
Int 1 N (%)[a]	465(39.8)	138(42.7)	121(40.2)	101(35.3)	105(40.5)	
Int 2 N (%)[a]	226(19.3)	51(15.8)	68(22.6)	62(21.7)	45(17.4)	
High risk N (%)[a]	91(7.8)	31(9.6)	19(6.3)	25(8.7)	16(6.2)	
Tx prior d N (%)[b]	505(32.6)	111(26.6)	121(32.6)	120(31.0)	153(41.2)	p=0.0002
CCI >0 (%)	900(53.3%)	206(45.7)	195(47.6)	243(57.9)	256(62.8)	p<0.0001
CCI restricted to score >0[c] (mean (SD))	2.256(1.519)	2.262(1.739)	2.210(1.540)	2.115(1.401)	2.418(1.412)	p=0.0065
Deceased N (%)	564(33.4)	121(26.8)	126(30.7)	145(34.5)	172(42.2)	

Missing values: [a]520, [b]142, [c]789 pts without CIRS-relevant comorbidity.

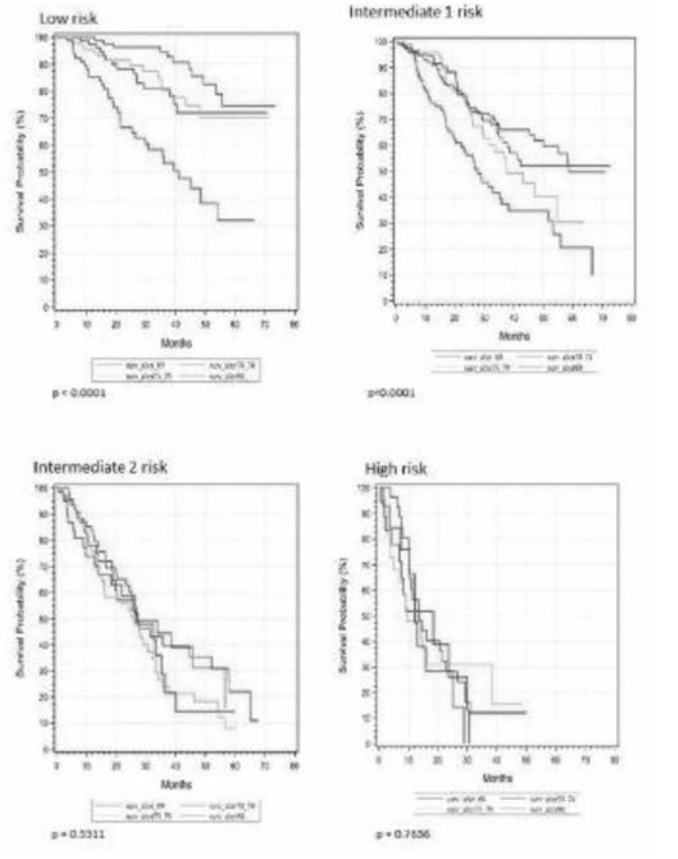

Figure 1. Survival stratified in IPSS and age (classified <=69, 70-74, 75-79, >=80).

Summary/Conclusions: The median age of MDS pts in the German regular care registry is higher than that of the cohorts, who generated the IPSS. Further analysis will clarify the impact of the enhanced rate of comorbidities and need for transfusion in the 80y+ cohort related to the survival in the low and Int-1 IPSS subgroups.
The MDS-registry is supported by an unrestricted grant from Celgene and Novartis.

P621

VALIDATION OF PROGNOSTIC SCORING SYSTEMS FOR MYELODYSPLASTIC SYNDROMES IN THE SWEDISH MDS-REGISTER

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Background: Since the survival in patients diagnosed with MDS varies from months to decades, prognostic models are important tools for estimating prognosis and making therapy-related decisions. Several scoring systems for risk stratification have been developed including the International Prognostic Scoring System (IPSS), the Revised IPSS (IPSS-R) and the World Health Organization (WHO) Classification-based Prognostic Scoring System (WPSS). The nationwide Swedish MDS-register was started in 2009 and includes newly diagnosed patients with Myelodysplastic syndrome (MDS) and disorders with combined features of myelodysplastic and myeloproliferative neoplasms (MDS/MPN). All hospitals in Sweden diagnosing these patients report to the MDS-register, including university hospitals, county and local hospitals. The completeness against the compulsory Swedish Cancer Register was 95% for the period under study (2009-2013).
Aims: The aim of this study was to validate and compare IPSS, IPSS-R and WPSS in a nationwide population-based setting. Furthermore, we present data on incidence, clinical characteristics at diagnosis and clinical outcomes such as overall survival and AML-transformation.
Methods: All patients aged 16 years or older diagnosed with MDS 2009-2013 and reported to the MDS-register were included (n=1334). Patients with MDS/MPN were excluded (n=291). Data included date of diagnosis, age, gender, WHO-diagnosis, laboratory parameters, transfusions-dependency, diagnostic procedures including information on bone marrow morphology and cytogenetics and information on previous treatment with chemotherapy or irradiation. By means of record linkage, information was obtained from the Swedish Cause of Death Register and the Swedish AML-register up to December 31, 2014 to calculate overall survival (OS) and transformation to AML.
Overall and AML-free survival was assessed using the Kaplan-Meier method. Prognostic scores were calculated for IPSS, IPSS-R and WPSS, respectively. The prognostic methods were evaluated using Harrell's concordance (C) index. The indices were internally validated by bootstrapping with 1000 samples.
Results: The crude yearly incidence of MDS was 3.5 per 100.000 persons. Median age at diagnosis was 76 years and 58% of the patients were male. At time of diagnosis, 49% of patients were dependent on red blood cell transfusions. The median OS for the whole population was 2.7 years and time to 25% AML-transformation was 3.8 years. For 848 (64%) patients data were complete, enabling risk score assessment for all three systems. The major part of missing data was attributed to that karyotyping was not performed, mainly in the older population. Compared to previous register-based studies we found that a larger proportion of patients had higher risk disease. Risk category in the different scoring systems, OS and time to 25% AML-transformation are presented in Table 1. Comparisons between the scoring systems show that all three had good prognostic power. However, IPSS-R and WPSS had significantly better prognostic properties compared to IPSS.

Table 1. Clinical outcome and discriminative power on the WPSS, IPSS and IPSS-R based risk categories.

	Total, N (%)	Overall survival		AML-risk	
	848 (100)	Median	C-index +	AML 25% *	C-index +
WPSS			0.72		0.79
Very low risk	108 (13)	NR		-	
Low risk	205 (24)	65.6		NR	
Intermediate risk	157 (19)	36.0		NR	
High risk	246 (29)	19.9		16.3	
Very high risk	132 (16)	9.8		9.7	
IPSS			0.71		0.78
Low risk	254 (30)	67.2		NR	
Interm. risk I	320 (38)	31.3		42.4	
Interm. risk II	202 (24)	13.5		13.3	
High risk	72 (8)	10.8		7.4	
IPSS-R			0.73		0.79
Very low risk	113 (13)	NR		NR	
Low risk	280 (33)	57.7		NR	
Interm. risk	168 (20)	30.2		31.7	
High risk	138 (16)	17.1		15.9	
Very high risk	149 (18)	9.5		9.0	

* Number of months after diagnosis when 25% of patients had developed AML.
+ The C-index measures the discriminative power of a score, ranging between 0.5 (no discriminative power) and 1 (perfect discrimination).
NR = Not reached

Summary/Conclusions: To our knowledge, this is the first nationwide population based validation of IPSS, IPSS-R and WPSS. While our findings confirm the prognostic power of all three systems, IPSS-R and WPSS appear to be slightly more powerful tools for prognostication than IPSS.

P622

EXPRESSION OF TUBB GENE IN MYELODYSPLASTIC SYNDROME WITH OR WITHOUT EVOLUTION TO LEUKEMIA

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Background: Myelodysplastic syndromes (MDS) are a heterogenous group of clonal hematopoietic stem cell disorders characterized by increased risk of leukemic transformation.Patients evolving into leukemia respond poorly to chemotherapy and die within a short time. Hence, it is very important to find detectable marker to predict the probability of leukemic transformation once MDS has been diagnosed. In our previous study, we establish a nested case-control study cohort in 435 MDS patients. With whole-genome expression chip, we analyzed the different gene expression profile of paired MDS patients. A subset of 6 genes was found to distinguish the leukemia group (MDS patients with evolution to leukemia) and control group (MDS patients without evolution to leukemia). These 6 genes were TUBB, PSMD1, SLC7A5, ATG3, TUBB2C and TIMM10.
Aims: Based on our previous study on feature genes related to leukemic evolution of myelodysplastic syndrome (MDS), we investigated the differential expression of TUBB gene in MDS patients with leukemic evolution and that without evolution. Also, we studied the effect of down-regulation of TUBB gene on SKM-1 cell line.
Methods: Based on our nested case-control study cohort of MDS patients, we chose paired patients in 1:1 ratio, according to age, gender, WHO subtype, IPSS cytogenetic subgroup and follow-up period (≥1 year). We examined TUBB gene expression changes in paired patients from case group (with evolution) and control group (without evolution), using quantitative real time PCR. We used small interfering RNA to down-regulateTUBB gene expression in SKM-1 cell line. The effect of TUBB-siRNA on the growth rate,colony formation percentage and the ultrastructure of SKM-1 were tested by CCk-8 kit, flow cytometry, soft agar and transmission electron microscope, respectively.
Results: We chose 11 patients in each group of our MDS nested case-control study cohort excluding patients underwent gene expression microarray test. TUBB gene expression of MDS patients in case group were significantly higher than that in control group (P<0.01).Satisfactory transfection efficiency (>80%) could be obtained withlipofectamine. Real time PCR showed the down-regulated expression of TUBB after transfection (P<0.05). Growth inhibition of SKM-1 cell was obvious 24 hours after TUBB-siRNA transfection (P<0.05). The cell-cycle analysis by flowcytometershowed the percentage of SKM-1 before transfection in S phase was higher than that after transfection. Colony formation percentage of SKM-1 was inhibited obviously with TUBB-siRNA transfection (P<0.01). The ultrastructure of SKM-1 cell after transfection exhibit several features, such as vacuole in cytoplasm and nuclear fragmentation (Figure 1).

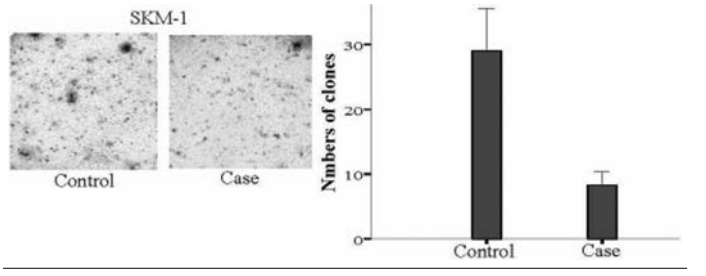

Figure 1.

Summary/Conclusions: TUBB gene expression was significantly higher in case group than that in control group of our MDS nested case-control study cohort. Growth and colony formation inhibition are the result of siRNA against TUBB.Our results indicate that TUBB gene may play a role in evolution of MDS into acute leukemia.

P623

HIDDEN MDS: A PROSPECTIVE STUDY TO CONFIRM OR EXCLUDE MDS IN PATIENTS WITH ANEMIA OF UNCERTAIN ETIOLOGY

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Background: Anemia is one of the most frequent abnormalities detected in hemograms and it can be the first sign of MDS, especially in the elderly patients. Diagnosis of MDS when anemia is the only abnormality can be complicated, so the diagnosis is delayed until the degree of anemia becomes severe (transfusion dependency) and/or other cytopenias/blasts appears.
Aims: To evaluate the impact of systematically review of all hemograms performed in a hematology department-lab selecting those with anemia and/or macrocytosis of undetermined significance. In these hemograms selected we performed a study trying to identify the underlying disease causing anemia.
Methods: We performed a prospective study within 4 months (Dec/12-Mar/13) in 3 hematology labs in Spain. Algorithm approach consisted of four steps (Figure 1). NGS analysis was performed on a MiSeq platform (Illumina) and IonReporter (Life Technologies) in MDS patients. Target-capture sequencing was performed across genes previously related to MDS. The statistical analysis was implemented using SPSS v21.0.
Results: From 290 hemograms candidates to MDS screening, 139 final diagnoses have been made (48%) (Figure 1). The main causes of anemia were iron deficiency (n=59) and megaloblastic anemia (n=39). A MDS was diagnosed in 14/25 hematological malignancies (56%). Regarding those MDS patients (Table 1) median age was 79 years (58-91); 12/14 patients had anemia [Hb=10,7g/dl] such as isolated cytopenias. All patients had macrocytosis (mean MCV=104fL). Most of patients (n=10) were low-risk MDS (IPSS-R≤3.5) and were treated according to guidelines. Table 1: Baseline characteristics of MDS cohort.

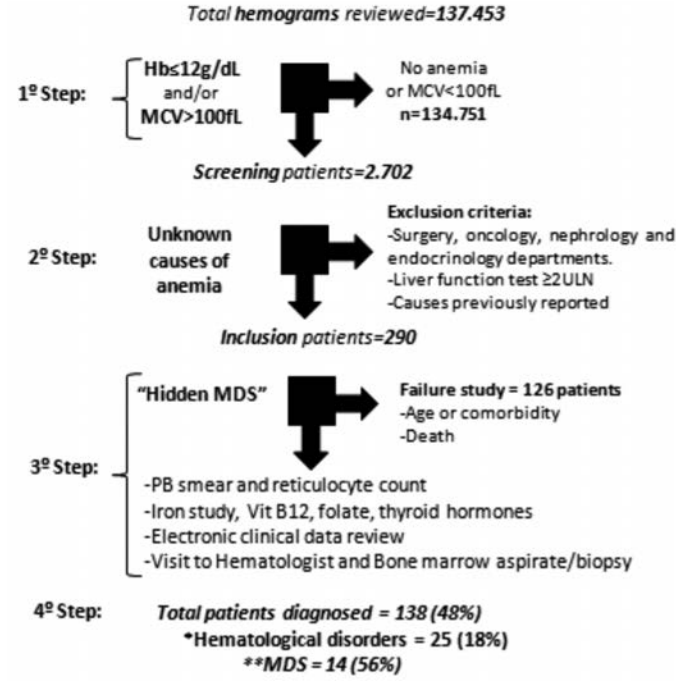

Figure 1.

Table 1.

Id	Age/Sex	WHO 2008	Hb (g/dl)	MCV (fL)	Platelets count (x10^9/L)	ANC (x10^9/L)	Cytog.	IPSS-R	Treatment	OS (Months)	Gene mutated
1	58/M	RCMD	8,5	112	379	2,3	N	2	Stimulating Ag.Chelation	28	*SF3B1*
2	71/M	MDS NOS	10	107	125	2,4	+8	2	Blood cells Transfussion	36	*WASF3*
3	81/M	RCMD	10,7	119	343	3,9	N	1	Stimulating Ag.Chelation	36	Any
4	79/M	RAEB-1	7,4	106	20	7,8	N	5,5	Blood cells Transfussion	10	*ZRSR2*
5	82/F	RAEB-1	6,8	102	424	2,8	del(5q)	4,5	Blood cells Transfussion	17	*TP53*
6	85/F	RCMD	10,3	104	227	2,2	N	1	Stimulating agents	30	*SF3B1*
7	91/F	RAEB-2	10,7	99	98	0,9	ND	ND	Blood cells Transfussion	3	No analyzed
8	84/F	RAEB-1	10,1	100	136	1	del(5q)	3	Stimulating Ag. IMiDs	28	*TET2*
9	85/M	RCUD	11,4	99	182	3	N	1	No	31	*SF3B1*
10	77/M	RCMD	11,7	100	262	3,5	N	1	No	31	*SF3B1, JAK2*
11	74/F	RARS	11,1	106	350	3,4	N	1	Stimulating agents	30	*SF3B1, DNMT3*
12	60/M	RCUD	12	111	307	1,7	N	1	No	30	*TET2*
13	76/M	RAEB-2	10,9	102	183	1,1	Complex	7	Hypometh. agents	5	*TP53*
14	81/F	RCUD	10,8	101	319	2	N	1	No	31	*SF3B1, DNMT3*

The K700E mutation in *SF3B1* was the most frequent (n=6) and was highly correlated to ring sideroblasts in bone marrow (100% of patients). The median follow-up MDS patients were 30 months [28-36]. KM estimated 3-year OS was 71%. Regarding impact on survival of mutational status, *TP53* and *ZRSR2* mutations were associated to poor prognostic and all these patients had died as compared to the presence of other mutations (*SF3B1* or *TET2*) where all patients are still alive (p=0.001).

Summary/Conclusions: This prospective approach is a reasonable screening procedure in the hematological laboratories and will probably allow us to diagnose early the more relevant causes of anemia, including MDS, and providing the appropiate intervention for the patients.
Celgene supported this study.

P624

MANAGEMENT OF IRON OVERLOAD WITH DEFERASIROX IN VARIOUS HEMATOLOGICAL MALIGNANCIES – REAL WORLD EXPERIENCES FROM THE GERMAN NON-INTERVENTIONAL STUDY EXSEPT

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Background: Iron overload (IO) resulting from chronic transfusion therapy is common in patients with various hematological disorders and may finally result in organ failure. Deferasirox is approved for the treatment of iron overload in patients with transfusion dependence.
Aims: The aim of the study was to evaluate the pattern of use of deferasirox in the daily routine setting.
Methods: Overall, 420 patients (pts) receiving deferasirox were analyzed across 107 German office- and hospital-based hematology centers: 280 with myelodysplastic syndrome (67%), 53 with myeloproliferative disorders (13%), 18 with AML (4%) and 69 with other acquired and inherited anemias (16%). The study was conducted during 2010-2014 with an ongoing 2-year follow-up period until the end of 2016. Serum ferritin, liver function parameters, dosing and adverse events were recorded at baseline and at month 1, 3, 6, 12, 18 and 24 following initiation of deferasirox therapy. Deferasirox was administered according to the summary of product characteristics and pts with at least 1 follow-up visit were eligible for analysis.
Results: Of a total of 420 pts, 243 were male (58%) and 177 female (42%) with a median age of 74 yrs., (range 2-93 yrs.). Mean time from first diagnosis of the hematological malignancy and transfusion therapy to initiation of IO therapy was 3.4 yrs. and 1.8 yrs. respectively. 227 pts (54%) showed a moderate need for blood transfusions (2-4 units/month) and 107 pts (25%) a mild need for blood transfusion (<2 units/month) at baseline. Deferasirox effectively reduced mean iron burden as measured by serum ferritin from 2230 µg/L (±1279 µg/L) at baseline to 1786 µg/L (±1390 µg/L) at 24 months, respectively. As expected, mean creatinine clearance decreased from 91 mL/min to 80 mL/min within the first 3 months and remained stable until the end of observation. Median daily dose of deferasirox at time of initiation was 17 mg/kg (range 2-30 mg/kg); dose adjustments were required in 162 pts (39%). Adverse events (AE) were reported in 364 pts (87%). The most common AEs were gastrointestinal disorders (29%), a decrease in renal creatinine clearance (22%) and an increase in blood creatinine (18%), presented as proportion of pts suffering from the AE.
Summary/Conclusions: These findings indicate that chelation therapy with deferasirox is feasible in treated patients with the capability to reduce iron burden. The safety profile shows no unexpected toxicities. The results of this interim analysis will further contribute to understand the management of IO therapy with deferasirox in the routine setting.

P625

CLASSIFICATION OF MYELODYSPLASTIC SYNDROMES (MDS) IN DAILY PRACTICE: A POPULATION-BASED STUDY AMONG 8912 PATIENTS DIAGNOSED IN THE NETHERLANDS FROM 2001 TO 2014

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Background: Specific morphological features in the bone marrow (BM)—eg, type and degree of dysplasia, as well as myeloblasts—constitutes a diagnostic hallmark of MDS. Careful assessment and subsequent documentation of these features are necessary to facilitate an accurate classification of MDS according to the World Health Organization (WHO) classification that was initially published in 2001 and updated in 2008.
Aims: In this population-based study, we sought to assess the implementation of the WHO classification of MDS in daily practice in the Netherlands according to time and health care region.
Methods: We selected all adult (≥18 years) MDS patients diagnosed between

2001-2014 (N=8912; median age, 75 years; 59% males and 19% previous malignancy) from the nationwide Netherlands Cancer Registry (NCR). All MDS subtypes were recorded in the NCR according to the International Classification of Diseases for Oncology Third Edition (ICD-O-3) that follows the WHO classification of MDS and includes: refractory cytopenia with unilineage dysplasia (RCUD), refractory anemia (RA) with ring sideroblasts (RARS), MDS with isolated del(5q), refractory cytopenia with multilineage dysplasia (RCMD), RA with excess blasts (RAEB) and MDS not otherwise specified (MDS NOS). The ICD-O-3 does not have separate codes for RAEB-1 or 2. Univariable and multivariable logistic regression models were constructed to identify variables associated with the diagnosis of MDS NOS. These variables included age at, and year of, diagnosis, sex, previous malignancy, as well as hospital type (non-academic *v* academic) and health care region of diagnosis (8 regions in total).

Results: The proportion of MDS NOS decreased from 58% in 2001 to 13% in 2014 (Figure 1A). There was regional variation noted in the distribution of MDS NOS (average in 2001-2014, 43%; range 30%>50%; *P*<0.001), which eventually leveled off with time (average in 2014, 13%; range 8%>24%; *P*=0.385; Figure 1B). Following multivariable logistic regression analysis with adjustment for variables with *P*<0.20 in univariable analysis, age per 10 year increase (odds ratio [OR], 1.18; *P*<0.001) was associated with having a MDS NOS diagnosis, whereas year of diagnosis per 1 year increase (OR, 0.87; *P*<0.001) and diagnosis in particular health care regions were associated with a lower odds of MDS NOS.

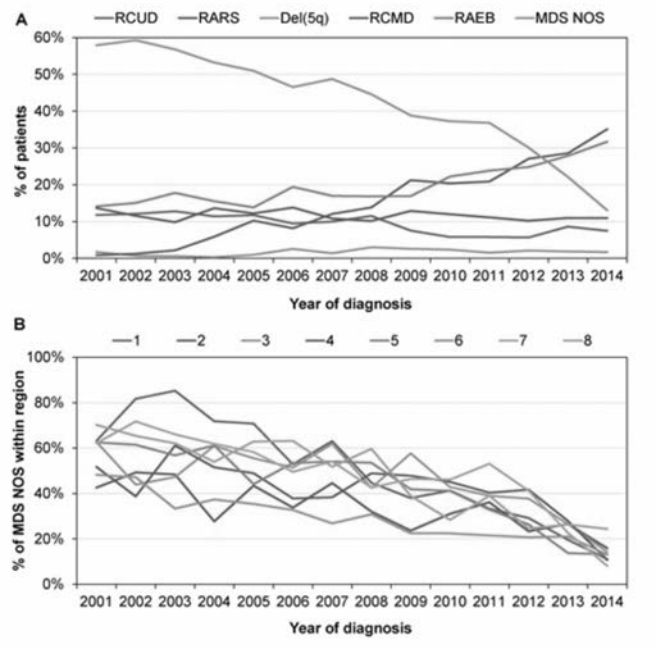

Figure 1.

Summary/Conclusions: These encouraging population-based results indicate that the classification of MDS improved markedly over time across all health care regions in the Netherlands. The reason behind this improvement may be twofold. First, clinicians, cytomorphologists and pathologists became increasingly aware with the WHO classification of MDS. This is mainly due to efforts by HOVON to create awareness for MDS by education and the design of MDS-specific clinical trials, especially since 2008. Secondly, the registration of MDS subtypes by registrars of the NCR improved due to better training in recognizing MDS subtypes in the medical records. Nevertheless, advance age is still associated with the diagnosis of MDS NOS. It is anticipated that specific morphological features in the BM will remain central for the classification of MDS in the upcoming revision of the WHO classification that will be expected in 2016. Therefore, careful analysis and proper documentation of morphological findings remain crucial to facilitate an accurate classification of MDS, which, in turn, is needed to accurately inform patients about their MDS. Well-established population-based registries are useful instruments to evaluate guideline adherence and registration practice.

P626

LEVELS OF TRANSFUSION BURDEN AND ASSOCIATED COSTS FOR PATIENTS WITH TRANSFUSION-DEPENDENT MYELODYSPLASTIC SYNDROMES

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Background: For patients (pts) with myelodysplastic syndromes (MDS), dependence upon red blood cell (RBC) transfusions has been long demonstrated to be a risk factor for progression-free survival. Recent studies have suggested that higher levels of transfusion burden are associated with worse prognosis (Chan LSA, *et al.* Leuk Lymphoma 2014;55:10:2296-2300; Pereira A, *et al*. Am J Hematol 2010;86:245-250). The increasing prognostic risk associated with more frequent transfusions poses the question of whether costs also vary with transfusion frequency.

Aims: To assess the costs associated with different levels of transfusion burden among pts with transfusion-dependent (TD) lower-risk MDS, from a US perspective based on claims data.

Methods: Pts were identified from a large US claims database (2008–2013) by ICD-9 codes for MDS, with the first such code determining the index date. Pts with ≥12 months pre- and ≥6 months post-index data were included if they met the criteria of TD post-index, defined as 2 consecutive 8-week periods each with ≥1 transfusion episode and no interim 56-day period without a transfusion. Pts with ICD-9 codes for high-grade MDS or acute myeloid leukemia at diagnosis or stem cell transplant anytime were excluded. Transfusion burden was measured as average days between RBC transfusions during the TD period. Pts were followed until they no longer met TD criteria or end of data. Total costs of care (medical and pharmacy) were evaluated in 2015 US dollars from a payer's perspective, based on paid claims for initial TD periods and averaged to determine monthly cost levels.

Results: 2,645 TD MDS pts met the inclusion criteria; 59% were male and 51% were ≥75 years old. 1,854 (70%) pts averaged ≤28 days between RBC transfusions during the TD period. When patients were categorized by the average time between transfusions, those transfused every 2–3 weeks comprised the largest group (23%) (Figure 1). The principal differences in baseline characteristics for TD pts transfused ≤28 days apart *vs* TD pts with less frequent transfusions were sex (60.7% female *vs* 53.6% male), age (median 74 years *vs* 78 years) and prevalence of thrombocytopenia (25.9% *vs* 16.8%). Average monthly total cost of care per pt was 53% higher for TD pts transfused ≤28 days apart *vs* TD pts with less frequent transfusions ($19,497 *vs* $12,717). Within the former group, costs were 58% higher among pts who transfused ≤14 days apart compared with those who transfused every 15–28 days ($25,268 *vs* $16,033). Inpatient services and pharmacy represented 45% and 8% of total monthly TD costs, respectively.

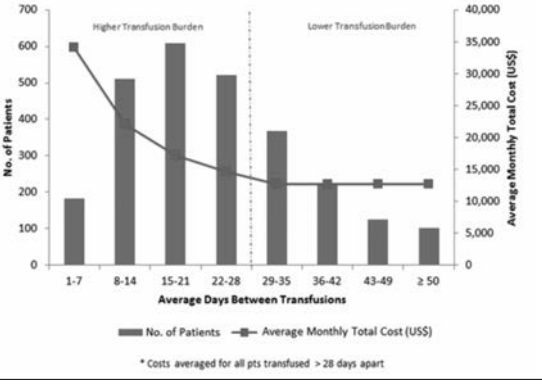

Figure 1. Average time between transfusions during TD period, and associated average total monthly cost/patient*.

Summary/Conclusions: The average time between transfusions is heterogeneous among TD MDS pts, with 27% having ≤14 days between transfusions and similar percentages transfused every 2-3 or 3-4 weeks. Pts with more frequent transfusions had higher average monthly total costs. This suggests that TD pts with higher transfusion burden face greater economic challenges as well as the established clinical challenges.

P627

THE INCORPORATION OF COMORBIDITIES IN THE PROGNOSTICATION OF PATIENTS WITH LOWER-RISK MDS

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Background: Patients (pts) with Lower-risk Myelodysplastic syndromes (LR-MDS) defined by having Low or Intermediate-1 IPSS score show a wide prognostic heterogeneity. In addition to age, the presence of comorbidities defined as other chronic advanced medical diseases, can impact on survival in MDS. However, the prognostic significance has not been evaluated specifically in the group of LR-MDS pts.

Aims: To evaluate extra-hematological comorbidities together with classic hematological parameters in a series of pts with LR disease by IPSS and non-unfavorable cytogenetic categories by IPSS-R.

Methods: 232 pts with LR-MDS were retrospectively analyzed between 2000 and 2015. Only pts who received best supportive care with blood transfusion, erythropoietic stimulating agents and/or just observation if no symptomatic cytopenias were included. Non-leukemic death (NLD) was analyzed according to the following variables: age, hematological parameters, IPSS and the MDS-comorbidity index (MDS-CI. Della Porta MG, *et al. Haematol.* 2011)
Results: Clinical and baseline characteristics are shown in Table 1. At last follow-up, 148 pts (64%) had died and 33 pts (14%) progressed to acute myeloid leukemia. Diabetes requiring treatment and cardiac disease were the most frequent comorbidities (30% and 29% respectively). Prevalence of comorbidities was higher in the group of pts aged >65 years (63.3% *vs* 38.4%; *P*=0.04) as well as the number of pts with more than one comorbidity (30.2% *vs* 9.6%; *P*<0.001). After a median follow-up of 60 months (range, 48-71), median OS for the whole series was 36 months (range, 30-41). In univariate analysis, the following variables significantly influenced on outcome (P<0.05): IPSS (low *vs* int-1), Hb <10 g/dL, platelet <50x10e^9/L, BM blasts 5-9%, transfusion dependence (TD), diabetes requiring treatment, cardiac, severe pulmonary and severe to moderate hepatic disease as defined by MDS-CI. Table 2 shows multivariate analysis. Age did not influence on survival. According to these data, an assigned weight was applied to each parameter with prognostic impact for OS. As shown in Figure 1, three sets of patients were categorized into different risk groups with significantly different risk of NLD (P<0.001): group 1 (pts with score 0-2; Low-risk group, n=110, 47.5%), group 2 (score 3-4; intermediate risk group, n=94, 40.5%) and group 3 (score 5-6; high risk, n=28, 12%). At last follow-up, 51 out of 110 (46.4%) patients in the low risk group and 69 out of 94 (73.4%) in the intermediate risk group had died from other causes non-leukemia related.

Table 1.

Table 2.

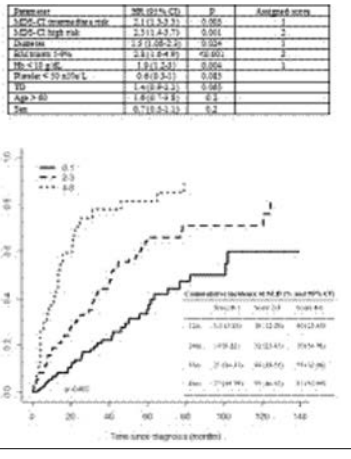

Figure 1.

Summary/Conclusions: This integrated score (clinical and comorbidity) stratifies patients in 3 groups with significant different risk of NLD. Diabetes and cardiac diseases are the most prevalent comorbidities. Interestingly, age, when considered together with clinical parameters and comorbidities, did not influence on outcome. In summary, this study confirms the importance of comorbidities at diagnosis, together with other well-known hematological parameters in pts with LR-MDS and may help to proper define therapeutic strategies in this set of pts.

Bone marrow failure syndromes incl. PNH - Biology

P628

STROMAL MICROENVIRONMENT COMPONENTS DAMAGE IN PATIENTS WITH GRAFT FAILURE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELLS TRANSPLANTATION

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Background: Graft failure following allogeneic hematopoietic cell transplantation (alloHSCT) may result from rejection by recipient T cell. The changes of stromal microenvironment (SM) in patients with graft failure were not described. It was demonstrated that multipotent mesenchymal stromal cells (MSC), the element of SM, are impaired by allogeneic lymphocytes. It was assumed that SM in patients with graft failure could be altered.
Aims: The aim of the study was to analyze SM in patients with graft failure and long-term bone marrow (BM) aplasia.
Methods: A randomized clinical trial (ClinicalTrials.gov NCT01941394) for acute graft-*versus* host disease prevention with MSCs has been on-going at the National Research Center for Hematology. BM MSCs have been developed individually for each patient from its own donor of HSC. Three patients with AML and graft failure after alloHSCT (1 from unrelated and 2 from related partially compatible donors) were observed. In an attempt to restore the SM donor MSCs were injected under local anesthesia in the iliac crest after obtaining informed consent from the patient. The cells were injected into the bone tissue in small portions 100-200 mkl through 2 skin punctures, and multiple periosteal punctures. In 2 patients bone marrow punctures were performed 5 and 3 months after the donors' MSCs implantation. It was withdrawn by 2-3 ml of bone marrow from 4 independent punctures from iliac crest of each patient. Colony-forming units fibroblast (CFU-F) from these BM samples were analyzed and MSCs were isolated and cultured by standard method.
Results: In 2 patients 2 weeks after MSCs administration their own hematopoiesis recovered. Analysis of BM MSCs in 5 months after donor MSCs implantation revealed significant decrease in cumulative MSCs production for 3 passages (1.2±0.2x10^6) compared with all AML patients after alloHSCT (4.2±0.6x10^6) and healthy donors (8.0±2.1x10^6). The efficiency of cloning, showing the number of MSCs with proliferative ability in the population, was also significantly decreased (0.101±0.014) compared with donors (0.34±0.07). In one patient leukocyte count reached 10^9/L only in 9 months after donor MSCs implantation. Analysis of BM samples from this patient after MSCs implantation revealed decrease of bone marrow cellularity (Table 1) per ml *versus* 16.7±0.7x10^6 in donors. Cumulative MSCs production in this patient was very low (Table 1) Cloning efficiency was also significantly lower than donors. Gradually, the total cell production increased and 3 months after the start was 2 times higher than the original and CFU-F concentration in the BM also began to recover. In 3 and 5 months after MSCs implantation donor MSCs were found in the bone marrow of 2 patients.

Table 1. Analysis of BM samples of one patient.

Days after MSCs implantation	Number of leukocytes per mkl	Number of BM cells/ml, x10^6	Cumulative MSC production for 4 passaged, x10^6	CFU-F per 10^6 BM cells	Efficiency of cloning
77	100	1±0.16	0.73±0.2		
99	700	2.76±1.02	1.19±0.34	8.0	
171	700	2.32±0.21	1.56±0.38	40.8±15.7	0.25±0.08

Summary/Conclusions: Thus it is possible that graft failure in patients after alloHSCT is not only connected with the immune response, but with severe damage of bone marrow stromal cells, which may partly be restored after administration of the donor MSCs.

P629

SPONTANEOUS REMISSION IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA - RETURN TO HEALTH OR TRANSITION INTO MALIGNANCY?

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired syndrome characterized by intravascular hemolysis, thrombosis and bone marrow failure. The disease is caused by a mutation on the *PIG-A* gene that leads to

a lack of glycosylphosphatidylinositol (GPI) -anchored complement regulatory molecules CD55 and CD59 from the surface of a clonal lineage of blood cells. The diagnosis of PNH is made by analyzing GPI-anchored molecules on blood cells by flow cytometry. The disease course is often unpredictable and the therapeutic options are limited. While in previous studies spontaneous remissions have been reported to occur in up to 15-30% of cases, there are no detailed case reports published of these patients. In particular, it would be important to distinguish true remissions from other developing disorders, especially from malignancy.

Aims: Because of the variable clinical course of PNH we wanted to explore whether true spontaneous remissions occur in PNH in the Finnish patient material. A nation-wide study of all patients in Finland since 1995 and an extended follow-up time (up to 20 years) have allowed a detailed analysis of the course of the disease in our patients.

Methods: In a nation-wide project we collected patients from all Health Care Districts in Finland. The sources of information included the Helsinki University Central Hospital Laboratory (HUSLAB) databases, flow cytometry analysis of red blood cells (RBC) and leukocytes, patients' medical records and a patient questionnaire. The patients were evaluated until September 2015. An ethical permission for the study was given by the co-ordinating ethical committee at the Helsinki University Hospital Health Care District. A research permission was also obtained from the National Institute of Health and Welfare (Helsinki, Finland) and the patients gave written informed consents for the study.

Results: In a cohort of 106 Finnish patients with a PNH clone we found six cases where the clone disappeared or clearly diminished (to a level equal or below 1.5%). Two of the six patients subsequently developed leukemia (acute myeloid leukemia and chronic myelomonocytic leukemia) while the other four are in clinical remission. The median time from the diagnosis until the remission was 12.5 years (range 6-15 years). For the two patients who developed leukemia the diagnosis was made 26 years and 12 years respectively after the initial PNH diagnosis. For one of the patients the CD59-deficient cell clone disappeared three years earlier and for the other one in the same year leukemia was diagnosed. For all patients the median CD59-deficient RBC clone size was 64% at highest (range 30-91%). Four of the patients had classic PNH with signs of hemolysis. All of the patients had underlying aplastic anemia.

Summary/Conclusions: According to our data spontaneous remissions are not as frequent as previously described and the disappearance of the PNH cell clone may have dramatically different outcomes. Nevertheless, true remissions occur, but the patients need to be carefully followed up for a potential emergence of malignancy. The remission may have an immunological background. In our cases immunosupressive treatment may have affected the PNH cell clone or the cell clone could have lost its growth advantage after repopulation of the bone marrow. Spontaneous remissions provide an interesting topic for further studies, which could bring along a better understanding of the natural history of PNH.

P630

UNAFFECTED MYELOID DIFFERENTIATION OF IPS CELLS DERIVED FORM A CYCLIC NEUTROPENIA (CYN) PATIENT WITH ELANE MUTATION

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Background: Cyclic neutropenia (CyN) is a hematologic disorder in which blood cell counts particularly granulocytic neutrophils, monocytes, platelets, and reticulocyte numbers show cycles at appr. 21 day intervals. The majority of CyN patients (ca 90%) harbor inherited mutations in the ELANE gene. Intriguingly, same *ELANE* mutations are present in two different bone marrow failure syndromes: congenital as well as in cyclic neutropenias. It is unclear how mutation in the same gene cause congenital (CN) or cyclic neutropenia (CyN). The pathomechanism of cycling hematopoiesis downstream of *ELANE* mutations is also unclear. Recent studies using inducible pluripotent stem cells (iPSCs) derived from severe congenital neutropenia (CN) patients harbouring ELANE mutations demonstrated markedly diminished granulocytic differentiation of these cells *in vitro* (Nayak RC, *et al.* JCI 2015; Hiramoto T, *et al.* PNAS 2013). Interestingly, correction of *ELANE* gene mutation in iPSCs from a CN patient using CRISPR/Cas9 technology restored defective granulopoiesis, suggesting the monogenic origin of the *ELANE* mutation caused congenital neutropenia (Nayak RC, *et al.* JCI 2015). These data suggest that additional gene mutations or epigenetic defects in combination with mutated *ELANE* might be responsible for the pathogenesis of cyclic neutropenia.

Aims: In the present study we evaluated the *in vitro* myeloid differentiation of iPSCs derived from the CyN patient harboring sporadic heterozygous *ELANE* mutation (c.761C>G p.W241L), in comparison to iPSCs derived from healthy individuals.

Methods: We used embryoid-body (EB)-based protocol of granulocytic differentiation from iPSCs described by N. Lachmann *et al.* (Stem Cell Reports 2015).

Results: we found that myeloid differentiation of iPSCs derived from CyN patient, was comparable to that of healthy individual iPSCs, as revealed by the analysis of the percentage and absolute numbers of $CD45^+/CD11b^+$, $CD45^+/CD16^+$ and $CD45^+/CD15^+$ cells as well as by the examinations of the cell morphology on cytospin preparations.

Summary/Conclusions: These data suggest that the *in vitro* hematopoiesis in CyN is normal as judged by iPSC technology and that *in vivo* additional humoral or bone marrow niche components might influence myeloid cell cycling in CyN.

P631

OUTCOME OF ADOLESCENT AND YOUNG ADULTS WITH ACQUIRED APLASTIC ANEMIA TREATED WITH IMMUNOSUPPRESSIVE THERAPY IN COMPARISON WITH CHILDREN AND OLDER ADULTS

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Background: Acquired aplastic anemia (AA) is a rare hematological disorder with a peak of incidence in the Adolescent and Young Adult (AYA) population. Younger age has been associated with better response after immunosuppressive therapy with anti-thymoglobulin and cyclosporine (ATG-CSA), as well as better survival. However, no study focused on the specific outcome of AYA patients so far.

Aims: This retrospective study assessed the impact of age on outcome of AA after ATG-CSA by comparing AYA with younger and older patients.

Methods: We retrospectively analyzed a cohort of AA patients treated frontline by the association of ATG (rabbit or horse) and CSA in Saint-Louis Hospital between January 2000 and June 2013. Patients were divided into three age groups: children (<15 years), AYA (15-25 years) and adults (>25 years). Eighty-four consecutive patients were included (29 children, 32 AYA and 23 adults). Children presented more frequently with very severe AA (55% *versus* 22% in AYA and 22% in adults, p=0.023). Time from diagnosis to ATG was longer in adults (69 days *versus* 26 in children and 28 in AYA, p=0.007). Children were more frequently treated with rabbit-ATG (86% *versus* 59% in AYA and 52% in adults, p=0.004). Patients were similarly transfused before ATG in the 3 groups. Rates of transplantation after failure/relapse were similar in these subgroups. Complete response (CR) was defined as a neutrophil count >1 G/L, a platelet count >100 G/L and a haemoglobin level >10 g/dl, according to the National Institutes of Health (NIH) criteria. Partial response (PR) was defined as transfusion independence with a neutrophil count >0.5 G/L, but not meeting the blood count criteria for CR.

Results: The overall response rate at 6 months was 48% in children, 69% in AYA and 30% in adults, and 76%, 78% and 49% respectively at 5 years. In multivariate analysis, along with the use of horse ATG (OR=3.398, p=0.045), age remained associated with better response rate. Thus, in comparison with AYA patients, the 6-month response rate was lower in adults (OR=0.179, p=0.008) and similar in children (OR=0.609 p=0.386). The 5-year relapse rate was 20.7%, with no impact of age. The only relapse risk factor identified was higher baseline lymphocytes count (OR=1.939, p=0.011). Twenty seven patients (12 children, 6 AYA and 9 adults) underwent allogeneic hematopoietic stem cell transplantation (HSCT), 4 from an HLA-identical sibling donor (adults over 40 years), 18 from an unrelated donor and 5 from cord blood. They were referred to HSCT after failure (16), relapse (8) or clonal evolution (3). With a median follow-up of 5.4 years, the 5-year overall survival (OS) was 86.5%. After censoring at the time of HSCT, the 5-year OS was 95.9%. Eleven patients died, all in failure/relapse after ATG-CSA, including 8 after HSCT (5 from infection, 2 from GVHD and 1 from graft failure). Adults over 25 years had shorter survival than AYA (HR=4.984, p=0.049) and showed a trend toward worse survival than children (HR=3.311, p=0.092). However, in multivariate analysis, younger age did not impact OS. The predictive factors associated with improved survival were a lower baseline lymphocytes count (HR=4.696, p=0.015), a lower baseline neutrophils count (HR=4.735, p=0.045), a higher baseline reticulocytes count (HR=0.897, p=0.023) and a shorter time from diagnosis to ATG (HR=7.341, p=0.001) (these 4 factors analyzed as continuous variables), as well as a better 6-month response rate (HR=0.008, p=0.015).

Summary/Conclusions: Response rate of AYA population treated with ATG-CSA for AA is similar to children's and better than adult's one. However, younger age has no influence on OS or relapse.

P632

A PHASE 1 SINGLE-ASCENDING-DOSE CLINICAL STUDY OF RA101495, A SUBCUTANEOUSLY ADMINISTERED SYNTHETIC MACROCYCLIC PEPTIDE INHIBITOR OF COMPLEMENT C5 FOR TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired, clonal hematopoietic stem cell disorder caused by a deficiency in glycosylphosphatidylinositol (GPI)-linked proteins on cell surfaces. Patients with mutations

in the phosphatidylinositol glycan class A gene are unable to produce functional, protective, GPI-linked proteins, resulting in the accumulation of specific complement proteins on the surface of red blood cells (RBCs) and subsequent RBC lysis by the membrane attack complex (MAC). Inhibition of complement activation at the level of complement C5 is a clinically validated approach for the treatment of PNH. RA101495, a synthetic macrocyclic peptide, binds to C5 at a unique site not targeted by currently available therapies, and allosterically inhibits C5 cleavage into C5a and C5b, preventing production of a key component of the MAC. RA101495 also inhibits the assembly of MAC by blocking the interaction between C5b and C6.

Aims: A Phase 1 single-ascending-dose clinical pharmacology study in healthy human volunteers designed to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of RA101495 following subcutaneous (SC) injection.

Methods: The study was randomized, double-blinded, and placebo (PBO)-controlled with 4 SC single-ascending-dose cohorts housed in a clinical pharmacology unit for 3 days. After obtaining written informed consent, all subjects received 1 dose of RA101495 on Day 1. Four subjects (2 RA101495 and 2 PBO) were administered the lowest dose level (0.05 mg/kg) and 6 subjects per cohort (4 RA101495 and 2 PBO) were sequentially administered the 3 higher dose levels (0.1, 0.2, and 0.4 mg/kg). Safety was assessed by intensive clinical monitoring, and frequent blood samples were obtained for determination of RA101495 concentrations by liquid chromatography/high resolution mass spectroscopy and ability to inhibit complement-mediated RBC lysis in an *ex vivo* antibody-sensitized sheep erythrocyte hemolysis assay. All subjects received prophylaxis for N. meningitides infection with ciprofloxacin and, for the highest dose, vaccination as well.

Results: A total of 22 subjects were enrolled into the study (14 RA101495 and 8 PBO). Plasma concentrations of a single SC injection showed linear dose-dependent exposure across all dose levels. Preliminary results show that at the maximum plasma concentration, the percent inhibition of hemolysis compared to baseline reached ≥90% for the 0.1, 0.2, and 0.4 mg/kg dose cohorts and 60% for the lowest dose (0.05 mg/kg) cohort. Suppression of hemolysis of up to 4 days (further time points are being analyzed) was observed for the 0.1, 0.2, and 0.4 mg/kg dose ohorts. Notably, a single injection of 0.4 mg/kg resulted in ≥90% suppression of hemolysis for at least 4 days. The only safety finding noted was mild cutaneous injection site erythema in 3 of 6 subjects on the highest dose (data are still blinded); there was no associated pain, tenderness, swelling, or induration and all events resolved rapidly following dosing.

Summary/Conclusions: RA101495 is a novel synthetic macrocyclic peptide inhibitor of C5-mediated hemolysis that is being developed as an alternative to intravenous monoclonal antibody therapy for the treatment of PNH. RA101495, currently being investigated for daily at-home SC self-administration, shows a rapid onset of activity, and appears to be safe and well tolerated. These preliminary data suggest low daily doses will achieve steady-state levels suitable for ≥90% suppression of hemolysis, and that once-weekly dosing should be achievable.

P633

THE PRESENCE OF A PNH CLONE INFLUENCES THE KINETICS OF RESPONSE TO IMMUNOSUPPRESSIVE THERAPY (IST) IN APLASTIC ANEMIA (AA) PATIENTS.

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Background: Immunosuppressive therapy (IST) is effective in improving the hematologic cytopenias in 65-75% of patients (pts) with aplastic anemia (Young NS, *Blood*, 2006). A clone of hematopoietic cells deficient in glycosylphosphatidylinsoitol membrane protein anchors - a PNH clone - can be seen in ~50% of AA (pts) (Socie G, *Sem Hematol*, 2000). The presence of a PNH clone in AA may predict for a favourable response to IST (Sugimori, *Blood*, 2006) although this remains controversial (Scheinberg, *Br J Haemtol*, 2009). The development of high-resolution PNH flow cytometric testing may be a mitigating factor explaining interstudy variability.

Aims: To determine whether the presence or the size of a PNH clone in AA pts affects the rate or degree of response to standard IST at a regional/provinical referral centre.

Methods: A retrospective chart and computer data base review was performed on all AA pts >age 16 years (yrs) referred to Vancouver General Hospital (VGH) between January 2008 and October 2015 for treatment with IST. Bone marrow pathology was centrally reviewed and all pts had metaphase cytogenetic analysis performed to exclude a clonal abnormality. PNH flow cytometry was done at VGH on the red cells, granulocytes and monocytes of all pts using an antibody panel that included FLAER, CD55 and/or CD59. IST was uniform during the study period and consisted of Cyclosporine (CSA) 2.5 mg/kg p.o. b.i.d., ATGAM 40 mg/kg/d IVx4 and IV Methylprednisolone 1 mg/kg/dx10 with a 10-day taper. CSA doses were adjusted to maintain whole blood trough CSA levels of 200-300 ug/L for a 12-month period followed by tapering based uoon hematologic tolerance. Severity of AA and criteria for response to IST were as previously published (Marsh J, *Br J Haematol*, 2009). Statistical comparisons were performed using the Fisher's exact test and differences in group medians were assessed with the Mann Whitney U test.

Results: During the 8-year study period, 57 pts with AA received IST at VGH; 2 pts died within 2 weeks of commencing treatment and 55 pts were evaluable for response. There were 28 males (M) and 27 females (F) with median age of 56 yrs (range 17-74). AA was very severe in 7 pts, severe in 28 pts and non-severe in 20 pts. Response to IST was seen in 39 pts (70.1%) - partial response (PR) in 26 pts (47.3%) and complete response (CR) in 13 pts (23.6%). Ten pts received a second cycle of ATGAM or rabbit ATG (Thymoglobulin), 3 pts for recurrent AA following CSA taper (all responded) and 7 pts for initial non-response (no responses at 12 mos). A PNH clone was found at diagnsois in 29 pts (52.7%; PNH+) with median clone size 0.9% (range 0.1% - 87%). M/F ratio was 0.7 in PNH+ pts and 1.6 in pts without a PNH clone (PNH-) (p=0.18). Median age was similar in the PNH+ (51 yrs) and PNH- (57 yrs) groups (p=0.49) but was significantly younger in PNH+ pts with clone size >1%, n=14 (33.5 yrs; p=0.04). Rate and degree of response to IST was similar in PNH+ pts (65.5%; 20.7% CR) and PNH- pts (76.9%; 26.9% CR) (p=0.38) and the size of the PNH clone did not influence the response rate. Kinetics of response to IST did differ notably between PNH+ and PNH- pts (Table 1).

Table 1.

	3 mos	6 mos	12 mos	24 mos
PNH+ (n=29)	51.7%	62.1%	65.5%	65.5%
PNH- (n=26)	42.3%	53.8%	53.8%	76.9%

All of the PNH+ responders achieved ≥PR by 12 months while 6/20 of the PNH- pts reached PR/CR between 12 and 24 mos (p=0.02). On serial testing, PNH clone size increased >10% in 3 pts (10%) and decreased by >10% in 2 pts (7%). Five pts in the PNH- group (19%) developed a persistent PNH clone on follow-up. With a median follow-up of 44 mos, 49 of 55 evaluable pts remain alive; 4 PNH+ pts and 2 PNH- pts have died.

Summary/Conclusions: IST is associated with a 70% response rate in AA pts. The presence of a PNH clone and the size of a PNH clone does not predict the degree or rate of response to IST. However, the kinetics of response differs between PNH+ and PNH- pts. PNH- pts may take longer to achieve response criteria with 20-25% of pts responding during the seond year of IST.

P634

A CLINICAL SIGNIFICANCE AND TIME-DEPENDENT CHANGE OF PNH CLONE SIZE IN PATIENTS WITH BONE BARROW FAILURE SYNDROME: JAPANESE MULTI-CENTRE PROSPECTIVE STUDY

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Background: In patients with bone marrow failure (BMF) syndromes, including aplastic anemia (AA) or low-risk types of myelodysplastic syndrome (MDS), a small population of GPI-AP-deficient blood cells (PNH-type cells) is often detectable through high-resolution flow cytometry. Some studies have shown that BMF patients with PNH-type cells have a favorable response to immunosuppressive therapy (IST), compared to BMF patients lacking PNH-type cells.

Aims: We conducted a multi-centre prospective observational investigation in Japan, the OPTIMA study, to determine the clinical significance and time dependent change of PNH clone size in BMF patients.

Methods: From July 2011, we recruited patients who were diagnosed with BMF syndromes. The primary endpoint was to determine the prevalence of increased PNH-type cells in BMF syndromes and to clarify the clinical significance of the presence and quantitative changes of these cells with regard to clinical features. Six university laboratories across Japan were assigned as regional analyzing centres and the percentage of PNH-type cells was measured by high-resolution flow cytometry, originally established in Kanazawa University. Cross validations were conducted twice per year at each regional centre to minimize inter-laboratory variations in detection sensitivities, cutoff values, and other variables. The liquid FLAER method (≥0.003%) and cocktail method (≥0.005%) with CD55 and CD59 antibodies were used for the detection of PNH-type granulocytes and erythrocytes.

Results: Between July 2011 and July 2015, 2411 patients were enrolled in the

study, and we analyzed 2299 patients who were eligible for this interim analysis. Based on high-resolution flow cytometry, 784 (34.1%) patients had ≥0.005% PNH-type erythrocytes and ≥0.003% PNH-type granulocytes; for each disease subset PNH-type cells were found in 73/73 (100%) patients with PNH, 377/716 (52.7%) with AA, 110/622 (17.7%) with MDS, 182/655 (27.8%) with undiagnosed BMF, and 42/233 (18%) with suspected PNH. Overall, 186 (8.1%) patients had ≥1% of both PNH-type erythrocytes and granulocytes: 69 (94.5%) patients with PNH, 69 (9.6%) with AA, 22 (3.5%) with MDS, 10 (1.5%) with undiagnosed BMF syndromes and 16 (6.9%) with suspected PNH. With regard to the WHO classification of MDS subtypes, 20.1% (58/288) of patients with RCMD, 17.5% (28/160) with RCUD, and 40% (2/5) with del (5q) MDS possessed PNH-type cells; no patients with RARS (n=27) or RAEB (n=63) had PNH-type cells. At 3-year follow-up, a total of 86 (3.7%) patients were able to be evaluated for time-dependent changes of PNH-type cells. Seventy-six of the 86 (11 with PNH, 48 with AA, 11 with MDS, 4 with undiagnosed BMF syndromes, and 2 with suspected PNH) patients had PNH-type cells at enrollment. In four of the 76 patients, PNH-type cells became undetectable at 3-years; 3 of the 4 patients had AA and the other had MDS; all but one AA patient received IST and achieved partial or complete response to the treatment. PNH-type cells were not detected at 3 years in 10 patients who did not show PNH type cells at enrollment.

Summary/Conclusions: PNH-type cells were detected in 52.7% of patients with AA and 17.7% of patients with low-risk types of MDS, but were not detected in any of RARS and RAEB patients. The results of the nation-wide study confirmed our previous finding that the presence of increased PNH-type cells represents a benign nature of bone marrow failure.

P635

HLA-HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION: A VALID ALTERNATIVE OPTION FOR PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: In the era of Eculizumab, indentifying patients with PNH who may benefit from allogeneic stem cell transplantation(SCT) is challenging, especially for those who have no HLA-matched donors. Several recent studies have shown that HLA-haploidentical SCT for patients with hematological malignancy can achieved comparable outcomes with HLA-identical sibling transplantation. There are very few reports on the use of HLA-haploidentical SCT for PNH.

Aims: The aim of the present study was to assess the long-term clinical outcome of HLA-haploidentical SCT in patients with PNH.

Methods: Total of 12 PNH patients received HLA-haploidentical SCT between Oct 2010 and Oct 2015 at our institution. The patients were aged 8 to 54(median 22.5 years). The median interval from the diagnosis to transplantation was 5 months (range 2-180). Of the 12 HLA-haploidentical donors, 6 were siblings, 2 fathers, 2 mothers, 1 son and 1 daughter. 11 patients received myeloablative conditioning regimen consisting of busulfan, cyclophosphamide and ATG (antithymocyte globulin), 1 patient who underwent salvage HLA-haploidentical SCT after the graft failure of double umbilical cord blood transplantation received conditioning including reduced-intensity total body irradiation, cyclophosphomide and ATG. G-CSF-mobilized bone marrow and peripheral blood stem cells were transplanted as graft. Prophylaxis for graft-*versus*-host disease(GVHD) consisted of cyclosporine or tacrolimus+mycophenolate mofetil+short-term methotrexate.

Results: All 12 patients were engrafted successfully. The median time of neutrophils (ANC) reached to 0.5×10^9/L and platelets (PLT) reached to 20×10^9/L were 12 (range 11-26) days and 15 (range 11-120) days, respectively. 2 patients developed grade II acute GVHD, 2 patients developed limited chronic GVHD. After a median follow-up time of 16.5 (range 2.0-40.0) months, the 3-year OS probability was 77.8±13.9%. 2 patients died of treatment-related mortality, including severe pulmonary infection (n=1) and transplant-associated thrombotic microangiopathy (n=1),respectively. No patients were documented to have a recurrence of PNH clone after SCT.

Summary/Conclusions: This study showed that long-term outcomes of HLA-haploidentical SCT in patients with PNH were comparable to that of HLA-matched donor SCT (the 3-year OS probability was 80.5±10.2%, P=0.02) at our institution. HLA- haploidentical SCT should be considered as a valid alternative therapeutic option for PNH patients without HLA-matched donors.

P636

GENE MUTATIONS IN INHERITED BONE MARROW FAILURE SYNDROME REVEALED BY NEXT GENERATION SEQUENCING: COMPARISON WITH CHROMOSOME BREAKAGE TEST

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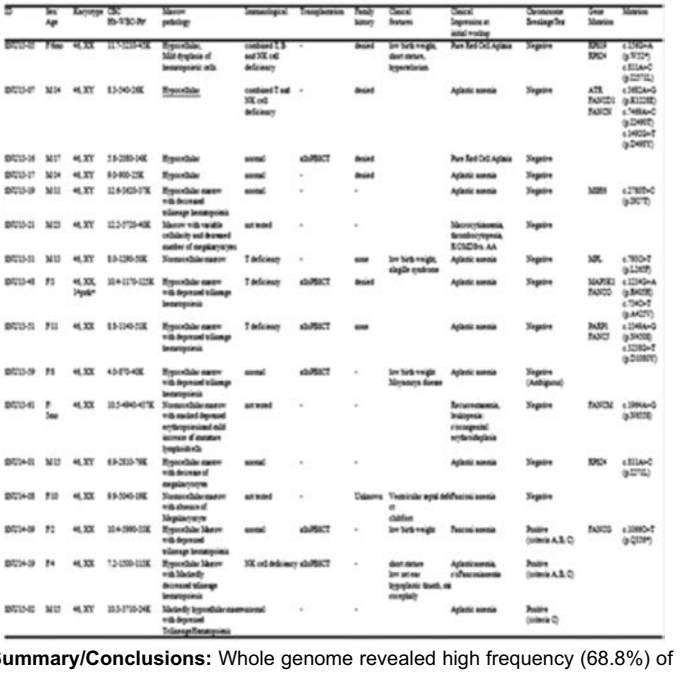
Background: Inherited bone marrow failure syndrome (IBMFS) is characterized by cytopenias, congenital physical abnormalities, and predisposition to malignancy. Fanconi Anemia (FA) is the most frequent cause among IBMFS and chromosome breakage test is routinely performed to diagnose FA, while gene tests for FA is not easily accessible due to large sized and multiple involved genes. We performed whole genome sequencing and compared the gene mutation with the results of chromosome breakage test.

Aims: The aim of our study was 1) to investigate the incidence of FA showing positive results for CBT among patients diagnosed with AA, and 2) to investigate the frequency of the gene mutations which are related to IBMFS in patients with AA. We collected the DNA of 16 patients who were suspected for the diagnosis of IBMFS and performed whole genome sequencing. For specimen in whom we could not acquire consent for genetic testing and research, we retrospectively rescored the results of CBT according to 3 different diagnostic criteria for FA.

Methods: In 16 patients with childhood Aplastic Anemia(AA), whole genome sequencing was performed in context with chromosome breakage test with mitomycin C and diepoxybutane. Additionally, the results of 67 consecutive chromosome breakage tests from 57 patients under suspicion of FA were retrospectively rescored based on widely used 3 different scoring systems and compared the concordance rate among the results of 3 different scoring systems.

Results: Mutation variants related with IBMFS were detected in 68.8% (11/16 patients); RPS19 and RPS24 in 2 patient, BLM in 2 patient and FA genes (FANCA, FANCD1, FANCG, FANCJ, FANCM, FANCN, FANCO) in 9 patients. Inherited predisposition to myeloid leukemia related genes (MSH6, ATR, MPL) were detected in 18.7% (3/16 patients). Coexisting somatic mutations of oncogene (MAP3K1) was detected in 1 patients among 9 patients with FANC mutation (11.1%, 1/9 FA patients). Among 9 patients with FA gene mutations, 2 patients showed positive results for chromosome breakage test and the other 7 patients showed negative results for chromosome breakage test. Concordance rate among 3 different scoring systems for chromosome breakage test was 91.2% (52/57 patients) (Table 1).

Table 1.



Summary/Conclusions: Whole genome revealed high frequency (68.8%) of IBMFS related genes in childhood aplastic anemia, suggesting that molecular testing is requisite for diagnosis of IBMFS. Considering that chromosome breakage test detected only 22.2% of FA patients who were confirmed by molecular test and significant portion of patients who showed positive chromosome breakage test did not harbor FA gene mutation, significance of chromosome breakage test for FA must be reconsidered.

P637

PROSPECTIVE STUDY ON THE USE OF PEGFILGRASTIM IN CHILDREN WITH SEVERE CONGENITAL NEUTROPENIA

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Background: Granulocyte-ColonyStimulating Factor (G-CSF), has radically changed the prognosis of Severe Congenital Neutropenia (SCN) patients who are at risk of lethal infections.The compliance to daily subcutaneous injections

is sometime scarce thus affecting the efficacy of treatment.The peghilated form of filgrastim (PEG) due to its long half-life should enable the frequency of injections.
Aims: To describe the efficacy, safety and PK profile of PEG in children affected with SCN.
Methods: SCN pediatric patients treated with G-CSF scarcely compliant to daily injections, were considered eligible to the study**.** After 72 hours wash out, PEG was administered at starting dose of 100 g/kg at an interval not shorter than 4 days; subsequent injections aimed to mantain median absolute neutrophil count (ANC) between 1000-5000/cmm and to control infections. Bone marrow morphology, cytogenetics, G-CSFR mutation analysis, abdomen ultrasound scan, and bone density assessment by dual energy X-ray absorptiometry (DEXA) were performed at baseline and then yearly. Blood count, biochemical parameters, serum concentration of pegfilgrastim (ELISA,Quantikine HS, R and D System, Inc, MN) were frequently evaluated within the first six months and afterward 3-4 times/year. Evaluation of QoL up by Short Form Health Survey questionnaire (SF-36) was performed at the beginning and at the end of the follow-up. Infectious load was calculated by infectious ratio (IR) which considers the number of documented infections over the period at risk/patient, normalized by 1000.
Results: From July 2006 to Oct 2015, 5 SCN patients (3males) diagnosed at a median age of 2 months (0-18) entered in the study. Four patients were ELANE and one HAX-1 mutated. G-CSF was given for a median of 36 months (0,23-89) and at a median dose of 7.5g/kg (5-25 g/day) before PEG. Median age at enrollment was 50 months (7-110) and median duration of PEG was 46 months (7-111). The median PEG dose was 60g/kg (50-100) given every 7-12 days. ANC rose from a median of 1000/cmm in G-CSF to 1515/cmm in PEG (p=ns). IR dropped from 9.5 in G-CSF to 5.5 in PEG (p=ns).Otitis, otomastoditis and skin abscesses were the most frequent type of infections seen during PEG.When PEG was given every 7-8 *vs* every 9-12 days ANC significantly increased (1283/cmm) over G-CSF phase (670/cmm; p=0.002). Also IR further declined with the more frequent PEG schedule (IR 6 *vs* 3.5; p=ns). The QoL questionnaire showed a marked overall improvement (100%) particularly in physical and mental performances and a relevant reduction of physical pain.PEG was never interrupted because of side effects.Biochemical parameters remained in the normal ranges, spleen size did not increase and in one patient osteoporosis developed. In the same patient a mutation of G-CSF receptor appeared after 4 years of PEG then disappeared. Peak serum concentration of PEG was achieved after 72 hr from administration, levels then declined to those of the washout phase (Pre-PEG) on day 7.
Summary/Conclusions: PEG was able to rise neutrophils and to protect patient toward severe infections enabling a better quality of life withoutside effects. PK data are probably in keeping with low risk of overexposure. Larger study and longer follow up are needed to confirm these findings.

Novel targets for MM

P638

IKAROS PROTEIN EXPRESSION IN BONE MARROW B AND T CELLS RATHER THAN MYELOMA CELLS PREDICTS OUTCOME AFTER LENALIDOMIDE-DEXAMETHASONE THERAPY

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Background: The identification of cereblon, ikaros and aiolos as central partners and targets of immunomodulatory drugs (IMiDs) represents a milestone in our understanding of the mechanisms of actions of these agents. However, although we and others noted a significant association between the expression of these molecules and response or survival, methods for a valid stratification of patients to IMiD-based therapies are still missing.
Aims: In the current study, we analysed IKAROS protein expression by using multicolour flow cytometry in myeloma cells as well as distinct cell subsets of the bone marrow microenvironment for its potential as predictive and/or prognostic marker for IMiD-based therapies.
Methods: IKAROS protein levels were analysed in stored BM samples of 16 MM patients before initiation of treatment with lenalidomide-dexamethasone. Cells were thawed, restored overnight and stained for CD3/CD38/CD45/CD56/CD138/HLA-DR or CD3/CD4/CD8/CD14/CD19/CD34/CD45. Intracellular staining of IKAROS (or the corresponding isotype control) was performed according to the BD Transcription Factor Buffer Set protocol. Live/dead cells were discriminated using BD Fixable Viability Stain 520. Analyses were performed on a FACS Canto II.
Results: IKAROS protein expression was detected in all cell subtypes analysed, including MM cells, B-lymphocytes, T-lymphocyte subsets (CD3+CD4+, CD3+CD8+, CD3+CD4+CD8+, CD3+CD4-CD8-), NK cells, monocytes and CD34+ cells. No association was observed between IKAROS protein levels and response. However, we detected a significant correlation of IKAROS protein levels and overall survival (OS) for the percentage of IKAROS positive B-lymphocytes (R=0.61, P=0.012) and CD3+CD8+ T cells (R=0.50, P=0.047). Similarly, IKAROS median fluorescence intensities (MFI) of CD3+ T lymphocytes (R=0.55, P=0.027), CD3+CD8+ T cells (R=0.53, P=0.035) as well as monocytes (R=0.55, P=0.029) significantly correlated with OS. No association was found between OS and the percentage of IKAROS positive MM cells (R=0.10 P=0.70) or MFI of MM cells (R=0.12, P=0.65). Univariate Cox regression analysis confirmed the association with survival for the percentage of IKAROS positive B-lymphocytes (P=0.014) and the MFI of CD3+ (P=0.029) and CD3+CD8+ (P=0.05) T lymphocytes as well as monocytes (P=0.017). When we stratified patients according to high or low IKAROS expression (cutoff=mean) in distinct cell subtypes we were able to define different prognostic subgroups. In particular, a higher percentage of IKAROS positive cells were associated with better outcome for B-lymphocytes (median OS 82.3 *vs* 33.6 months, P=0.01) and CD3+CD8+ T lymphocytes (median OS 82.3 *vs* 31.8 months, P=0.05). Again, no association was observed between the percentage of IKAROS positive MM cells and OS (median OS 35.6 *vs* 45.3 months, P=0.43).
Summary/Conclusions: In conclusion, our results demonstrate a significant association between IKAROS protein levels in bone marrow CD3+CD8+ T lymphocytes, B-cells and monocytes with overall survival. In contrast, no association was noted between IKAROS expression in myeloma cells and OS. This suggests that the activity of IMiDs is mainly mediated via IKAROS expression in bone marrow immune cells, which therefore could serve as prognostic marker for patients assigned to IMiD containing regimens. Based on these initial results, prospective analysis of IKAROS protein expression in an enlarged patient cohort is in progress.

P639

CXCL13 CHEMOKINE – A NOVEL TARGET IN MULTIPLE MYELOMA MICROENVIRONMENT

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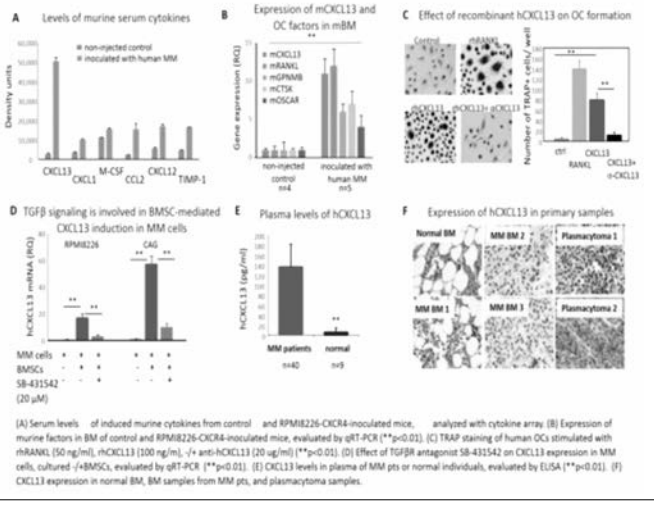
Background: Chemokine and cytokine network plays an important role in multiple myeloma (MM) pathogenesis. Studying the underlying mechanisms may help to identify new therapeutic targets to treat MM. Here we reveal B-cell chemo-attractant CXCL13 (BCA-1) being novel factor expressed in the tumor microenvironment of MM, supporting osteoclastogenesis and being associated with extramedullary progression.
Aims: To characterize the expression of CXCL13 and define its functional sequel in the MM milieu.

Methods: We utilized our recently established *in vivo* xenograft model of BM-disseminated human myeloma (using engineered CXCR4-expressing RPMI8226 cells), as well as analysis of MM cell lines, stromal components and primary samples from patients (pts) with MM.
Results: Evaluation of the cytokines in sera of MM-inoculated mice in comparison to control mice detected increased levels of the CXCL13 chemokine being the highest factor among the broad panel analyzed. Elevated mCXCL13 was also detected in bone marrow (BM) samples from the MM-bearing mice, and correlated with induced expression of murine factors associated with osteoclast (OC) activation (RANKL, NFATc, GPNMB, CTSK, OSCAR). IHC analysis of MM-occupied murine BM revealed myeloid cells being the main source of increased mCXCL13, while human RPMI8226 cells in murine BM milieu also expressed detectable levels of hCXCL13. In addition, hCXCL13 mRNA was found to be expressed by MM cell lines (n=8), BM stromal cell lines and peripheral-blood generated Mϕ. Strong induction of CXCL13 expression in both MM and stromal cells was detected upon their co-culture. Furthermore, CXCL13 expression in BMSCs and Mϕ was significantly induced following RANKL treatment; in turn, addition of CXCL13 up-regulated RANKL levels, demonstrating a positive regulation loop between CXCL13 and RANKL. Functional tests revealed the ability of CXCL13 to induce *in vitro* formation of TRAP+ OCs, while CXCL13 neutralizing antibodies blocked this effect. Furthermore, CXCL13 neutralization markedly decreased RANKL expression in BMSCs. Of note, CXCR5, cognate CXCL13 receptor, was expressed predominantly by stromal and myeloid cells, suggesting the paracrine effects of MM-generated CXCL13. Mechanistically, we found that TGFβ signaling was involved in CXCL13 induction in both MM and stromal cells. Addition of TGFβ receptor kinase inhibitor SB-431542 interfered with the activation triggered by the interaction between MM cells and stromal components and prevented the increase in CXCL13 expression. Finally, we evaluated the presence of hCXCL13 in primary MM samples. CXCL13 transcript was detected in BM aspirates from MM pts (n=20), its expression was significantly upregulated upon co-culture with BM stromal cells and correlated with expression of osteoclastogenic factors, including RANKL and MT1-MMP, an important component of OC fusion machinery. In addition, plasma level of CXCL13 was significantly higher in MM pts (n=44) (148 pg/ml±136) in comparison to normal individuals (n=9) (19 pg/ml±7.6) (p<0.001). Furthermore, IHC analysis of BM biopsies from MM pts (n=7) and plasmacytoma samples (n=6) demonstrated the expression of CXCL13 in malignant plasma cells. Importantly, CXCL13 showed markedly increased expression within plasmacytoma tissues, suggesting that elevated CXCL13 levels may be associated with extramedullary disease (Figure 1).



(A) Serum levels of induced murine cytokines from control and RPMI8226-CXCR4-inoculated mice, analyzed with cytokine array. (B) Expression of murine factors in BM of control and RPMI8226-CXCR4-inoculated mice, evaluated by qRT-PCR (**p<0.01). (C) TRAP staining of human OCs stimulated with rhRANKL (50 ng/ml), rhCXCL13 (100 ng/ml), -/+ anti-hCXCL13 (20 ug/ml) (**p<0.01). (D) Effect of TGFβR antagonist SB-431542 on CXCL13 expression in MM cells, cultured -/+BMSCs, evaluated by qRT-PCR (**p<0.01). (E) CXCL13 levels in plasma of MM pts or normal individuals, evaluated by ELISA (**p<0.01). (F) CXCL13 expression in normal BM, BM samples from MM pts, and plasmacytoma samples.

Figure 1.

Summary/Conclusions: Altogether, our data define a previously unrecognized role of CXCL13 in MM, unravel its involvement in the osteoclastogenic process and suggest CXCL13 as potential novel target for the diagnosis and treatment of MM.

P640

MESENCHYMAL STEM CELLS (MSC) PROMOTES TUMOR MICROENVIRONMENT TRANSFORMATION DRIVING GRANULOCYTE-LIKE MYELOID DERIVED SUPPRESSOR CELLS (G-MDSC) ACTIVATION IN SMOLDERING AND MULTIPLE MYELOMA PATIENTS

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Background: A well-recognized feature of multiple myeloma (MM) is the intimate relationship between plasma cells (PC) and BM microenvironment, which is mainly composed of mesenchymal stromal cells (MSC), endothelial cells, immune cells and extracellular matrix. Granulocytic-Myeloid-derived suppressor cells (G-MDSC) accumulate in the tumor microenvironment during tumor development. MDSC promote tumor growth and invasion, immunosuppression and host immune evasion by suppressing lymphocyte activation and antigen recognition. Even though it has been demonstrated that G-MDSC are increased in MM microenvironment, the role of MSC in promoting immunosuppressive microenvironment through activation of G-MDSC remains unexplored.
Aims: Analyzing MSC from MGUS, Smoldering myeloma (SMM) and MM patients in promoting tumor microenvironment transformation.
Methods: Human peripheral blood mononucleated cells (PBMC) isolated from healthy subjects (HS) were cultured alone, with HS- (n=8), MGUS- (n=6), SMM- (n=4) or MM-MSC (n=12, 8 patients at diagnosis and 4 relapsed) at 1:100 ratio. After one week, PBMC were collected. G-MDSC were isolated using anti-CD66b magnetic microbeads and the phenotype (CD11b+CD33+CD14-HLADR-) was confirmed by cytofluorimetric analysis. Immunosuppression was analyzed after incubation with autologous T cells CFSE+ stimulated by phytohaemagglutinin (PHA-P).
Results: G-MDSC educated by SMM- and MM-MSC co-cultures (MSCed-G-MDSC) exhibited suppressive effect with a reduction of T cell proliferation (p<0.001) compared to G-MDSC control (isolated from PBMC cultured in medium alone). Notably, neither MDSC control nor HS- or MGUS-MSCed-G-MDSC showed suppressive ability. Before incubation with T cells, the expression of immunomodulatory factors was investigated by real-time PCR in SMM- and MM-MSCed-G-MDSC compared to MGUS-MSCed-G-MDSC. SMM- and MM-MSCed-G-MDSC up-regulated Arg1 (56.4±18.2 and 24.9±13, p<0.001), NOS2 (82±35 and 21±18, p<0.001), TNFα (10±3 and 45.7±28.8, p<0.05) and CEBPA (90±23 and 65±19, p<0.001), a transcription factor promoting suppressive phenotype. Adding Bortezomib (5 nM) to co-culture of SMM- and MM-MSC with PBMC, isolated G-MDSC lost immunosuppressive ability. Analysis of MM-MSC from 4 patients revaluated after 3 bortezomib-based therapy followed by autologous stem cell transplantation showed that their immunological dysfunction was reverted after therapy. Since it has been reported that neutrophils can acquire monocytic characteristics in response to inflammatory signals, G-MDSC control and MSCed-G-MDSC were plated onto dentine disks (DDs) for 3 days. A significant digestive activity was observed only in DDs with MM-MSCed-G-MDSC (p=0.002) and was lost by MM-MSCed-G-MDSC isolated from co-culture with Bortezomib. Moreover, compared to MGUS-MSCed-G-MDSC, SMM- and MM-MSCed-G-MDSC up-regulated PROK2 expression (5.2±1.2 and 7.6±2, p<0.05), a chemotactic and pro-angiogenic factor. Investigating effect on angiogenesis *in vitro*, MM-MSCed-G-MDSC induced tube formation. On the contrary, this effect was not observed in the condition with MM-MSCed-G-MDSC isolated from co-culture with Bortezomib.
Summary/Conclusions: MSC from SMM and MM but not MGUS patients are able to activate G-MDSC favoring indirectly transformation of microenvironment in a "tumor" milieu with consequent immune escape and PC growth and survival. Their immunological dysfunction can be reverted by bortezomib exposure.

P641

TARGETING OF BMI-1 WITH PTC-209 AFFECTS MYELOMA CELL GROWTH & SURVIVAL AND IMPAIRS THE TUMOUR MICROENVIRONMENT

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Background: The polycomb complex protein BMI-1 was previously reported to be overexpressed in multiple myeloma (MM) and silencing of BMI-1 was shown to impair the proliferation and survival of MM cells. However, therapeutic agents specifically targeting BMI-1 are not available so far.
Aims: Here we investigated PTC-209, a novel transcriptional repressor of BMI-1, for its activity in MM.
Methods: *BMI-1* expression was analysed by using publically available gene expression (GEP) data. The anti-MM activity of PTC-209 was examined by viability testing, cell cycle analysis, annexin V and 7-AAD staining, quantification of cleaved PARP, JC-1 as well as colony formation assays. Gene and protein expression was studied by quantitative PCR and flow cytometry, respectively. The impact of PTC-209 on osteoclast, osteoblast and tube formation was analysed *in vitro* by using cell type specific differentiation assays.
Results: Overexpression of *BMI-1* in MGUS, SMM and MM patients was confirmed by using publically available GEP-datasets. Of note, *BMI-1* expression was further increased at relapse which translated into significantly shorter overall survival in relapsed/refractory patients treated with bortezomib or dexamethasone (median OS 22.2 months *vs* 13.7 months, *P*=0.003).
Treatment with PTC-209 induced downregulation of BMI-1 protein levels and significantly impaired viability of all HMCLs analysed with IC50 values <2 μM in 6 of 8 MM cell lines (range: 0.21-5.68 μM). Mechanistically, PTC-209 led to an accumulation of MM cells in the G1 phase of the cell cycle and induced apoptosis. The latter was confirmed by annexin V/7-AAD staining, detection of cleaved PARP and depolarization of the mitochondrial membrane. These alterations were

observed in conjunction with the deregulation of central myeloma genes, such as downregulation of *CCND1* (up to 0.67±0.04 fold reduction, *P*<0.001), *MYC* (up to 0.50±0.07 fold reduction, *P*<0.001) as well as upregulation of p21 (up to 3.4±0.4 fold increase, *P*<0.001), p27 (up to 2.1±0.6 fold increase, *P*=0.03) and *NOXA* (up to 3.6±1.2 fold increase, *P*=0.009). In addition, PTC-209 significantly reduced the number and size of colonies formed by myeloma cells in colony formation assays (OPM-2: 215±50 *vs* 105±12 colonies with PTC-209 at 1 μM, *P*=0.005; KMS-12-BM: 59±12 *vs* 17±3, *P*<0.001). PTC-209 likewise decreased the survival propagating effects of IGF-1 and IL-6. In co-culture with BMSCs, PTC-209 was found to uphold its anti-MM activity and enhanced the activity of pomalidomide and carfilzomib. Additive and synergistic drug activities of PTC-209 and pomalidomide/carfilzomib were confirmed in six MM cell lines. Analysing the impact of PTC-209 on cells of the myeloma microenvironment demonstrated a significant reduction of *in vitro* osteoclast and tube formation. No signs of TRAP-positive osteoclasts as well as a significantly decrease of total tube length (*P*=0.005) were observed with PTC-209 at 1 μM. Unfortunately, PTC-209 displayed a likewise negative impact on the formation of osteoblasts (68±4% reduction in alkaline phosphatase activity with PTC209 at 1 μM, *P*<0.001). However, this was associated with a significant induction of DKK1 expression upon PTC-209 treatment and concurrent treatment with an anti-DKK1 antibody was found to overcome (43±6% *vs* 21±12% decrease in ALP activity, *P*=0.02), at least in part, the osteoblast inhibitory properties of PTC-209.

Summary/Conclusions: In conclusion, we confirmed overexpression of BMI-1 in MM highlighting its role as attractive drug-target and reveal therapeutic targeting of BMI-1 by PTC-209 as a promising novel therapeutic intervention for MM.

P642

LENALIDOMIDE MAINTENANCE COUNTERACTS PD-1 ELEVATION ON LYMPHOCYTES IN MYELOMA PATIENTS

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Background: Lenalidomide (Len) has become an integral component of multiple myeloma (MM) therapy with its tumoricidal and immunomodulatory effects, the latter being of particular interest in continuous treatment. However, the precise mode of action and the consequences of Len on lymphocyte subset composition and function are still incompletely understood.

Aims: Here, we analyzed the composition and PD-1 expression of lymphocytes from MM patients undergoing Len maintenance (LenMT) and compared those to the respective parameters in control groups of MM patients (at primary diagnosis/PDX, at relapse/RD or in complete remission/CR; according to IMWG criteria) and healthy donors.

Methods: We performed multi-parameter FACS analysis on peripheral blood (PB) of 71 MM patients to identify CD4+ and CD8+ naïve (45RA+62L+), central (45RO+62L+) and effector (45RO+62L-) memory T cells, B cells (CD19+), NK cells (CD3-CD56+), NKT cells (CD3+CD56+) and γ/δ T cells (γ/δ TCR+) and their PD-1 surface expression. Corresponding bone marrow (BM) samples from the same individuals were equally analyzed for lymphocytic PD-1 surface expression when available (n=15). Analysis was performed in the following subgroups of patients: at PDX (15), at RD (12), in sustained CR without recent anti-MM therapy (18) and during LenMT (20). The fifth subgroup comprised patients without exposure to Len and a disease burden that was comparable to the cohort of patients during LenMT who were not in CR (6). All patients provided written informed consent according to the local regulatory boards.

Results: At PDX, absolute numbers of lymphocytes were found within the normal ranges for T, B and NK cells, while the administration of LenMT significantly reduced CD3+ T cells counts. This was predominantly due to a loss of CD4+ naïve T cells (p=0.004), whereas CD8+ naïve T cells and the entire central and effector memory T cell compartments were largely preserved. B cell counts were highest in the CR group, with an approximately fourfold increase in regard to the LenMT group. Absolute numbers of NK cells were significantly higher in patients undergoing LenMT as compared to all other previously treated MM patients. Analyzing PD-1 surface expression, we found highest levels on CD3+ T cells, no matter whether the lymphocytes were of BM or PB origin. We further observed increased PD-1 expression on all PB lymphocytes from patients with active MM disease (PDX, RD), whereas optimal disease control (CR) brought PD-1 expression down to ranges observed in healthy donors. Similarly, the LenMT cohort displayed lower PD-1 levels than patients with active disease, which was most pronounced for CD8+ T cells. In order to rule out the influence of disease burden on PD-1 levels, we compared patient cohorts with equivalent residual BM and serological disease burden with and without exposure to Len and found a significant reduction of PD-1 surface expression on CD8+ T cells during LenMT (p=0.038). Longitudinal data of PD-1 levels before, during and after LenMT further confirmed this observation.

Summary/Conclusions: Our data show that LenMT modulates lymphocyte subset composition in MM patients, underlining its potent immunomodulatory capacity. Furthermore, this first *in vivo* observation of low PD-1 expression levels associated with LenMT suggests a potential for combined use of Len with novel therapeutic approaches, such as chimeric antigen receptor modified T cells or bispecific T cell engaging antibodies. Whether additional checkpoint blockade might further enhance anti-MM efficacy of Len by decoupling the PD-1/PD-L1 axis will have to be studied in future clinical trials.

P643

CIRCULATING SERUM MIRNA PROFILE IN AL AMYLOIDOSIS

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Background: Circulating serum microRNAs (miRNAs) are emerging biomarkers in cancer as well as a minimally invasive diagnostic solution due to their high stability and association with the disease. Previously, circulating miRNAs with diagnostic and prognostic potential in a broad range of monoclonal gammopathies have been described. Nevertheless, circulating miRNAs profile in immunoglobulin light chain amyloidosis (ALA) is still not elucidated. ALA is a plasma cell dyscrasia characterized by deposition of amyloid fibrils in various organs and tissues, derived from monoclonal immunoglobulin light chains (LC), leading to organ dysfunction.

Aims: To reveal ALA specific profile of circulating miRNAs

Methods: MiRNAs profiling using TaqMan Low Density Arrays was used to find differently expressed miRNAs between 15 ALA, 6 MGUS, 10 MM patients and 11 healthy donors (HD). Data normalization was performed using geometric mean of three most stably expressed miRNAs (miR-126, miR-24, miR-484). Kruskal-Wallis and Mann-Whitney U test were used to define significance. Receiver operating characteristic (ROC) analysis of chosen miRNAs was performed to describe their predictive potential.

Results: In general, 50 miRNAs were differentially expressed in ALA compared to MM, MGUS and HD (p<0.05) and 12 of them have met significance criteria after Bonferroni correction (p<0.0004). Based on high specificity and sensitivity values (≥80%), five miRNAs were defined to have prognostic value: miR-21, miR-25, miR-328, miR-451, miR-134 (Figure 1). Additionally, in ALA samples, four following miRNAs were detected as significantly overrepresented compared to the rest of tested cohorts: miR-134 (sensitivity 93.3% and specificity 81.5%, p=0.0005), miR-133a (sensitivity 73.3% and specificity 74.5%, p=0.0052), miR-342 (sensitivity 100% and specificity 59.3%, p=0.0001) and let-7b (sensitivity 66.7% and specificity 77.8%, p=0.0044) (Figure 1). We suppose that these overrepresented miRNAs could have a biological relevance in ALA pathobiology. Serum miR-134 and miR-133a were described as markers of acute coronary syndrome (Gacoń *et al.*, 2015); let-7b was found to be serum marker for primary IgA nephropathy (Serino *et al.*, 2015), while serum miR-342 was described as a biomarker for the diagnosis of Alzheimer's disease (Tan *et al.*, 2014). It is postulated that extracellular amyloid beta (Aβ) deposits are the fundamental cause of this disease. This finding raises the intriguing possibility that all amyloid diseases, irrespective of the nature of the amyloid forming protein, share common biomarkers.

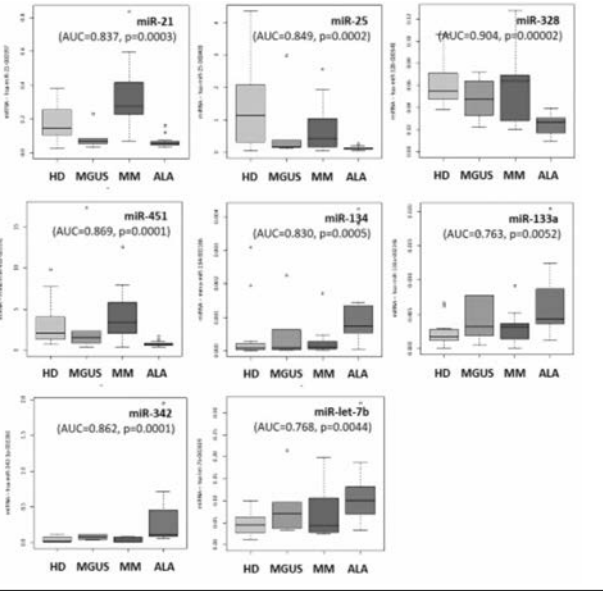

Figure 1.

Summary/Conclusions: This study identified five serum miRNAs with a potential to distinguish ALA patients from MM, MGUS and healthy donors with high sensitivity and specificity. Moreover, we described increased level of miR-134, miR-133a and let-7b that could serve as biomarkers of end-organ damage in ALA patients. Level of serum miR-342 can be suggested as a potential amyloid-specific biomarker and needs to be validated on the other amyloid diseases.

Acknowledgments: This work was supported by the Ministry of Health (15-29667A), Institutional Development Plan of University of Ostrava (IRP201550) and MH CZ - DRO - FNOs/2015-2016.

P644

PRECLINICAL EVALUATION OF ABBV-838, A FIRST-IN-CLASS ANTI-CS1 ANTIBODY-DRUG CONJUGATE FOR THE TREATMENT OF MULTIPLE MYELOMA

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Background: ABBV-838 is an antibody-drug conjugate (ADC) directed against CS1 (SLAMF7/CD319), a cell-surface glycoprotein that is expressed with high levels and prevalence on multiple myeloma (MM) cells, with normal expression limited to leukocyte subsets (including plasma cells, NK cells, and activated monocytes). This ADC uses monomethyl auristatin E (MMAE) with a cathepsin-cleavable linker, and it binds to a unique epitope on CS1.
Aims: We evaluated the preclinical anti-tumor activity and toxicology of ABBV 838, with a goal to enable Phase 1 clinical development of this novel anti-CS1 ADC.
Methods: Tumor efficacy studies of ABBV-838 monotherapy and in combination with standard-of-care agents were conducted in mouse xenograft models. Tolerability studies were conducted in rodents and non-human primates (NHP), and the IND-enabling toxicology studies were conducted in NHP.
Results: ABBV-838 showed significant antitumor activity and induced complete tumor regressions in mouse models using human myeloma xenografts, including disseminated bone marrow models (OPM-2 and LP-1). Enhanced efficacy was observed when a sub-optimal dose of ABBV-838 was combined with standard-of-care agents such as bortezomib and pomalidomide. Tumor regressions were also observed in xenograft-bearing mice that were pretreated for two weeks with bortezomib, then switched to treatment with ABBV-838 alone, or pomalidomide plus ABBV-838. To optimize efficacy and to minimize safety issues, an additional process step was used to isolate ABBV-838 product containing primarily two MMAE molecules per antibody (identified as E2). Efficacy and tolerability studies were conducted to compare different drug-to-antibody ratios (DAR) and distributions of loading; the greatest therapeutic index (maximum tolerated dose/minimum effective dose) observed in non-clinical studies was achieved with E2-enriched ADC. In a GLP-compliant toxicology study, primary toxicities of ABBV-838 were typical of other MMAE-based ADCs and consisted of bone marrow toxicity and lymphocyte decreases in lymphoid tissues, and were mostly resolved or consistent with a regenerative response at the end of a 3-week recovery period. A single-dose no-observed-adverse-effect level (NOAEL) was established in cynomolgus monkey at 12 mg/kg (1340 µg/m² of conjugated MMAE), which is the equivalent conjugated MMAE dose for the single-dose max non-lethal dose of 6 mg/kg as published for brentuximab vedotin (Figure 1).

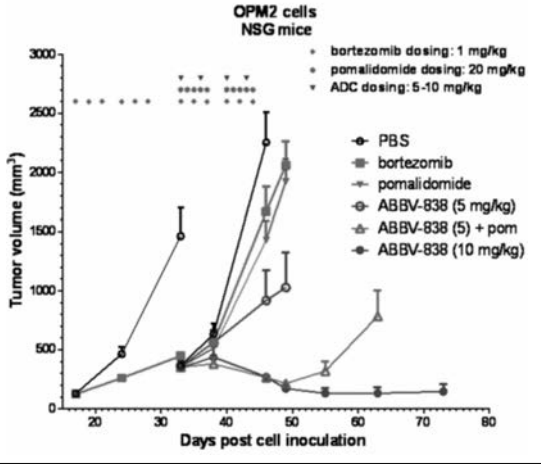

Figure 1.

Summary/Conclusions: Targeting myeloma cells via the CS1 protein with ABBV-838 results in potent preclinical anti-tumor activity as a monotherapy, and in combination with multiple approved therapies, with complete tumor regressions against multiple xenograft models of myeloma. ABBV-838 is currently being investigated in a Phase 1 first-in-human safety study.
Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

P645

SOLUBLE BCMA BINDS ITS LIGAND BAFF AND PREVENTS NORMAL ANTIBODY PRODUCTION IN MULTIPLE MYELOMA PATIENTS

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Background: A hallmark of multiple myeloma (MM) is the low levels of uninvolved immunoglobulin (Ig) levels. B-cell maturation antigen (BCMA) is a receptor expressed in mature non-malignant and malignant B lymphocytes, including plasma cells. We previously demonstrated that BCMA is present in the serum of MM patients and that its levels predict overall survival (Sanchez *et al.* Br J Haematol 2012).
Aims: We hypothesized that soluble BCMA binds BAFF, preventing normal plasma cell development and antibody production in MM patients.
Methods: BCMA-Fc and control Ig were obtained (R&D Systems). Human BCMA and mouse BAFF, IgG, IgA and IgM were measured with ELISA (R&D Systems & Bethyl). Human IgA and IgG levels were determined using nephelometry (Immage 800, Beckman Coulter). Hevylite Assays (Binding Site) were used to quantify the levels of heavy0light chain isoform pairs.
Results: hBCMA-mBAFF complexes were detected with an ELISA at high levels in plasma of SCID mice with human MM xenografts; these mice showed reduced levels of free BAFF. rhBCMA or control Ig-Fc (100 µg) was injected into immune competent C57 Bl or Balb/c mice, and resulted in decreases in IgA, IgG and IgA levels. Decreases in IgA levels were observed when compared to baseline levels on days 4 and 6 (P=0.0031 and P=0.0064, respectively),, and the controls (P=0.0087 and P=0.0221). Mouse IgG levels also showed a reduction compared to baseline (P=0.0023), the Ig-Fc (P=0.0014) and the control (P=0.0129). IgM levels showed similar decreases when compared to the untreated (P=0.0001) and Ig-Fc (P=0.0088) groups. MM patients' serum containBCMA-BAFF complexes, and showed reduced free BAFF levels compared to controls (P=0.0004). Raji B-cells were then incubated with serum from a healthy subject (0.02 µg/ml BCMA) of a MM patient with serum containing high levels of BCMA (0.75 µg/ml) or low levels of BCMA (0.02 µg/ml) or rhBCMA-Fc (3.0 µg/ml) in the presence of rhBAFF (500 ng/ml). Serum from the MM patient with high BCMA and rhBCMA decreased rhBAFF binding by 71% (from 96.8 to 25.6%) and 74% (from 96.8 to 22.9%), respectively, whereas serum from the healthy subject and MM patient with low BCMA did not affect BAFF binding. Using antoher human B-cell line (TB94), serum containing high BCMA and rhBCMA decreased rhBAFF detection on these cells from 94% to 62.1% and from 98.6% to 48.7%, respectively. Given the reduction in BAFF binding to B-cells and reduction in serum free BAFF levels in MM patients, we determined whether the relationship of serum BCMA levels to uninvloved Ig levels in MM pts. Serum BCMA levels inversely correlated with uninvolved IgG in IgA MM (n=134) and uninvolved IgA in IgG MM (n=313, P <0.0001). Using Hevylite Assay, similar results were observed in BCMA levels compared to uninvolved IgG isoforms in both pts with involved IgG lambda (n=62, P=0.0006) and IgG kappa (n=117, P <0.0001).
Summary/Conclusions: We demonstrate BCMA-BAFF complexes in MM xenografts resulting in reduced BAFF levels, and administration of rhBCMA to normal mice leads to marked reductions in antibody levels. We also show these complexes in MM patients, resulting in reduced serum free BAFF levels which prevent this ligand from binding B-cells. As a consequence, serum BCMA levels inversely correlate with uninvolved Ig levels in MM pts. Thus, the lack of normal antibody production in MM pts results in part from corculating BCMA binding its ligands, preventing production of normal antibody-producing cells.

P646

DIFFERENTIAL EFFECTS OF ELOTUZUMAB (ANTI-SLAMF7) AND ANTI-CD38 MONOCLONAL ANTIBODIES IN PRECLINICAL MODELS

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Background: Monoclonal antibodies (mAbs) have recently presented a new treatment paradigm for patients with multiple myeloma (MM). Two such approaches, anti-SLAMF7 antibody (elotuzumab) and anti-CD38 antibodies (daratumumab and SAR650984), were primarily developed based on the rationale of high expression of target protein on myeloma cells. Expression of these protein targets (SLAMF7 and CD38), however, is not limited to tumor cells and is also found on cells of the innate and adaptive immune systems. Non-tumor-targeted effects mediated by these mAbs may not only contribute to the overall anti-tumor response, but may also provide the rationale for new combination regimens.
Aims: To further define the mechanism of action of elotuzumab and anti-CD38 mAbs on immune cell subsets.

Methods: The effect of elotuzumab and anti-CD38 mAbs on antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity, and natural killer (NK) and T-cell activation was assessed. Surrogate antibodies of daratumumab (daratumumab-s) and SAR650984 (SAR650984-s) were generated on the basis of protein sequences, as published in their corresponding patent families, and were expressed as recombinant hIgG1 for in-vitro studies with human cells or mIgG2a for in-vivo mouse tumor studies. Mutant versions of mAbs that eliminated Fc:FcγR binding were also generated to assess the contribution of FcR binding for mAb efficacy in preclinical models.
Results: SLAMF7 and CD38 were highly expressed on myeloma cells. *In vitro*, elotuzumab and anti-CD38 mAbs were equally potent in inducing ADCC of MM1S cells, whereas only daratumumab-s was able to fix complement. SLAMF7 and CD38 were also highly expressed on human NK cells. In a co-culture of myeloma cells and NK cells, elotuzumab induced more pronounced NK-cell activation than did daratumumab-s. SLAMF7 and CD38 were differentially expressed on T-cell subsets: SLAMF7 expression was highest on $CD8^+$T cells and memory subsets, whereas CD38 expression was highest on naïve cells. While both SLAMF7 and CD38 levels were induced following anti-CD3 stimulation, neither elotuzumab nor anti-CD38 mAbs mediated ADCC towards activated T cells. Elotuzumab and anti-CD38 mAbs, however, led to inhibition of cytokine production by naïve T cells isolated from healthy donors, and these effects were reversed in the presence of lenalidomide. Ongoing studies are characterizing the effects of elotuzumab and anti-CD38 mAbs on T cells from bone marrow aspirates from patients with MM.
Summary/Conclusions: Elotuzumab enhanced activation of NK cells *in vitro*, whereas anti-CD38 mAbs were cytolytic against NK cells. Engagement of both targets on myeloma cells led to similar augmentation of ADCC. SLAMF7 and CD38 engagement on T cells may also have non-overlapping effects based on the differential expression of these proteins between naïve and memory subsets, which may inform the rationale for future clinical use.
Funding: Bristol-Myers Squibb. Medical writing assistance: M Thomas, Caudex, funded by Bristol-Myers Squibb

P647

NRF2 ACTIVITY IN BONE MARROW MESENCHYMAL STROMAL CELL PROTECTS MULTIPLE MYELOMA FROM CARFILZOMIB AND BORTEZOMIB INDUCED CELL DEATH

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Background: Bone marrow mesenchymal stromal cells (BM-MSC) interact with multiple myeloma (MM) cells and modify their viability and drug sensitivity. Recent studies identify various oncogenic pathways which are involved in modulating the BM-MSC/MM cell microenvironment. The transcription factor NRF2 is a key regulator for the maintenance of intra-cellular redox homeostasis and is negatively regulated by proteasome degradation through its inhibitor KEAP1. NRF2 pathways have been shown to contribute to the malignant phenotypes of several cancers through effects on proliferation and drug sensitivity.
Aims: To determine if NRF2 signalling in BM-MSCs affects the redox homeostasis of MM and promotes survival and resistance to proteasome inhibitors.
Methods: Primary human MM and BM-MSC cells were obtained under UK ethical approval (LREC ref 07/H0310/146). Transwell co-culture system was used to characterize the MM-BMSC interactions. NRF2 protein expression and its target genes were examined by Western blotting and qRT-PCR. CellTiter-Glo luminescent cell viability assay and apoptosis assay (PI/ Annexin V staining by flow cytometry) were performed. Lentiviral mediated shRNA knockdown (KD) of NRF2 in the BM-MSC.
Results: To study the cell-cell communications between BM-MSC and MM cells we established a co-culture system using primary MM derived from patients BM and the equivalent stromal cells. We also used MM cell lines. NRF2 was found to be highly expressed in primary BM-MSC derived from patients with MM. We found that both bortezomib and carfilzomib further increased NRF2 activity in both primary MM cells and BM-MSC. In addition treatment with proteasome inhibitors enhanced NRF2 target gene expression as measured by qRT-PCR and Western blotting. We showed that BM-MSC protect MM from bortezomib and carfilzomib induced cell death. Next we knocked down NRF2 (NRF2-KD) in both MM and BM-MSC and then performed a co-culture with and without bortezomib and carfilzomib. We found that BM-MSC derived NRF2 was essential for MM protection from bortezomib and carfilzomib induced cell death in-vitro.
Summary/Conclusions: NRF2 driven cytoprotective responses are activated in BM-MSC and MM by bortezomib and carfilzomib. Furthermore, NRF2-KD in BM-MSC reverses MM resistance to bortezomib and carfilzomib. This highlights the importance of NRF2 in regulating MM drug resistance within the bone marrow microenvironment through independent actions in both the tumour and the non-malignant stromal cells which support it.

Innovative therapies for MM 3

P648

PHASE 1B STUDY OF AN ALTERNATIVE LIQUID FORMULATION OF ACY-1215 (RICOLINOSTAT) IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR RELAPSED-AND-REFRACTORY MULTIPLE MYELOMA

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Background: Ricolinostat (ACY-1215), a liquid, selective HDAC6 inhibitor, potently synergizes with bortezomib (Btz), lenalidomide (Len) and pomalidomide (Pom) in models of multiple myeloma (MM) (Quayle Blood 2013). Pan-HDAC inhibitors are active in MM in combination with Btz and Len, but toxicities (thrombocytopenia, fatigue, and GI events) limit dosing exposure. By contrast, ricolinostat is well tolerated in clinical studies as monotherapy (Raje Blood 2012) and in combination with Btz/dexamethasone (Dex) (Vogl, Blood 2015), Len/Dex (Yee, Blood 2015) and Pom/Dex (Raje, Blood 2015) and acts synergistically even in refractory patients. ACE-MM-104 is a dose-escalation study of an alternative liquid formulation (ALF) of ricolinostat at a concentration of 10 mg/mL in combination with Pom/Dex in patients with relapsed or relapsed-and-refractory MM (RRMM). This formulation has an improved excipient profile over earlier formulations.
Aims: Determine safety, tolerability and preliminary efficacy of ricolinostat ALF in combination with Pom/Dex.
Methods: Dose escalation of ricolinostat ALF was in combination with Pom (4 mg) for 21/28 day cycle and weekly Dex (40 mg). Patients (pts) had measurable disease, ≥2 prior therapies including ≥2 cycles of Len and proteasome inhibitor and relapsed or RRMM. Adequate bone marrow, hepatic and renal function were required. Pts with non-secretory MM, prior Pom or HDAC inhibitor therapy were excluded. Blood samples were obtained for PK and pharmacodynamics (PD) assessment of acetylated tubulin and histones. Starting dose of 120 mg BID was based on preclinical data and experience with ricolinostat in either 20 or 12 mg/mL formulations currently used in clinical trials. Median age was 64 years and median number of prior regimens was 2 (1-4). Ten pts were refractory to prior therapies (8 to Dex, 6 to Btz, 6 to Len, and 2 to both BTz and Len).
Results: Dose escalation comprised 16 pts. No DLTs were observed in the first 4 pts dosed at 120 mg ricolinostat BID. Emerging data with the 12 mg/mL formulation suggested that BID dosing was less well tolerated. Therefore, subsequent pts were dosed on a QD schedule: 4 at 120 mg, and 8 at 180 mg. Toxicities were mostly low grade and treatment was tolerated at QD dosing of 21/28 day cycles. Common toxicities of safety-evaluable pts (n=15) included fatigue (60%), URI, pruritus, dyspnea, dizziness (27% each), diarrhea, constipation, nausea (20% each). Grade 3/4 related toxicities included neutropenia (5 pts, 33%), anemia (27%), febrile neutropenia and neutropenia (20%). Few TEAEs were attributed to study drug. One DLT (neutropenia) was observed at 180 mg QD. Cohort expansion to 6 pts showed no further DLT. PK/PD demonstrates selective inhibition of HDAC6 *vs* HDAC1,2,3 at therapeutic doses. PK shows 120 mg of ricolinostat ALF and 160 mg in the 12 or 20 mg/mL formulations achieved equivalent exposure which did not increase with increased dosing. Hence, the 180 mg QD schedule was declared the highest dose level tested. There was no evidence of ricolinostat accumulation or drug-drug interaction with Pom. With a median F/U of 7 cycles for the 11 response-evaluable patients there were 6 ≥PR and DCR was 82%.
Summary/Conclusions: Ricolinostat ALF is well-tolerated in combination with Pom/Dex at doses up to 180 mg QD without major toxicities. This formulation of ricolinostat is used in ongoing clinical studies in combination with paclitaxel and abraxane to treat solid tumors.

P649

DEVELOPMENT OF A PREDICTIVE MODEL TO IDENTIFY PATIENTS WITH MULTIPLE MYELOMA NOT ELIGIBLE FOR AUTOLOGOUS TRANSPLANT AT RISK FOR SEVERE INFECTIONS USING DATA FROM THE FIRST TRIAL

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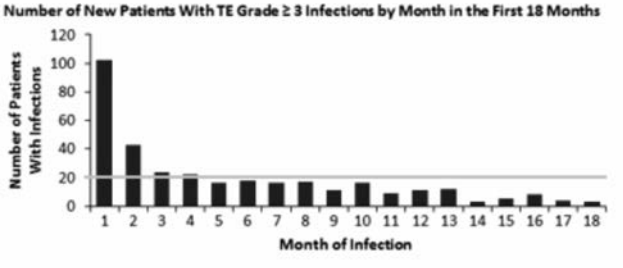
Background: Patients (pts) with multiple myeloma (MM) have an increased risk of infections due to toxicities of therapy, immunosuppression by the underlying disease, comorbidities, and old age (Nucci & Anaissie, *Clin Infect Dis*, 2009). Furthermore, MM demonstrates a low immune response to prophylactic vaccination and to both viral and bacterial infections, highlighting the need for preventative or early treatment measures (Karlsson *et al., Clin Vaccine Immunol*, 2011; Blimark *et al., Haematologica*, 2015). The pivotal, phase 3 FIRST trial compared lenalidomide plus low-dose dexamethasone (Rd) to melphalan, prednisone, and thalidomide (MPT) in transplant-ineligible pts with newly diagnosed MM (NDMM).
Aims: To identify factors predictive of infections in the first 4 months of the FIRST trial and develop an infection risk scoring system.
Methods: In the FIRST trial, pts were randomized 1:1:1 to receive Rd until disease progression (Rd continuous), Rd for 18 cycles (Rd18), or MPT. For this analysis, data from the Rd continuous and Rd18 arms were pooled. Demographics, medical history, and baseline characteristics including co-medications were analyzed to identify risk factors of treatment emergent (TE) grade ≥3 infection during the first 4 months. Pts with both progression-free survival ≤4 months and no TE grade ≥3 infection within the first 4 months were excluded. A subgroup discovery algorithm was used to identify univariate prognostic factors associated to high or low risk of infection. The most clinically and biologically relevant variables were used in a multivariate logistic regression model with an iterative variable selection process. Pts with missing data on at least one input variable were excluded from the model (n=9). From the resulting predictive model, a scoring system was developed by allocating -2 to 2 points to factors of low or high risk based on their influence in the model. The cumulative score classified pts into high (2 to 5 points) or low (-3 to 1 points) infection risk groups.
Results: Of the 1623 pts enrolled in the FIRST trial, 340 pts had TE grade ≥3 infections, and 56.2% of these 340 pts had their first infection in the first 4 months (Figure 1A). Risk of infection was similar regardless of therapeutic arm (*P*=.53). Risk factors were assessed in 1378 pts and the final model retained 4 variables: (Eastern Cooperative Oncology Group [ECOG] performance status, serum β2-microglobulin [Sβ2M], lactate dehydrogenase [LDH], and hemoglobin) that independently associate with TE grade ≥3 infections occurring in the first 4 months (Figure 1B). The high- and low-risk groups defined by the scoring system were associated with significantly different rate of TE grade ≥3 infections during the first 4 months (24% and 7%, respectively).



B Multivariate Logistic Regression Model for TE Grade ≥ 3 Infection Within the First 4 Months

Model	Pr(>|z|)	Points	Infection Risk
Sβ2M ≤ 3 mg/L	0.02135	−2	Lower
ECOG = 0	0.06231	−1	Lower
Hemoglobin ≤ 11 g/dL	0.07660	1	Higher
ECOG ≥ 2	0.01583	1	Higher
LDH ≥ 200 U/L	0.00299	1	Higher
Sβ2M ≥ 6 mg/L	3.09E-06	2	Higher

Figure 1.

Summary/Conclusions: The risk scoring system described here can identify pts at risk for infections during the first 4 months of treatment and may be used to implement risk-adapted strategies for the treatment or prevention of infections.

P650

OUTCOME OF MYELOMA PATIENTS WITH DELETION 17P – IMPACT OF BASELINE CHARACTERISTICS, TREATMENT AND ADDITIONAL CHROMOSOMAL CHANGES

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Background: Deletion 17p13, del(17p), is associated with poor outcome in patients with multiple myeloma (MM) but some patients show long-term survival.
Aims: With the current study we intended to identify prognostic factors for survival in patients with del(17p). Analyzed factors included baseline characteristics, treatment modalities and additional chromosomal aberrations.
Methods: Between 05/2002 and 10/2015 total of 4906 interphase FISH (iFISH) studies have been performed in patients with plasma cell diseases at the University Hospital of Heidelberg, Germany. We identified 110 patients with del(17p) in newly diagnosed, symptomatic MM according to CRAB criteria of the International Myeloma Working Group. FISH was performed on CD138-purified plasma cells using probes for: 1q21, 5p15, 5q35, 8p21, 9q34, 11q23, 13q14.3, 15q22, 17p13 and 19q13, translocations t(11;14), t(4;14) and t(14;16). Hyperdiploidy (HD) was assessed by the score of Wuilleme *et al.* (gains of at least two of the chromosomes 5, 9 and 15). Survival times were estimated with the Kaplan-Meier method and compared using log-rank test. Multivariable Cox regression analysis was performed to assess impact of baseline characteristics, treatment modalities and additional cytogenetic abnormalities on outcome.
Results: Age >65 years (n=42, 38%) was associated with adverse outcome (PFS: 13 *vs* 26 months, p=0.001; OS: 21 *vs* 56 months, p<0.001) as well as higher ISS score (ISS I/II/III PFS: 35/ 20/ 16 months, p=0.02; OS: 72/ 71/ 27 months, p=0.004) and elevated baseline LDH (PFS: 14 *vs* 26 months, p<0.001; OS: 18 *vs* 53 months, p=0.001). Patients with subclonal (10-60% of plasma cells, n=47, 42.7%) del(17p) had longer PFS than patients with del(17p) in a major >60% of plasma cells (26 *vs* 19 months, p=0.03). Additional gain of 1q21 (n=41, 37.3%) was associated with shorter PFS (17 *vs* 25 months, p=0.01, Figure 1A). Hyperdiploidy (n=36, 37.9%) did not ameliorate impact of del (17p) (Figure 1B), but gain 19q13 (n=41, 37.6%) predicted longer PFS (30 *vs* 18 months, p=0.01) and OS (50 *vs* 29 months, p=0.01, Figure 1C). Multivariate analysis in transplant eligible patients (≤65 years, n=68, 61.8%) revealed better survival for patients treated with upfront autologous transplantation (hazard ratio, [95% confidence interval]: 0.15 [0.04, 0.58], p=0.006) and a trend for better survival for bortezomib induction therapy (0.35 [0.11, 1.08], p=0.07). Application of maintenance therapy was associated with better survival in transplant-eligible patients (0.3 [0.09, 0.99], p=0.05).

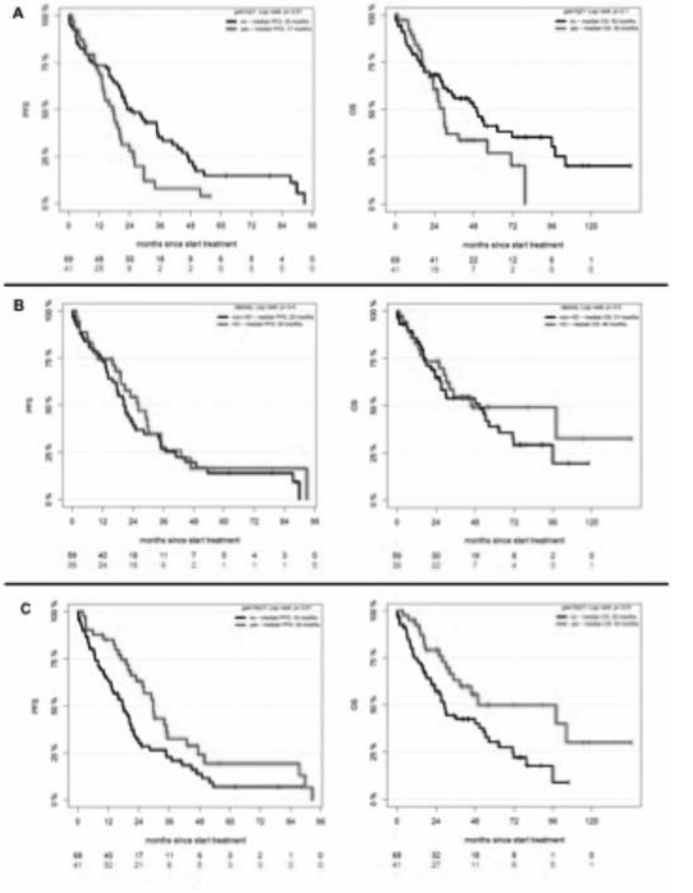

Figure 1.

Summary/Conclusions: We demonstrate heterogeneous outcome of patients with del(17p) according to baseline characteristics and treatment. 19q13 should be included in routine FISH panel, since gains were associated with better survival. Bortezomib-based induction therapy followed by autologous stem cell transplantation and maintenance therapy might be the best treatment option for patients with del(17p).

P651

CIRCULATING TUMOR DNA IN MULTIPLE MYELOMA

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Background: For decades, diagnosis and therapy for multiple myeloma has been guided by sequential measurements of monoclonal immunoglobulin (M-protein) as a marker for total tumor mass. However, the increasing availability of mutation specific drugs and insight into the complex genetic architecture of cancer raises a need for disease monitoring at the clonal and mutational levels. Circulating tumor DNA (ctDNA) has shown promise to this end in solid tumors, but its biology and dynamics over time are poorly understood and only preliminary reports are available for myeloma.
Aims: To evaluate the properties of ctDNA as a tumor marker in myeloma by comparison with M-protein levels and sequential whole exome sequencing (WES) of bone marrow plasma cells.
Methods: We measured ctDNA levels in stored Serum (n=278), EDTA-plasma (n=8) and citrate-plasma (n=2) samples from 22 patients by digital droplet (dd) PCR. Target mutations for each patient had been previously identified by WES or PCR of bone marrow plasma cells and were mainly in the NRAS, KRAS and BRAF genes. Blood samples were processed and frozen at -80° C within 1.5 hours of acquisition and then stored for a median of 5 years (range 0-23) before DNA isolation. Fragment length analysis by Bioanalyzer 2100 (Agilent technologies) was performed to ensure sample quality. DdPCR was performed using the QX100/200-system with Prime PCR mutation detection assays according to manufacturer's instructions (BioRad). Samples were called mutation-positive if they contained more than 1 single-positive mutant droplet, yielding a specificity of 98.4% based on control experiments.
Results: There was no correlation between sample storage time and the quantity of amplifiable DNA, and fragment length analysis revealed the presence of circulating DNA in 30 representative samples. Mutation levels in 6 time-matched serum and EDTA-plasma samples were similar. Taken together, these observations validate our material. Known tumor mutations were detected by ddPCR of ctDNA in all 14 patients analyzed at diagnosis and in 11/12 patients with relapsed disease. Compared with the average 55% sensitivity in early stage solid tumors by similar methods, newly diagnosed myeloma behaves like an advanced solid tumor with regards to ctDNA. Mutated allele frequency measured by WES of bone marrow plasma cells correlated with the level of ctDNA at the same time (Spearman´s rho=0.507, p=0.003). Furthermore, we detected two mutations in ctDNA that were not present in time-matched bone marrow aspirates, indicating that a single bone marrow aspirate may not be representative with respect to the heterogeneity of the tumor. We found a striking co-variation between ctDNA and M-protein for periods up to seven years in 9 out of 11 patients where sequential samples were available. In many cases, ctDNA reacted more rapidly to changes in tumor mass compared to M-protein although this could not be systematically assessed in a retrospective material. Peak mutation levels in ctDNA started out low at diagnosis and gradually increased with each relapse in 7 out of 9 patients. This observation was particular evident in three patients where ctDNA levels increased to around 400 times diagnostic levels at the end of the disease course as illustrated by patient 292 (Figure 1). Interestingly, in this patient we also observed increasing levels of ctDNA immediately following the initiation of effective chemotherapy, including a previously undetectable IRF4 mutation.

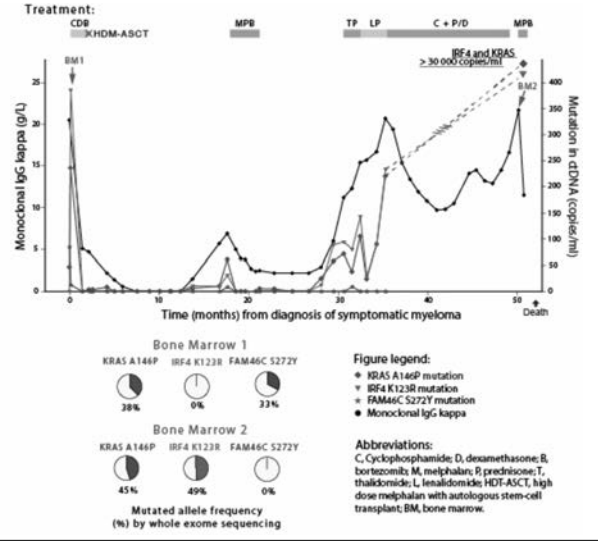

Figure 1. Co-variation of serum mutations and M-protein in patient 292.

Summary/Conclusions: We show that ctDNA is a marker for tumor mass in myeloma, as evidenced by the co-variation with M-protein. The good performance of ctDNA in this study, despite long storage time and sub-optimal samples (serum rather than EDTA-plasma), adds to its robustness as a tumor marker. Our data suggest that myeloma is particularly suited for using ctDNA to access the tumor genome, as tumor mutations can be detected in almost all patients. Increased ctDNA-levels immediately following the initiation of effective therapy may be exploited for very early evaluation of treatment response and to increase the sensitivity of ctDNA analysis.

P652

SINGLE-AGENT IBRUTINIB IN RITUXIMAB-REFRACTORY PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA (WM): UPDATED RESULTS FROM A MULTICENTER, OPEN-LABEL PHASE 3 SUBSTUDY (INNOVATETM)

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Background: Single-agent ibrutinib, a once-daily oral inhibitor of Bruton's tyrosine kinase (BTK), demonstrated durable responses in previously treated patients (pts) with WM (Treon, *N Engl J Med.* 2015), leading to its FDA and EU approval in WM. *MYD88 L265P* is a commonly identified mutation in pts with WM; the resulting mutated protein signals through interleukin-1 receptor-associated kinase 1 and BTK, leading to constitutive activation of the NF-κB pathway (Yang, *Blood.* 2013). Ibrutinib attenuates the MYD88-BTK interaction, thus blocking BTK-dependent downstream signaling and inducing apoptosis of WM cells (Treon, *N Engl J Med.* 2012).
Aims: To evaluate the efficacy and safety of single-agent ibrutinib in WM pts considered refractory to their last rituximab-containing therapy (defined as relapse at <12 months or failure to achieve at least a minor response).
Methods: After informed consent, pts with confirmed diagnosis of WM, symptomatic disease requiring treatment (Kyle, 2003), refractory to rituximab, hemoglobin ≥8 g/dL, IgM ≥0.5g/dL, platelets ≥50,000 cells/mm^3, absolute neutrophil count ≥750 cells/mm^3, adequate hepatic function, and ECOG ≤2 received oral ibrutinib 420 mg daily until progression or unacceptable toxicity. Pts with central nervous system involvement, clinically significant cardiovascular disease, known bleeding disorder, treatment for WM ≤30 days prior to first dose were excluded. Primary endpoints were PFS, ORR, hemoglobin improvement, and OS.
Results: Baseline characteristics are shown in the Table 1 (N=31). Common reasons for treatment initiation were fatigue unrelieved by rest (74%), constitutional symptoms (42%), anemia (42%), and lymphadenopathy (23%); 4 pts started therapy due to peripheral neuropathy. Most recent therapies included monotherapy (32%), combination therapy without antibody (29%), and combination therapy with antibody (39%). At a median follow-up of 12.7 months, ORR was 84%, with a major response rate (MRR; defined as ≥PR) of 68%, and the estimated 1-year PFS rate was 93%. Initial analysis of 23 baseline samples identified MYD88^{MUT}/CXCR4^{WT} in 16 pts and MYD88^{MUT}/CXCR4^{WHIM} in 6 pts. The ORR and MRR were 88% *vs* 83% and 75% *vs* 83%, respectively. One of the 23 pts had MYD88^{WT}/CXCR4^{WT} and achieved a best response of stable disease. Four of 5 pts were able to discontinue required plasmapheresis for disease control at the end of cycle 1. Baseline median hemoglobin of 10.3 g/dL increased to 11.4 g/dL after 1 cycle and reached 12.8 g/dL at Week 49. Median IgM of 3830 mg/dL declined by >50% after 1 cycle, with continued improvement over time (median 920 mg/dL at Week 49). Any-grade adverse events (AEs; >15%) included diarrhea (42%); upper respiratory tract infections, hypertension, increased tendency to bruise (23% each); nausea, thrombocytopenia, neutropenia (19% each); and pyrexia, arthralgia, back pain (16% each). Common grade ≥3 AEs included neutropenia (13%), hypertension (10%), anemia and diarrhea (6%

each). All pts with Grade ≥3 cytopenias had received at least 4 prior therapies. Serious AEs occurred in 9 (29%) pts. Four (13%) pts had dose reductions. Four pts discontinued ibrutinib—2 pts due to progressive disease and 2 due to an AE (gastrointestinal AL amyloidosis and diarrhea*)*. Overall, 87% continue on ibrutinib, with no events of IgM flare, atrial fibrillation or major bleeding (Figure 1).

Table 1. Baseline characteristics.

	N=31
Median age, years (range)	67 (47-90)
Age ≥ 70 years, n (%)	11 (35)
ECOG, n (%)	
0-1	25 (81)
2	6 (19)
IPSSWM, n (%)	
Low	7 (23)
Intermediate	11 (35)
High	13 (42)
Median serum IgM, mg/dL (range)	3830 (740-10700)
Median β2-microglobulin, mg/L (range)	3.6 (1.7-24)
Median hemoglobin levels, g/dL (range)	10.3 (6.4-14.6)
Median platelet count, 10^9/L (range)	218 (51-896)
Median absolute neutrophil count, 10^9/L (range)	2.9 (0.7-15.4)
Median number of prior therapies (range)	4 (1-8)
Types of prior therapy (> 50%) , n (%)	
Rituximab	31 (100)
Corticosteroid	25 (81)
Alkylating agent	25 (81)
Prior autologous stem cell transplant, n (%)	2 (6)

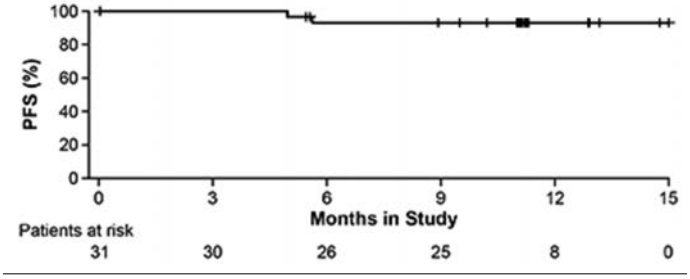

Figure 1. Kaplan-Meier analysis of PFS (all treated patients).

Summary/Conclusions: Single-agent ibrutinib is highly active in this heavily pretreated rituximab-refractory WM population, with a high ORR and manageable safety profile consistent with previous studies.

P653

POMALIDOMIDE, BORTEZOMIB, AND LOW-DOSE DEXAMETHASONE IN PROTEASOME INHIBITOR–EXPOSED AND LENALIDOMIDE-REFRACTORY MYELOMA: RESULTS OF A MULTICENTER, DOSE-ESCALATION, PHASE 1 TRIAL (MM-005)

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Background: The combination of an immunomodulatory drug with the proteasome inhibitor (PI) bortezomib (BORT) and low-dose dexamethasone (LoDEX) has demonstrated preclinical synergy and considerable clinical activity in relapsed and refractory multiple myeloma (RRMM) (Mitsiades *et al., Blood*, 2002;99:4525-30; Richardson *et al., Blood*, 2014;123:1461-9). Treatment (Tx) with pomalidomide (POM)+LoDEX has been shown to delay disease progression and extend survival in patients (pts) with myeloma previously treated with lenalidomide (LEN) and BORT (Richardson *et al., Blood*, 2014;123:1826-32; San Miguel *et al., Lancet Oncol*, 2013;14:1055-66). Preliminary results of MM-005 showed that POM+BORT+LoDEX (PVd) was effective and well tolerated in LEN-refractory and BORT-exposed pts. Previously, subcutaneous (SC) BORT demonstrated non-inferiority to intravenous (IV) BORT and an improved safety profile in pts with RRMM (Moreau *et al., Lancet Oncol,* 2011;12:431-40). Thus, MM-005 included a cohort that received PVd with SC BORT.
Aims: To identify the optimal dose of PVd for a phase 3 trial in pts with RRMM, to assess the efficacy and safety of PVd, and to evaluate SC administration of BORT within the PVd regimen.
Methods: Pts must have provided informed consent and received 1-4 lines of prior Tx, with ≥2 consecutive cycles of LEN plus a PI. Pts had to be PI exposed and refractory to LEN but not to BORT. A 3+3 design with 21-day cycles was used to determine the maximum tolerated dose (MTD). In cycles 1-8, dose-escalation cohorts received POM (1-4 mg/day on days 1-14), IV or SC BORT (1-1.3 mg/m^2 on days 1, 4, 8, and 11), and LoDEX (20 mg/day, or 10 mg/day for pts aged >75 years, on days 1, 2, 4, 5, 8, 9, 11, and 12) until progressive disease (PD) or unacceptable adverse event (AE). After cycle 8, BORT was given on days 1 and 8, and LoDEX was given on days 1, 2, 8, and 9. The primary endpoint was MTD.
Results: Of the 34 pts enrolled, 59% were male, and median age was 58.5 years (range, 36-76 years). The median number of prior anti-myeloma Tx lines was 2 (range, 1-4). All pts were refractory to LEN, and all were exposed to prior PI (97% received prior BORT and 6% received prior ixazomib). All pts discontinued Tx, mostly due to PD (n=23) but none due to Tx-related AEs. No dose-limiting toxicities were reported in the dose-escalation cohorts or at the maximum planned dose (MPD) of POM 4 mg, BORT 1.3 mg/m^2, and LoDEX 20 mg (10 mg for pts aged >75 years). The median number of Tx cycles received was 9 (range, 2-36) for all pts and, in the MPD cohorts, was 11 (range, 2-19) with IV BORT (n=10) *vs* 8 (range, 3-15) with SC BORT (n=12). The overall response rate (≥partial response [PR]) for all pts was 65% (n=22), with 2 complete responses (CRs), 1 stringent CR, 11 very good PRs, and 8 PRs; median duration of response was 7.4 months. All pts achieved at least stable disease. In the MPD cohorts, grade 3/4 AEs were more frequent with IV BORT *vs* SC BORT (90% *vs* 75%), including neutropenia (80% *vs* 25%), thrombocytopenia (40% *vs* 17%), and pneumonia (30% *vs* 8%). There were no reports of grade 3/4 peripheral neuropathy (PN) or deep vein thrombosis (DVT).
Summary/Conclusions: PVd was highly effective in pts with LEN-refractory and PI-exposed myeloma. PVd was well tolerated, with no grade 3/4 PN or DVT and no Tx discontinuation due to Tx-related AE. AEs were generally less frequent with SC *vs* IV BORT. Thus, the favorable tolerability and efficacy of PVd, a potential new therapeutic option in pts with RRMM, is being further evaluated in a large phase 3 trial (MM-007).

P654

PROSPECTIVE COMORBIDITY AND FUNCTIONAL GERIATRIC ASSESSMENT (CF-GA) IN MULTIPLE MYELOMA (MM) PATIENTS (PTS): RESULTS FROM A MULTICENTER GERMAN STUDY GROUP MM (DSMM) TRIAL

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Background: Cancer pts present with a highly heterogeneous health status and today treatment choices are numerous. Therefore, careful assessment of individuals' condition is relevant. Moreover, in order to define best tolerable treatment options, novel metrics for non-disease variables are needed. Albeit impairment in the Karnofsky Performance Status (KPS), Activities of Daily Living (IADL or ADL) and quality of life (QoL) are predictive for outcome in cancer pts, the prognostic variables within a defined and prospectively assessed battery of established functional tests have rarely been meticulously assessed in MM.
Aims: Our aim is to establish a prospective comorbidity and functional geriatric assessment (CF-GA) to more objectively rate fitness and pts' biological age, rather than the chronological age alone in MM. We assessed a battery of defined tests in German DSMM study centers in newly diagnosed (ND) MM pts.
Methods: This trial of DSMM centers performed a thorough, prospective comorbidity and functional geriatric assessment (CF-GA) in consecutive ND MM pts. This GA was performed prior to initiation of anti-myeloma treatment and reflected pts' baseline health status. The CF-GA included the IADL, ADL, Timed Up and Go Test (TUGT), malnutrition, pain, rating of fitness, SF12-QoL and geriatric depression scale (GDS). Moreover, established comorbidity (CM) scores, namely: ß2MG/eGFR (EJH 2009;83:519-27), Kaplan Feinstein (KF), Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), Charlson Comorbidity Index (CCI), initial-Myeloma Comorbidity Index (I-MCI) and revised-MCI (R-MCI) were assessed. The CF-GA was performed as a screening tool to assess pt fitness as well as to predict survival and toxicities. The trial protocol was approved by the ethics committees of the Freiburg University. All patients provided written informed consent and all procedures were conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization and Guidelines for Good Clinical Practice.
Results: Characteristics of a total of 238 pts, currently included in this CF-GA, were typical for tertiary centers with a median age of 62 years (27-84) and 94% with symptomatic disease: the median hemoglobin was 11.0g/dl (5-17), eGFR 72ml/min/1.73qm (7-163), ß2-MG 3.8mg/l (0.8-38.4) and BM infiltration 40% (0-95). The baseline frailty assessment revealed a median KPS of 80% (30-100) and physicians'- and patients'-scored fitness was rated with 3 (1-6). Median functional results for the IADL were 8 (1-8), for the ADL 5 (2-6), for pain 2

(0-10), for malnutrition 4 (0-14) and for cognitive deficiency via Mini Mental State Examination (MMSE) 28 (15-30). The median GDS was 2 (0-13) and TUGT 10 (4-80). Median CM scores were substantially different with an I-MCI of 1 (0-3), ß2MG/eGFR of 1 (0-2), KF of 1 (0-3), CCI of 2 (0-8), HCT-CI of 2 (0-8) and R-MCI of 4 (0-9). In age subgroups >60 and >70 years, mean R-MCI and IMWG did increase the most and much lesser with HCT-CI and KF use, confirming the latter's lesser usefulness in MM (Figure 1). According to our current results in 238 pts, highly relevant CF-GA-tools in MM are the TUGT, R-MCI and IMWG score. Since any CF-GA is time-consuming, we have created a web account that allows easy and prompt assessment of the R-MCI (https://myelomacomorbidityindex.org) within 1-2 minutes.

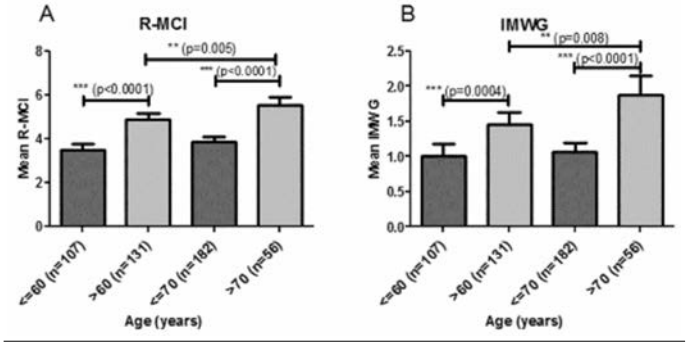

Figure 1. Mean results in different comorbidity indices in age groups.

Summary/Conclusions: To the best of our knowledge, this is the first prospective multicenter GA that accumulates most valuable MM-specific disease risks, frailty tests and comorbidity scores. Our current results in 238 pts showed that the most relevant and compromised parameter in MM are: KPS, renal function, osteolyses, pain, malnutrition, TUGT, frailty, ADL and fitness, whereas lung function, MMSE, GDS, ADL seem less informative nor substantially impaired. As combined CM test, most discriminative are the R-MCI and IMWG that reveal intermediate and unfit pts and increase with age, whereas almost all pts via HCT-CI and KF appeared fit. Most predictive CF-GA tools should be included in future clinical trials.

P655

IMPACT OF CYTOGENETIC RISK ON PFS2 OF PATIENTS TREATED WITH DIFFERENT THERAPY SEQUENCES: RESULTS OF A POST-HOC ANALYSIS

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Background: Pending the availability of second generation new drugs in clinical practice, currently patients with MM who relapse after induction therapy can be treated with bortezomib or lenalidomide-based combinations besides up front therapy. However, the impact of the different therapy sequences induction-first salvage therapy on PFS2 is not well known and even less it has been defined which is the most appropriate therapy sequence according to cytogenetic risk at diagnosis.
Aims: To analyze the impact on PFS2 of the therapeutic sequence induction-first salvage therapy in patients who were enrolled up front in 2 prospective trials (VMP *vs* VMPT-VT, MPR *vs* Mel-200) stratified according to cytogenetic risk by FISH.
Methods: Among patients included in the above prospective trials, 366/523 (70%) had available FISH analysis at diagnosis. One hundred and eight patients (29.5%) were classified at high cytogenetic risk (HR), defined by the presence of t(4;14) and/or del17p, whereas all the remaining 258 patients were considered as having a standard risk (SR). Overall, 45 patients (12%) were retreated with the same agent used up front (R-group), 94 (26%) received bortezomib as induction and lenalidomide at first relapse (B-L group), 159 (43.5%) received lenalidomide up-front followed by bortezomib at first relapse (L-B group) and 68 patients (18.5%) underwent bortezomib up-front followed by chemotherapy or thalidomide (B-suboptimal group).
Results: Both in HR (PFS2=22 months) and in SR group (PFS2=28 months), B-suboptimal sequence resulted in a significantly shorter PFS2 compared with all others treatment groups. Among patients receiving B-L sequence, HR patients had a PFS2 comparable with that of SR group (PFS2=42 *vs* 58 months; p=0.138). On the contrary, in the group treated with L-B sequence, adverse cytogenetics resulted in a significant shorter PFS2 if compared with SR group (PFS2=39 *vs* 48 months; p=0.061). Similar findings were observed in the R-group in which PFS2 of HR and SR patients was 39 and 64 months, respectively (p=0.008). Considering HR patients, no significant difference in term of PFS2 was seen between R-group and B-L group whereas PFS2 of L-B group resulted significantly shorter compared both with R-group (p=0.029) and B-L group (p=0.043).
Summary/Conclusions: The choice of appropriate therapy up-front and in first relapse is of utmost importance to maximize PFS2 in patients with MM. Cytogenetic risk should be take into account in this regard since outcome of patients with standard cytogenetic risk features seems to be not affected by therapy sequences whereas, patients with high-risk features, had a better outcome only if Bortezomib was used upfront.

P656

A FRAILTY SCALE PREDICTS OUTCOMES IN TRANSPLANT-INELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH CONTINUOUS LENALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE IN THE FIRST (MM-020) TRIAL

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Background: For patients (pts) with multiple myeloma (MM), older age and the presence of comorbidities have been associated with a poorer prognosis. A frailty scale was recently described that categorized pts with MM as fit, intermediate, or frail based on age, comorbidities, and physical and cognitive functioning (Palumbo *et al., Blood*, 2015). The frailty score was a predictor of death and toxicity. The pivotal phase 3 FIRST trial showed that treatment with lenalidomide plus low-dose dexamethasone (Rd) until disease progression (Rd continuous) prolonged progression-free survival (PFS) and overall survival (OS) *vs* melphalan, prednisone, and thalidomide (MPT) in transplant-ineligible pts with newly diagnosed MM (NDMM).
Aims: To examine the outcomes in pts in FIRST based on frailty score severity.
Methods: Pts in the FIRST trial provided informed written consent and were randomized to receive Rd continuous, Rd for 18 cycles (Rd18), or MPT. An algorithm based on the previously described frailty scale was used to categorize pts into 3 severity groups: fit, intermediate, and frail. Baseline pt characteristics included in the algorithm were age, Charlson Comorbidity Index score, and self-care and usual activities from the EQ-5D questionnaire (as proxies for the Activities of Daily Living and Instrumental Activities of Daily Living scales). Pts missing data on ≥1 variable were excluded (n=106). This analysis evaluated PFS and OS outcomes between treatment arms within each severity group using a data cutoff of March 3, 2014.

A

Breakdown of Severity Groups by Treatment Arm			
Severity Group	Rd Continuous (n = 506)	Rd18 (n = 504)	MPT (n = 507)
Frail, %	56.3	50.6	54.0
Intermediate, %	26.5	33.9	28.2
Fit, %	17.2	15.5	17.8

B

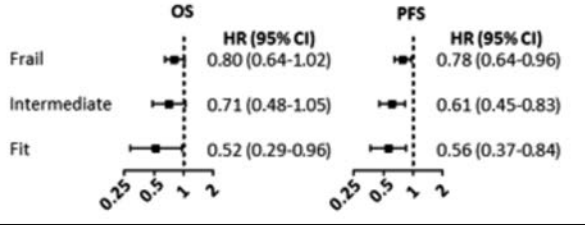

Figure 1.

Results: Of 1623 total pts, 1517 were included in the analysis, and the majority of these pts were frail compared with fit or intermediate (54% *vs* 17% *vs* 30%, respectively). Breakdowns based on severity group were similar across treatment arms (Figure 1A). Compared with fit or intermediate pts, frail pts were older and had higher Eastern Cooperative Oncology Group performance status scores, higher International Staging System (ISS) stage, higher lactate dehydrogenase levels, and worse renal function. OS was improved in fit *vs* frail pts (hazard ratio [HR], 0.42; *P* <.0001) and intermediate *vs* frail pts (HR, 0.62; *P* <.0001). Treatment with Rd continuous prolonged PFS and OS compared with

MPT for all severity groups (Figure 1B). Rd continuous reduced the risk of progression or death *vs* MPT by 44%, 39%, and 22% in fit, intermediate, and frail groups, respectively. Fit pts had a lower risk of grade 3 or higher (grade 3+) non-hematologic adverse events (AEs) compared with frail pts (HR, 0.77; *P*=.0021). The risk of grade 3+ hematologic AEs was similar for all levels of frailty. Pts treated with Rd continuous had a similar risk of grade 3+ non-hematologic AEs compared with those treated with MPT for all severity groups. The risk of grade 3+ hematologic AEs was lower with Rd continuous than with MPT in the fit, intermediate, and frail groups. Pts within each severity group were further divided based on ISS stage: fit pts were subdivided into stage I *vs* II/III and intermediate and frail pts into stage I/II *vs* III. Combining ISS stage with frailty classification further improved prognostic assessment of pts within each severity group for both PFS and OS.

Summary/Conclusions: This analysis of the FIRST trial population supports the use of the frailty scale for predicting risk of death in pts with NDMM. PFS and OS benefits were seen with Rd continuous *vs* MPT across all levels of frailty, with the greatest benefits seen in fit pts. These data support the use of continuous therapy with Rd as a standard of care for pts with transplant-ineligible NDMM.

Innovative therapies for MM 4

P657

PROSPECTIVE EVALUATION OF GERIATRIC ASSESSMENT TOOLS IN REAL-WORLD, UNSELECTED, ELDERLY PATIENTS WITH SYMPTOMATIC MYELOMA

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Background: Geriatric assessment (GA) collects data related to the medical, psychosocial and functional capabilities of the elderly patients, and provides tools for treatment and care decisions. Based on data from patients who participated in clinical trials, a "Frailty score" was developed by the IMWG, by using a simplified GA with 3 tools (Katz Activity of Daily Living (ADL), the Lawton Instrumental Activity of Daily Living (IADL) and the Charlson Comorbidity Index (CCI)) (Palumbo *et al.,* Blood 2015).

Aims: To prospectively evaluate different GA tools and comorbidity indices in consecutive, unselected, "Real-World", patients >65 years.

Methods: The following tools were used: G8 geriatric assessment screening tool (G8-GAS), VES-13, GDS, Katz ADL, Lawton IADL, MMSE, KPS (%), ECOG PS, number of falls in the past 1 & 6 months, lower-extremity function and disability in elderly tool, nutritional assessment tools (DETERMINE and Mini Nutritional Assessment), social support score, cognition evaluation tools (MMSE), Geriatric Depression Scale and comorbidity indices (CCI, CIRS-G, ACE-27 tool).

Results: Since January 2012, 144 consecutive patients >65 years diagnosed with symptomatic MM in the Department of Clinical Therapeutics, University of Athens, had a GA. The median age was 76 years (range 66-92); 55% were males; 26% had ISS-1, 24% ISS-2 and 50% ISS-3 and 19% (of 121 with available) had high risk cytogenetics (del17p or t(4;14)) while 22% had eGFR<30 ml/min/1.73 m^2. Treatment was IMiDs-based in 47% (thalidomide in 13% and lenalidomide in 34%) and proteasome inhibitor-based in 53%. PR or better was achieved by 78% of evaluable patients. Two year overall survival (OS) was 71%. Advanced age was associated with shorter OS and risk of early death (<3 months) (early death rates were 3%, 8% and 20% for patients ≤70 *vs* 71-80 *vs* >80 years). ISS stage was associated with OS (p=0.004) but not high risk cytogenetics (p=0.714) or different types of primary therapy (p=0.593).
Per the IMWG "frailty score", 29% were fit, 17% intermediately fit and 54% frail with 2-year OS of 77%, 81% and 62% respectively. The distribution in frailty scores was different than that of patients in the IMWG cohort (39%, 31% & 30%), probably because our patients were unselected and older. Several different GA tools showed prognostic significance in univariate analysis, such as the number of falls in the past 6 months (p=0.002), lower extremity function (p=0.014), mini nutritional assessment (p=0.014), G8-GAS (p<0.001), KPS <50% (p<0.001), ECOG PS >2 (p=0.04) and MMSE (p=0.024). There was an association of early death with KPS ≤50% (p=0.003), ECOG PS >2 (p=0.05), Geriatric depression score (p=0.018) and G8-GAS score (p=0.015). IMWG "frailty score" was not associated with early death. In multivariate analysis, which included ISS and age, the number of falls in the past 6 months (HR: 4.7, p=0.007) and the score of the G8-GAS tool (HR: 4.7, p=0.004) were independent factors for survival. Addition of cytogenetics did not improve the multivariate model. IMWG "frailty score" in a multivariate analysis, which included ISS stage, did not have independent statistical significance for OS.

Summary/Conclusions: In elderly myeloma patients, GA with G8-GAS tool provides prognostic information related to the risk of early death and overall survival, independently from disease characteristics and the treatment type. GA can be simple and provides a strong tool which may be useful for prognostication and treatment decisions in elderly patients.

P658

IS THERE A PROGNOSTIC IMPACT OF THE CEREBLON EXPRESSION IN MULTIPLE MYELOMA?

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Background: The introduction of new treatment modalities have changed significantly the perspective of multiple myeloma (MM) patients. The factors that

are associated with inferior treatment response and possible drug-resistance are currently in the focus of investigation.

Aims: The aim of the study was to analyze the prognostic significance of CRBN expression in MM patients ineligible for autologous stem cell transplantation (ASCT).

Methods: The study included 92 newly diagnosed MM patients (median age 67 years, range 35-80 years; 44 male/48 female) with following distribution: IgG myeloma had 55 patients (59.8%), IgA 18 (19.6%), light chains 16 (17.4%), and IgD 2 patients (2.2%). Advanced III clinical stage (CS, Durie&Salmon) had 68 patients (73.9%), II 18 (19.6%), and 6 (6.5%) had symptomatic I CS. Of 92 patients, 24 (26.1%) had ISS score 1, 28 (30.4%) ISS 2 and 40 (43.5%) ISS 3. Elevated LDH level was present in 18/92 patients (19.6%). High risk chromosomal abnormalities (CA, iFISH): t(4; 14), del(17p), were detected in 14 patients (15.2%). Renal impairment existed in 16 patients (17.4%). The CRBN expression was analyzed on the samples of bone marrow aspirate by RT-PCR method. All patients were treated with novel agents within three-drug combinations with alkylating agents (melphalan or cyclophosphamide) and corticosteroids (dexamethasone or prednisolone). Thalidomide based combinations were applied in 77 patients (83.7%) while 15 patients (16.3%) were treated with bortezomib based combinations. The patients were ASCT ineligible due to the age, high comorbidity index, progressive disease or personal attitude.

Results: Overall treatment response (CR/VGPR/PR/MR) was achieved in 77 patients (83.7%). Median CRBN expression for patients with CR, VGPR, PR, MR and PD, in the group treated with thalidomide- or bortezomib based combinations, was 3.13, 2.65, 2.08, 1.85 and 0.97 respectively; and 2.63, 3.00, 3.04, and 1.35, respectively. Treatment response correlated with high CRBN expression (p=0.011), mainly in the group treated with thalidomide based combinations (p=0.013), while in the group treated with bortezomib based combinations such correlation was not found (p=0.665). The most discriminative cut-off value of CRBN expression selected by the ROC analysis was 1.39 (sensitivity 41% and specificity 81.8%, AUC value 0.643, 95% CI 0.502–0.785, p=0.05). Low CRBN expression (≤1.39) had 22/92 patients (23.9%), 19/77 of thalidomide group (24.7%), and 3/15 (20%) of bortezomib group. The presence of high risk CA wasn't associated with lower CRBN expression (p=0.35). Patients with low CRBN expression (≤1.39), treated with thalidomide based combinations, had significantly shorter progression free survival (PFS) compared to patients with high CRBN expression (median of PFS 22 months *vs* median not reached, Log Rank=5.70, p=0.017). In this group, low CRBN expression wasn't associated with inferior OS (Log Rank=2.93, p=0.087). Regarding bortezomib treated patients, there wasn't observed impact of CRBN expression neither on PFS (Log Rank=0.063, p=0.80), nor on OS (Log Rank=0.036, p=0.85). Among significant variables (ISS, LDH, CA, and CRBN), Cox regression analysis has confirmed that low CRBN expression has the most significant prognostic influence on PFS (HR, 3.5; 1.40-8.77; p=0.008) in patients treated with thalidomide based combinations.

Summary/Conclusions: Indicating possible suboptimal treatment outcome, the level of CRBN expression may represent additional prognostic tool in personalized treatment approach to individual myeloma patient.

P659

CARFILZOMIB AND DEXAMETHASONE *VS* SUBCUTANEOUS BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: SECONDARY ANALYSIS FROM THE PHASE 3 STUDY ENDEAVOR (NCT01568866)

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Background: Subcutaneous (SC) delivery of bortezomib (BTZ) has been shown non-inferior to intravenous (IV) delivery in terms of efficacy while offering improved safety (Moreau, *et al.* Lancet Oncol 2011;12:431-40). The phase 3 ENDEAVOR study demonstrated a significant improvement in progression-free survival (PFS) for carfilzomib and dexamethasone (Kd) *vs* IV/SC BTZ and dexamethasone (Vd) in patients with relapsed or refractory multiple myeloma (RRMM) (Dimopoulos, *et al.* Lancet Oncol 2016;17:27-38). Currently, most BTZ use in MM is SC, and most relapsed MM patients had prior exposure to BTZ.

Aims: We present results of a subset analysis of the efficacy and safety of Kd *vs* SC Vd in the ENDEAVOR study consistent with current standard of care; the effect of prior exposure to BTZ was also investigated.

Methods: After providing informed consent, patients with RRMM (1-3 lines of therapy) were randomized 1:1 to Kd or Vd. The analysis compared Kd patients who had selected SC BTZ delivery pre-randomization if randomized to Vd arm with Vd patients who used SC BTZ. Kd arm received carfilzomib (30-min IV infusion) on days 1, 2, 8, 9, 15, and 16 (20 mg/m^2 on days 1 and 2 of cycle 1; 56 mg/m^2 thereafter) and dexamethasone (d) 20 mg on days 1, 2, 8, 9, 15, 16, 22, and 23 of a 28-day cycle. Vd arm received BTZ 1.3 mg/m^2 on days 1, 4, 8 and 11 and d (20 mg) on days 1, 2, 4, 5, 8, 9, 11, and 12 of a 21-day cycle. The primary endpoint was PFS. Secondary endpoints included overall survival (OS), overall response rate (ORR), rate of grade ≥2 peripheral neuropathy (PN), and safety.

Results: A total of 929 patients were randomized to Kd (n=464) or Vd (n=465); of these, 360 Vd patients received SC BTZ. Among 464 Kd patients, 356 patients selected SC route of BTZ administration if randomized to Vd arm. Median PFS has not been reached for Kd but was 9.5 months (mo) for Vd patients treated with SC BTZ (hazard ratio [HR]: 0.58; 95% confidence interval [CI], 0.46–0.72). Median PFS for Kd *vs* SC Vd was 13.4 mo *vs* 8.4 mo for patients with prior BTZ exposure (HR: 0.66; 95% CI, 0.50–0.87). Median OS has not been reached for Kd but was 24.3 mo for SC Vd (HR: 0.75; CI, 0.53–1.08). ORR was 76.1% (Kd) *vs* 64.4% (SC Vd), and 70.4% (Kd) *vs* 62.1% (SC Vd) for patients with prior BTZ use. Grade ≥2 PN rates were 6.5% (Kd) *vs* 33.3% (SC Vd), and 6.2% (Kd) *vs* 29.1% (SC Vd) for patients with prior BTZ. Grade ≥3 adverse events (AEs) were 74.4% (Kd) *vs* 67.5% (SC Vd), and 71.8% (Kd) *vs* 64.5% (SC Vd) for patients with prior BTZ. Results are shown in Table 1. Figure 1 shows Kaplan–Meier PFS curves.

Table 1. Outcomes

			Prior BTZ	
	Kd (n=356)	SC Vd (n=360)	Kd (n=196)	SC Vd (n=203)
Median PFS, mo	Not reached	9.5	13.4	8.4
HR for Kd *vs* SC Vd (95% CI)	0.58 (0.46–0.72)		0.66 (0.50–0.87)	
ORR, % (95% CI)	76.1 (71.3–80.4)	64.4 (59.3–69.4)	70.4 (63.5–76.7)	62.1 (55.0–68.8)
Grade ≥2 PN rate, %	6.5	33.3	6.2	29.1
Odds ratio (95% CI)	0.139 (0.09–0.22)		0.160 (0.08–0.31)	
Grade ≥3 AEs[a], %	74.4	67.5	71.8	64.5

[a]*Safety population was 355 (Kd) and 360 (SC Vd), and 195 (Kd) and 203 (SC Vd) in the prior BTZ group.*

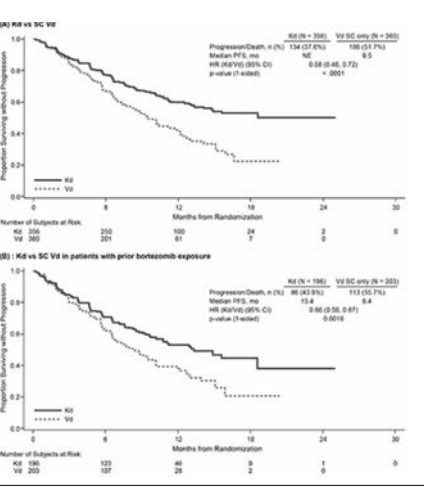

Figure 1. Kaplan-Meier PFS curves.

Summary/Conclusions: Treatment with Kd led to prolonged progression-free survival *vs* Vd patients who were administered SC BTZ. The use of Kd also led to higher response rates, a trend for prolonged OS, and lower rate of grade ≥2 PN *vs* SC Vd. In patients with prior BTZ exposure, Kd treatment resulted in longer PFS, greater ORR, and decreased PN *vs* SC Vd. These results suggest that Kd has a favorable benefit-risk profile and delivers superior efficacy and improved clinical outcomes compared with SC Vd for RRMM regardless of prior BTZ treatment.

P660

PATIENT-REPORTED QUALITY OF LIFE WITH IXAZOMIB-LENALIDOMIDE-DEXAMETHASONE (IRD) *VS* PLACEBO-RD IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS IN THE GLOBAL, PLACEBO-CONTROLLED TOURMALINE-MM1 STUDY

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Background: In the double-blind, placebo-controlled, phase 3 TOURMALINE-MM1 study (NCT01564537) in 722 patients with relapsed/refractory multiple myeloma (RRMM) following at least one prior therapy, IRd was associated with a significant improvement in PFS *vs* placebo-Rd (HR 0.742, p=0.012), together with limited additional toxicity (Moreau *et al.,* ASH 2015). Data from this study led to approval by the US FDA of ixazomib, in combination with Rd, in this setting.
Aims: Despite recent advances in treatment, no quality-of-life (QoL) data are available from double-blind, randomized, placebo-controlled studies in RRMM. A secondary endpoint of TOURMALINE-MM1 was to assess patient-reported QoL using the EORTC Quality of Life Questionnaire (EORTC QLQ-C30) and MM-specific MY-20 module.
Methods: Patients with RRMM were randomized 1:1 to receive IRd or placebo-Rd (ixazomib 4 mg or matching placebo on days 1, 8, and 15, plus lenalidomide 25 mg on days 1–21 and dexamethasone 40 mg on days 1, 8, 15, and 22, in 28-day cycles) until disease progression or unacceptable toxicity. Patients completed the EORTC QLQ-C30 and MY-20 questionnaires at screening, the start of cycles 1 and 2 and every other cycle, the end of treatment, and every 4 weeks until progression.
Results: Over a median follow-up of 23 months, QoL was maintained from baseline with both treatment regimens. Mean EORTC QLQ-C30 global health status scores for the intent-to-treat population were similar for the IRd *vs* placebo-Rd arms, suggesting the addition of ixazomib did not impact QoL (Figure 1). For the EORTC QLQ-C30 functioning scales, linear mixed model analysis of least squares (LS) mean change from baseline showed similar results over time for the IRd and placebo-Rd arms: 0.4 *vs* -1.3 for physical, -1.3 *vs* -3.0 for role, 1.0 *vs* -1.6 for emotional (p<0.05), -3.5 *vs* -5.7 for social, and -4.2 *vs* -4.6 for cognitive functioning scales (higher values indicate better functioning). For the physical, emotional, and social scales, significantly higher mean scores (p<0.05) were seen in the IRd *vs* placebo-Rd arm at several time points, suggesting better QoL with IRd. In line with the limited additional toxicity seen with IRd *vs* placebo-Rd (74%/47% *vs* 69%/49% of pts had grade ≥3 adverse events [AEs]/serious AEs), EORTC QLQ-C30 symptom scale scores were similar for IRd *vs* placebo-Rd, LS mean change from baseline was 1.8 *vs* 4.2 for appetite loss, 3.5 *vs* 5.1 for constipation, 14.5 *vs* 10.4 (p<0.05) for diarrhea, 1.0 *vs* 2.0 for dyspnea, -0.3 *vs* 0.9 for financial difficulties, 0.4 *vs* 2.4 for fatigue,1.1 *vs* 0.5 for insomnia, 1.6 *vs* 0.6 for nausea/vomiting, and -2.8 *vs* -2.3 for pain scales (lower scores indicate lower symptom burden). Diarrhea (which was mostly grade ≤2) did not appear to impact patient perception of overall QoL. Consistent results were also seen with the MY-20 measure: LS mean change from baseline in the IRd *vs* placebo-Rd arms was -3.3 *vs* -4.3 for body image, 8.1 *vs* 4.9 for future perspective (p<0.05) (higher scores indicate better QoL), -5.8 *vs* -5.5 for disease symptoms, and 3.8 *vs* 3.4 for side effects of treatment (lower scores indicate lower symptom burden). Post-hoc analyses showed that, in both arms, changes in global health status and several functioning and symptom scores were significantly correlated with response to treatment.
Summary/Conclusions: In the double-blind, placebo-controlled, phase 3 TOURMALINE-MM1 study in patients with RRMM, patient-reported QoL was maintained with the addition of ixazomib to Rd, which also provided a significant clinical benefit, thus improving patient outcome.

P661

WEEKLY CARFILZOMIB WITH DEXAMETHASONE FOR PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS FROM THE PHASE 1/2 STUDY CHAMPION-1 (NCT01677858)

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Background: Carfilzomib, an irreversible proteasome inhibitor, is approved as a single agent and in combination with dexamethasone in the United States (US) for the treatment of relapsed/refractory and relapsed multiple myeloma (MM), respectively and in the US and Europe in combination with lenalidomide (LEN)/dexamethasone for the treatment of relapsed MM. The approved dose/schedule of carfilzomib is a twice-weekly, 10-min intravenous (IV) infusion on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle (starting dose: 20mg/m^2 [cycle 1: days 1 and 2], escalating to 27mg/m^2 thereafter).
Aims: A phase 1/2 study (CHAMPION-1; NCT01677858) to determine the maximum tolerated dose (MTD) of carfilzomib (phase 1), the overall response rate (ORR [≥partial response]) (phase 2) and safety of once-weekly carfilzomib with dexamethasone (Cd) in patients with relapsed or refractory MM.
Methods: Patients with relapsed or refractory MM (1-3 prior lines of therapy) received carfilzomib (30-min IV infusion) on days 1, 8, and 15 every 28 days. Phase 1: patients received carfilzomib 20mg/m^2 on day 1 of cycle 1 followed by 45, 56, 70, or 88 mg/m^2 beginning day 8 of cycle 1 in successive dose-level cohorts until the MTD was reached (using a standard 3+3 dose-escalation scheme). All patients received dexamethasone 40mg (IV or oral) days 1, 8, 15, and 22 (cycles 1-8); dexamethasone was omitted on day 22 cycles ≥9. Phase 2: patients received carfilzomib 20mg/m^2 on cycle 1, day 1, escalating to the MTD for subsequent doses. Dexamethasone was given as previously. Cd was administered until unacceptable toxicity or disease progression. Blood samples were collected for pharmacokinetic and pharmacodynamic analyses.
Results: Patients with relapsed or refractory MM (1-3 prior lines of therapy) received carfilzomib (30-min IV infusion) on days 1, 8, and 15 every 28 days. Phase 1: patients received carfilzomib 20mg/m^2 on day 1 of cycle 1 followed by 45, 56, 70, or 88 mg/m^2 beginning day 8 of cycle 1 in successive dose-level cohorts until the MTD was reached (using a standard 3+3 dose-escalation scheme). All patients received dexamethasone 40mg (IV or oral) days 1, 8, 15, and 22 (cycles 1-8); dexamethasone was omitted on day 22 cycles ≥9. Phase 2: patients received carfilzomib 20mg/m^2 on cycle 1, day 1, escalating to the MTD for subsequent doses. Dexamethasone was given as previously. Cd was administered until unacceptable toxicity or disease progression. Blood samples were collected for pharmacokinetic and pharmacodynamic analyses (Table 1).

Table 1. Adverse events in any grade occurring in ≥20% of patients and grade ≥3 adverse events occurring in ≥5% of patients treated at the carfilzomib MTD.

Adverse event, n (%)	Patients (n=104)	
	Any grade	Grade ≥3
Fatigue	55 (53)	11 (11)
Nausea	38 (37)	1 (1)
Insomnia	33 (32)	2 (2)
Headache	32 (31)	3 (3)
Diarrhea	32 (31)	4 (4)
Upper respiratory tract infection	31 (30)	1 (1)
Anemia	29 (28)	6 (6)
Cough	29 (28)	1 (1)
Dyspnea	28 (27)	5 (5)
Pyrexia	26 (25)	0
Thrombocytopenia	23 (22)	6 (6)
Peripheral edema	22 (21)	0
Back pain	18 (17)	5 (5)
Hypertension	14 (13)	7 (7)
Asthenia	11 (11)	5 (5)
Pneumonia	8 (8)	6 (6)
Acute kidney injury	7 (7)	6 (6)
Chronic obstructive pulmonary disease	5 (5)	5 (5)

Summary/Conclusions: Once-weekly carfilzomib (70 mg/m^2) with dexamethasone for patients with relapsed or refractory MM has an acceptable safety and tolerability profile with promising efficacy. The dose and schedule of carfilzomib used in this study (20/70mg/m^2) is currently being compared with the regulatory-approved carfilzomib dose and schedule (20/27mg/m^2 twice-weekly) in an ongoing phase 3, superiority study (ARROW; NCT02412878).

P662

DO MULTIPLE MYELOMA R/R PATIENTS BENEFIT FROM EARLY TREATMENT AT BIOLOGICAL RELAPSE? PRELIMINARY RESULTS OF A SPANISH OBSERVATIONAL PROSPECTIVE REGISTRY

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Background: Over the past decade, numerous drug therapies have emerged for the treatment (Tx) of multiple myeloma (MM). However, eventually all patients (pts) relapse or progress with heterogeneous clinical pattern (Alegre *et al., Haematologica*, 2002). According to the International Myeloma Workshop Consensus Panel (IMWCP; Rajkumar *et al., Blood,* 2011), salvage Tx for MM relapse should begin at symptomatic clinical relapse (clinR) and earlier at asymptomatic biological relapse (BR) when paraprotein increases significantly. In the case of asymptomatic BR/progression, rescue Tx could be delayed in a subset of pts (Fernández de Larrea *et al., Bone Marrow Transplant,* 2014), particularly in low-risk pts with stable M-protein and hemoglobin. Progressive increases of M-spike in blood and/or urine could lead to rescue Tx, even in the absence of clinical symptoms, to avoid complications (*e.g.*, renal failure, plasmacytomas, or hypercalcemia).
Aims: This Spanish observational prospective Registry aims to describe MM relapse patterns, comparing the impact in terms of time to progression (TTP) on Tx decisions (starting Tx at BR [TxBR] *vs* delaying Tx until clinR progression [TxClinR]).
Methods: Adult pts with MM who received 1 or 2 lines of Tx and had achieved ≥partial response (PR) to the last Tx and were in (or prior to) BR according to the IMWCP(Rajkumar *et al., Blood,* 2011)were included. 41 Spanish sites are participating, with 410 pts registered. Data recorded included, among others, demographic, relapse clinical characteristics, TTP, and time from BR to clinR (TTP-ClinR). All pts provided their informed written consent.

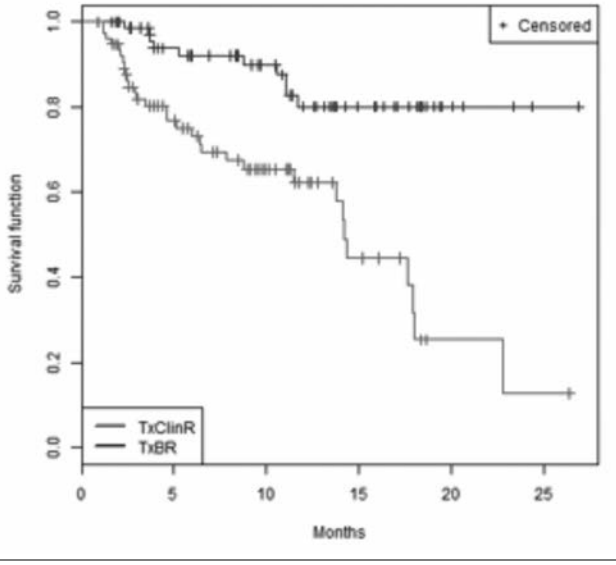

Figure 1. Time from biological relapse to clinical relapse, months.

Results: The cohort evaluated 184 pts (97 male/87 female; mean age of 68.7 years [SD, 10.7]). Heavy chain isotypes were IgG κ (39.0%), IgG λ (23.6%), IgA κ (14.3%), IgA λ (9.9%), IgM λ (1.1%) and IgD λ (0.5%). Prognostic stage at diagnosis according to the ISS was II (28.0%), I (26.9%), and III (24.7%). 47.8% of pts had received autologous stem cell transplant. In 49 pts with available cytogenetic data, 31 (63.3%) were standard-risk and 18 (36.7%) were high-risk. First-line Tx was based mostly on bortezomib (74.4%). After first-line Tx, pts achieved stringent complete response (17.9%), complete response (27.4%), very good partial response (29.6%), or PR (25.1%). Median follow-up was 8.5 months. After BR, 100 pts (55.2%) started Tx (lenalidomide, 68.4%; bortezomib, 28.9%) at BR (65% with significant paraprotein relapse), while Tx was delayed until clinR in 81 (44.8%) pts. Overall median time to progression from BR to clinR (the primary objective) was 22.8 months (95% CI, 17.7-not estimable [NE]). A statistically significant difference was found between Tx groups, median TTP-ClinR was 14.2 months (95% CI, 11.5-18.0) for TxClinR pts and NE for TxBR pts (*P* <.0001; Figure 1). Additional details regarding pattern of BR will be presented.
Summary/Conclusions: Although longer follow-up is needed, in this updated preliminary cohort of pts with MM BR, we found that starting Tx at BR delays the time to clinical relapse, suggesting that pts may benefit from early Tx after first or second relapse.

P663

EFFICACY AND SAFETY BY CYTOGENETIC RISK STATUS: PHASE 3 STUDY (ASPIRE) OF CARFILZOMIB, LENALIDOMIDE AND DEXAMETHASONE *VERSUS* LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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Background: Single-agent carfilzomib has demonstrated activity in patients with relapsed and refractory multiple myeloma with high-risk cytogenetic abnormalities. In the phase 3 study (NCT01080391; N=792 patients) carfilzomib, lenalidomide and dexamethasone (KRd) significantly improved progression-free survival (PFS) *versus* lenalidomide and dexamethasone (Rd) in patients with relapsed multiple myeloma (RMM).
Aims: A pre-planned subgroup analysis of the efficacy and safety of KRd *versus* Rd according to patients' baseline cytogenetic risk status.
Methods: Adult patients with RMM who had received 1-3 prior lines of therapy were randomized (1:1) to receive KRd or Rd in 28-day cycles. Patients in the KRd arm received carfilzomib as a 10-minute IV infusion on days 1, 2, 8, 9, 15, and 16 (cycle 1, 20mg/m^2 on days 1 and 2; escalated to target 27mg/m^2 thereafter) during cycles 1-12. For cycles 13-18, carfilzomib was omitted on days 8 and 9 and discontinued after 18 cycles. Lenalidomide 25mg was administered days 1-21 and dexamethasone 40mg days 1, 8, 15, and 22. The primary end point was PFS; secondary end points included overall survival, overall response rate (ORR), duration of response (DOR), health-related quality of life and safety. The high-risk group was defined as patients with the genetic subtype t(4;14) or t(14;16) or deletion 17p in ≥60% of plasma cells (as assessed using fluorescence *in situ* hybridization). The standard-risk group consisted of all other patients with known baseline cytogenetics.
Results: A total of 396 patients were randomized to each arm. For patients with known baseline cytogenetics, risk status was similar across treatment arms (high-risk: KRd, 24.6%; Rd, 23.4%; standard-risk: KRd, 75.4%; Rd, 76.6%). Efficacy outcomes by cytogenetic risk status are presented in the Table 1. In the high-risk group (n=100), median PFS was 23.1 months (95% confidence interval [CI]: 12.5–24.2) for KRd *vs* 13.9 months (95% CI: 9.5–16.7) for Rd (hazard ratio [HR]:

0.639; 95% CI: 0.369–1.106). In the standard-risk group (n=317), median PFS was 29.6 months (95% CI: 24.1–not estimable) and 19.5 months (95% CI: 14.8–26.0), respectively (HR: 0.657; 95% CI: 0.480–0.901). In the high-risk group, ORRs were 79.2% (KRd) *vs* 59.6% (Rd) and in the standard-risk group, 91.2% (KRd) *vs* 73.5% (Rd). In the high-risk group, rates of grade ≥3 adverse events were 89.1% (KRd) *vs* 78.4% (Rd) and in the standard-risk group 85.6% (KRd) *vs* 84.5% (Rd).

Table 1. Efficacy outcomes and AEs of interest by baseline cytogenetic risk status.

Outcome	High-Risk		Standard-Risk	
	KRd (n=48)	Rd (n=52)	KRd (n=147)	Rd (n=170)
Median PFS, months	23.1	13.9	29.6	19.5
HR for KRd vs Rd (95% CI)	0.639 (0.369–1.106)		0.657 (0.480–0.901)	
Best overall response, n (%)				
Stringent complete response	8 (16.7)	2 (3.8)	22 (15.0)	6 (3.5)
Complete response	6 (12.5)	1 (1.9)	34 (23.1)	5 (2.9)
Very good partial response	15 (31.3)	11 (21.2)	55 (37.4)	66 (38.8)
Partial response	9 (18.8)	17 (32.7)	23 (15.6)	48 (28.2)
Minimal response	3 (6.3)	4 (7.7)	4 (2.7)	15 (8.8)
Stable disease	0	6 (11.5)	3 (2.0)	15 (8.8)
Progressive disease	2 (4.2)	6 (11.5)	2 (1.4)	2 (1.2)
Not evaluable	5 (10.4)	5 (9.6)	4 (2.7)	13 (7.6)
ORR, % (95% CI)	79.2 (65.0–89.5)	59.6 (45.1–73.0)	91.2 (85.4–95.2)	73.5 (66.2–80.0)
Median DOR, months	22.2	14.9	30.4	20.4
Grade ≥3 AEs of interest, n (%)[a]				
Dyspnea[b]	2 (4.3)	0	5 (3.4)	5 (3.0)
Hypertension[b]	1 (2.2)	0	9 (6.2)	3 (1.8)
Acute renal failure[c]	3 (6.5)	1 (2.0)	6 (4.1)	3 (1.8)
Cardiac failure[c]	0	0	8 (5.5)	4 (2.4)
Ischemic heart disease[c]	0	1 (2.0)	7 (4.8)	2 (1.2)
Peripheral neuropathy[c]	0	1 (2.0)	6 (4.1)	4 (2.4)

[a]In the high-risk group, 46 (KRd) and 51 (Rd) patients were evaluable for safety; in the standard-risk group, 146 (KRd) and 168 (Rd) patients were evaluable for safety.

[b]Preferred term.

[c]Grouped term.

AE, adverse event; CI, confidence interval; DOR, duration of response; HR, hazard ratio; KRd, carfilzomib with lenalidomide and dexamethasone; NE, not estimable; ORR, overall response rate; PFS, progression-free survival; Rd, lenalidomide and dexamethasone.

Summary/Conclusions: In patients with high-risk cytogenetics, treatment with KRd improved median PFS by 9 months *versus* Rd (median PFS of nearly 2 years *vs* 13.9 months). KRd was also associated with a 10-month improvement in median PFS *vs* Rd in patients with standard-risk cytogenetics. Treatment with KRd resulted in higher response rates, greater depth of response and a longer DOR *versus* Rd, regardless of patients' baseline cytogenetic risk status. The triplet regimen of KRd had a favorable benefit–risk profile in patients with RMM and improved outcomes in patients with high-risk disease.

P664

A SUBSTUDY OF THE PHASE 3 ENDEAVOR STUDY: SERIAL ECHOCARDIOGRAPHIC ASSESSMENT OF PATIENTS WITH RELAPSED MULTIPLE MYELOMA (RMM) RECEIVING CARFILZOMIB PLUS DEXAMETHASONE OR BORTEZOMIB PLUS DEXAMETHASONE

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Background: In ENDEAVOR (NCT01568866; N=929), carfilzomib plus dexamethasone (Cd) was superior to bortezomib/dexamethasone (Bd), with a 2-fold improvement in median PFS (18.7 *vs* 9.4 months; HR=0.53; 95% CI, 0.44, 0.65; p<.0001) in patients with RMM.

Aims: To evaluate change from baseline in left ventricular ejection function (LVEF), right ventricular (RV) function and pulmonary artery systolic pressure (PASP) via echocardiogram (ECHO) in a subset of patients from the ENDEAVOR study.

Methods: Adults with RMM (1-3 prior regimens) with a LVEF ≥40% and no evidence of New York Heart Association class III or IV symptomatic heart failure (HF), symptomatic ischemia, uncontrolled arrhythmias or recent myocardial infarction within 4 months before randomization. Patients received either carfilzomib (30-min IV infusion on days [D] 1, 2, 8, 9, 15, 16 [20 mg/m^2 on D1, 2 of cycle 1; 56 mg/m^2 thereafter]) and dexamethasone (20mg on D1, 2, 8, 9, 15, 16, 22, 23 of a 28-day cycle) or bortezomib (1.3 mg/m^2; IV or SC on D1, 4, 8, 11) and dexamethasone (20mg on D1, 2, 4, 5, 8, 9, 11, 12 of a 21-day cycle). All eligible patients were assessed at baseline and every 12 weeks on D1 of treatment cycles and at the end-of-treatment visit (using 2D transthoracic ECHO). ECHOs were centrally read. An independent cardiac events adjudication committee (CEAC) reviewed results in conjunction with adverse events (AEs) to assess clinical significance. Primary endpoint was reduction (≥10% absolute decrease from baseline in patients with LVEF ≤55% or a reduction <45% for patients with baseline LVEF >55%) or no change in LVEF (≤24 weeks from baseline).

Results: A total of 151 patients (Cd: 75; Bd: 76) were included in this substudy; 74 in each arm were evaluable for safety. More patients in the Cd arm (*versus* the Bd arm) were aged ≥75 years (21.3% *vs* 14.5%), had prior cardiac-related medical history (26.7% *vs* 14.5%) and received drugs for obstructive airway disorders (28.0% *vs* 17.1%). Patients in the Cd arm had a higher incidence of HF reported as an AE *vs* Bd (10.8% [n=8] *vs* 4.1% [n=3]), consistent with the overall safety population (8.2% *vs* 2.9%). History of cardiac disorders was associated with an elevated (not significant) risk of HF (3/8 Cd pts; 0/3 Bd pts). Patients receiving Cd had a higher incidence of hypertension (HTN) *versus* those treated with Bd (20.3% *vs* 8.1%). Twenty-three patients (15.2%) discontinued treatment due to AEs); 8 due to non-fatal cardiac-related AEs (6 Cd [2 due to LV dysfunction, and 1 each due to HF, decreased EF, pulmonary embolism, and ischemic stroke] and 2 Bd [1 each due to decreased EF and RV failure]). Only 1 patient (Bd) had significant LVEF reduction as defined within the first 24 weeks. Six patients had a significant LVEF reduction at any time during the study (Cd: 3; Bd: 3). All but 1 had resolution to normal LVEF. CEAC evaluation found 14 patients (Cd: 8; Bd: 6) had on-study CEAC-defined clinically meaningful changes in ECHOs; 11/14 (79%) did not meet the ECHO criteria for decreased LVEF. The CEAC reported more Cd patients (*vs* Vd) had evidence of HF (n=4 *vs* n=0) or pulmonary HTN (n=4 *vs* n=1) (Figure 1).

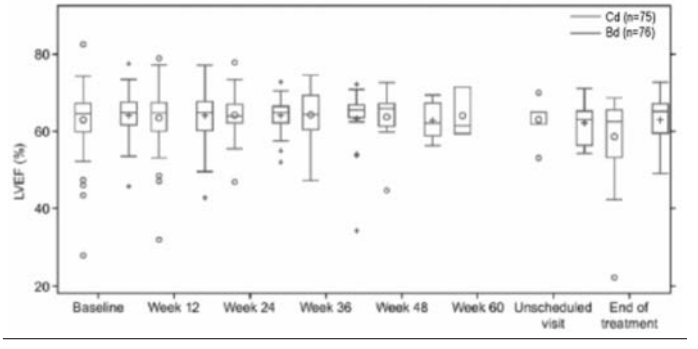

Figure 1. Left ventricular ejection fraction (LVEF) by week.

Summary/Conclusions: In the overall and cardiac substudy, HF and pulmonary HTN events occurred more frequently with Cd *vs* Vd although ECHO-detected significant decline in LVEF was low on both treatment arms and with similar frequency. The substudy found limited utility for serial screening with ECHOs to mitigate cardiac risk for unselected patients receiving carfilzomib. Alternative surveillance strategies are needed to detect early cardiotoxicity and prevent treatment interruption or discontinuation.

P665

PROGNOSTIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA AND STANDARD-RISK CYTOGENETICS

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Background: Cytogenetic evaluation using fluorescence *in situ* hybridization (FISH) at the time of diagnosis is essential for initial risk stratification and the employment of risk-adapted treatment strategies in multiple myeloma. Despite

the absence of cytogenetic high-risk abnormalities a subset of patients does experience poor overall survival.
Aims: To identify demographic, clinical, and cytogenetic characteristics associated with poor three-year overall survival in patients with standard-risk cytogenetics.
Methods: We studied 514 patients who were diagnosed with multiple myeloma between January 2004 and December 2015 at Mayo Clinic Rochester and underwent FISH evaluation within six months of diagnosis. Patients with high-risk cytogenetics and those who were lost to follow-up within three years were excluded. High-risk cytogenetics were defined as the presence of del(17p), t(4;14), t(14;16), or t(14;20). Bone marrow aspirates were evaluated for deletions, monosomies, trisomies, and tetrasomies using chromosome- or centromere-specific FISH probes. IGH rearrangements were evaluated using an IGH break-apart probe and evaluating up to five potential partners (FGFR3, CCND1, CCND3, MAF, and MAFB). Multivariable-adjusted logistic regression models were used to assess the associations between the parameters of interest and three-year overall survival.
Results: The median age at diagnosis was 65 years (31-95), 316 (62%) of the patients were male. The median overall survival was 6.4 years (5.5-7.7) for the entire cohort (n=514), 9.2 years (8.3-10.4) for those who survived at least three years (n=361, 70%), and 1.2 year (1.0-1.5) for those who did not survive three years after diagnosis (n=153, 30%). The factors associated with poor three-year overall survival are summarized in Table 1.

Table 1. Factors associated with poor three-year overall survival (n=514).

Parameter	Value	OR (95% CI)	p
Age	Older than 65 years at diagnosis	2.80 (1.80-4.36)	<0.001
Plasma cells	Less than 20% in bone marrow	2.00 (1.12-3.60)	0.020
Karyotype	Hyperdiploid clone present	0.51 (0.33-0.79)	0.003

The model was adjusted for sex, β_2-microglobulin, and albumin at diagnosis.

Summary/Conclusions: Patients with multiple myeloma and standard-risk cytogenetics are a heterogeneous group. One third of the patients are experiencing less than three years of overall survival after diagnosis. Advanced age, extent of bone marrow involvement, and specific clonal abnormalities were associated with poor three-year overall survival. These findings emphasize the importance of further risk stratification and the need for reliable predictors of poor clinical outcomes in this patient population.

Myeloproliferative neoplasms - Clinical 2

P666

CLINICAL CHARACTERISTICS AND LONG-TERM OUTCOME ON IMATINIB IN PATIENTS WITH MYELOID/LYMPHOID NEOPLASMS AND ASSOCIATED PDGFRB FUSION GENES IN CHRONIC OR BLAST PHASE

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Background: A distinct subcategory of the WHO 2008 classification of myeloid neoplasms comprises myeloid/lymphoid neoplasms with eosinophilia (MLN-eo) and rearrangements of PDGFRA, PDGFRB or FGFR1. Patients with PDGFRA or PDGFRB fusion genes are exquisitely sensitive to imatinib.
Aims: We sought to evaluate the clinical characteristics and long-term outcome on imatinib in MLN-eo patients (pts) with PDGFRB fusion genes.
Methods: Within the "German Registry on Disorders of Eosinophils and Mast Cells" 22 MLN-eo pts with PDGFRB fusion genese were retrospectively analysed.
Results: Fifteen different fusion genes were identified; three partner genes were recurrent (ETV6, n=5; CDCC88C, n=3; H4, n=2). Median age was 49 years (range 20-80) with a striking male predominance (20/22, 91%). Significant eosinophilia ≥0.5x10^9/l and ≥1.5x10^9/l was present in 15/19 (79%) and 11/19 (58%) pts, respectively, monocytosis >1.0x10^9/l (median 0.5x10^9/l, range 0-89) was seen in 5/15 pts (33%). Splenomegaly was observed in 12/15 (80%) pts, organ involvement in 5/22 (23%) pts including cytologically or histologically confirmed infiltration of pleura (n=2), central nervous system (n=1), endo-/myocard (n=1), liver (n=1) and skin (n=1). Five pts were initially diagnosed in blast phase (BP): myeloid BP (n=1), extramedullary myeloid sarcoma (n=1), T-cell lymphoblastic lymphoma (n=2) and B-cell acute lymphoblastic leukemia (n=1). Four pts (chronic phase, CP, n=2; BP, n=2) presented with a complex karyotype. All 17 CP pts were treated with imatinib (400mg/d, n=12, 71%; 100 mg/d, n=5, 29%) and achieved complete hematologic remission (CHR); no primary resistance was observed. Complete cytogenetic remission was achieved in 10/11 (91%) evaluated pts after median 11 months (range 3-34) and complete molecular remission (CMR), as detected by negative nested RT-PCR, in 10/12 pts (83%) after median 23 months (range 8-110). Imatinib was reduced from 400 mg/d to 100mg/d in 7/12 pts and to 3x100mg/week in two pts. After a median treatment of 7 years (range 0-11), 15/17 pts are alive. One CP patient with a complex karyotype achieved a CHR for 13 months but no cytogenetic remission and died due to progressive disease. The second CP patient died because of comorbidity while in CHR. One patient in BP with secondary T-cell lymphoma received intensive chemotherapy followed by imatinib and died due to comorbidity at month 27, while the second patient is alive on imatinib monotherapy 97 months after diagnosis. Three pts (complex karyotype, n=2) received an allogeneic stem cell transplantation (SCT) after intensive chemotherapy or a short course of imatinib median 3 months (range 1-7) after diagnosis. Two pts were re-exposed to imatinib because of residual disease. Two pts are alive 3 and 42 months after SCT, respectively. The third patient died due to progressive leptomeningeal involvement while in CHR on imatinib 9 months after SCT. Overall, 4/22 pts (18%) have died (BP, n=2; CP, n=2) due to comorbidity while in remission (n=2) or progressive disease (n=2). Of note, 3 of these 4 pts had an additional complex karyotype at diagnosis.
Summary/Conclusions: We here confirm known and report several new clinical and genetic characteristics in pts with MLN-eo and PDGFRB fusion genes: i) significant male predominance, ii) significant eosinophilia is not present in all pts (in contrast to FIP1L1-PDGFRA, but similar to FGFR1 fusion genes), iii)

except splenomegaly, organ involvement/dysfunction is rather uncommon, iv) pts can present in BP of myeloid or lymphoid origin, v) an additional complex karyotype may be associated with adverse prognosis, vi) in CP, no primary resistance to imatinib was observed and vii) pts can be treated with imatinib 100mg/d.

P667

THROMBOTIC RISK IN IDIOPATHIC ERYTHROCYTOSIS IS LOWER THAN IN POLYCYTHEMIA VERA BUT HIGHER THAN IN GENERAL POPULATION

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Background: Absolute erythrocytosis is a relatively rare finding in the clinical practice, characterized by persistently raised hemoglobin (Hb) and hematocrit (Ht) levels. The most extensively studied form of absolute erythrocytosis is Polycythemia Vera (PV), a primary neoplastic disease characterized by the presence of JAK2 mutations, risk of vascular complications and of evolution in myelofibrosis or acute leukemia. Secondary erythrocytosis are represented by rare congenital diseases (due to EPO-receptor or oxygen sensing pathway genes mutation), acquired forms, [erythropoietin (EPO)-mediated] or idiopathic erythrocytosis (IE) an absolute and isolated polyglobulia, without any definite cause, not JAK2 mutations, not EPO-mediated. These patients are rarely observed in clinical practice, thus few is known regarding their clinical characteristics, natural history and best management.
Aims: In this work, we report clinical features of a large cohort of patients with IE focusing on their thrombotic risk compared with a cohort of patients with PV.
Methods: We report 145 patients with IE (not carrying any JAK2 mutation nor affected by congenital/familial or secondary erythrocytosis) studied between 1980 and 2015 in two centers of Venetian region Italy. As controls we used 145 patients with PV all carrying JAK2 mutations, diagnosed in the same period and matched for Ht values. All patients were treated with phlebotomies, to maintain Ht below 45%. In all these patients, we collected disease-relevant parameters, including blood count at diagnosis and major events during follow-up. Statistical analysis was performed by using the Mann-Whitney test, the $\chi 2$ test or Fisher's exact test and the Kaplan-Meier method.
Results: Results are summarized in the following Table 1.

Table 1.

	IE	PV	p
Patients, n	145	145	-
Males, n (%)	127 (87.6)	83 (57.2)	<0.001
Median age at diagnosis, years (Percentile range, 5th to 95th)	56.5 (21.2-75.8)	61.7 (30.3-81.9)	0.006
Median Follow up, years (Percentile range, 5th to 95th)	6.6 (0.3-23.2)	7.4 (0.3-22.1)	n.s.
Median WBC, x10^9/L (Percentile range, 5th to 95th)	7.2 (5-11.9)	8.7 (5.7-17.3)	<0.001
Median Hb, g/L (Percentile range, 5th to 95th)	179 (164-195)	175 (149-213)	0.03
Median Ht, % (Percentile range, 5th to 95th)	52.8 (48.3-57.8)	53.1 (46.2-66.2)	n.s.
Median plts count, x10^9/L (Percentile range, 5th to 95th)	217 (132-320)	485 (209-882)	<0.001
Patients with at least a thrombosis, n (%)	21 (14.5)	39 (26.9)	0.01

During follow-up, 15 patients with IE (10.3%) and 27 with PV (18.6%) experienced at least a thrombotic complication (p=0.04), with a dramatically different Thrombosis Free survival (p=0.005). Arterial thromboses were 13 among patients with IE and 12 in patients with PV. The thrombotic incidence rate was 3% patients/year in PV and 1.5% patients/year in IE (IRR=2).
Summary/Conclusions: IE, is a poorer studied disease. The clinical picture (*i.e.* vascular complications during follow-up and final outcomes) are not fully investigated. PV, a well-studied disease, has an estimated cumulative thrombosis rate of about 4% patients/year not different than our finding in the present cohort. In this study, we demonstrate that patients with IE develop fewer thrombotic complications than patients with PV. However, IE, in spite of a stringent program of phlebotomy, absence of leukocytosis and/or thrombocytosis, maintain higher thrombosis rate (1.5% patients/year) when compared to general population (around 0.8% persons/year). The present data suggest that IE has an increased thrombotic risk even if lower than PV, showing that the rheological alteration due to high Ht play a relevant role even in the absence of a clonal disease. Moreover, taking into account that most cardiovascular complications are represented by arterial thrombosis, prophylaxis with aspirin should be considered, in absence of contraindications.

P668

AZACITIDINE IN THE TREATMENT OF PH- MYELOPROLIFERATIVE NEOPLASMS IN BLASTIC PHASE: THE EXPERIENCE OF GRUPPO LAZIALE FOR THE STUDY OF PH- SMPC

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Background: Prognosis of patients with myeloproliferative neoplasms (MPN) developing blast phase (MPN-BP) is very poor. Median overall survival (OS) of these patients is about 2-3 months and nowadays no drug can induce a durable complete response (CR) in patients who are not candidate to Bone Marrow Transplantation (BMT).
Aims: We retrospectively evaluated 27 patients (M/F 19/8, median age 62.5 years, range 30.8-80.6) with MPN-BP according to international criteria treated with azacytidine (AZA) in the last 7 years, to highlight the role of AZA in this subset. Data were obtained from database of Gruppo Laziale for the study of Ph- SMPC.
Methods: Primitive MPN diagnosis was Essential Thrombocythemia in 9 cases, Primary Myelofibrosis in 9, Polycythemia Vera in 5 and MPN-U in 4; the JAK-2 V617F mutation was present in 17/25 patients tested (68%); all but 2 patients received a previous treatment during chronic myeloproliferative phase [18 patients with Hydroxyurea (HU) alone, 1 with HU and Anagrelide, 4 with HU and Pipobroman, 1 with Melphalan followed by allo-BMT, 1 with Pipobroman alone]. Median time from diagnosis to BP evolution was 59.6 months (range 1-312). All patients were treated with 5-AZA at the dosage of 75 mg/m^2: as to the schedule, 24 patients received a 7-day schedule and 3 patients a 5-day schedule every 28 days. At the time of evolution, median WBC value was 12.8x10^6/L (range 2.2-89.5 X 10^6/L), median Hb level 9.8 g/dl (range 6.6-12.7 gr%) and median PLT value 183.5x10^6/L (range 9-846x10^6/L).
Results: Two pts were too early (only 1 cycle still performed) while 8/25 evaluable pts (32%) achieved a CR after a median time of 5 months (range 3-12) from AZA start, 2 pts (8%) had a partial response (PR), 4 pts (16%) had an hematological improvement (HI), with a 56% global response rate: the remaining 11 pts (44%) had no response (9 pts had a disease progression and 2 died early after AZA initiation from pulmonary fungal infection and respiratory failure respectively). The median OS from BP evolution of the whole cohort was 9.9 months (95% CI 7.0-12.8): the median OS of responding pts (CR+PR+HI) was 18.6 months (95% CI 8.4-28.8) compared with 4.9 months (95% CI 0.9-8.8) of resistant pts (p<0.001).
Summary/Conclusions: Our data confirm the relative efficacy and safety of AZA compared to historical controls in this group of patients with otherwise dismal prognosis, underlining the possible achievement of long-lasting responses in a sizeable portion of them.

P669

COMPARISON OF GENOMIC DNA-BASED *VS* MESSENGER RNA-BASED KIT D816V MUTATION ANALYSIS REVEALS LARGE DIFFERENCES BETWEEN BLOOD AND BONE MARROW IN SYSTEMIC MASTOCYTOSIS

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Background: Mastocytosis is a heterogeneous group of diseases characterized by growth and accumulation of neoplastic mast cells (MCs). Mastocytosis often represents a diagnostic challenge. The vast majority of adults with mastocytosis carry the activating somatic *KIT* D816V mutation. Recent advances in *KIT* D816V mutation analysis allow detection of the mutation in peripheral blood (PB) in most patients. Mutation analysis of PB is therefore increasingly used for screening in suspected mastocytosis. A very high sensitivity is needed to produce clinical meaningful diagnostic yields in indolent SM (ISM) in both PB and bone marrow (BM) aspirate. *KIT* D816V mutation analysis may be based on genomic DNA (gDNA) or the expressed messenger RNA (mRNA). Depending on *KIT* gene expression levels of the D816V-positive cells, mRNA-based analysis is potentially more sensitive than gDNA-based analysis. However, very limited experimental evidence comparing these two approaches presently exists.
Aims: To systemically compare gDNA-based and mRNA-based *KIT* D816V mutation analysis of PB and BM aspirate in SM patients to determine the optimal technical approach.
Methods: In 82 SM patients (76 ISM (including 3 BMM and 4 SSM) and 6 SM-AHNMD), gDNA-based and mRNA-based *KIT* D816V mutation analysis of PB was performed. In 65 patients, BM aspirate was also analyzed.
A highly sensitive *KIT* D816V mutation-specific qPCR assay was used for both gDNA-based and mRNA-based analysis. Importantly, gDNA-based analysis

was performed with gDNA from 50.000 cells per reaction and mRNA-based analysis was performed with cDNA from mRNA from 50.000 cells, thus allowing non-biased comparison of the two approaches.
Results: In mRNA-based analysis, PB from 24 of the 82 patients (29%) fulfilled criteria of positivity. In gDNA-based analysis, 77 of the 82 (94%) samples tested D816V-positive, thus demonstrating a higher diagnostic yield with gDNA in PB samples (chi^2-test: P<0.001). The gDNA-based D816V-positive allele burden was significantly higher in PB samples that tested positive in the mRNA-based analysis compared to samples that tested negative in mRNA-based analysis (median allele burden 2.8% *vs* 0.051%; Mann-Whitney U-test: P<0.001). In BM aspirate, 64 of 65 samples tested D816V-positive in mRNA-based analysis, whereas all 65 samples tested positive in gDNA-based analysis (chi^2-test: P=0.32). While the diagnostic yield was thereby high in BM aspirates in both gDNA-based and mRNA-based analysis, the confidence of mRNA-based results was significantly higher than gDNA-based in samples with low mutation levels as calculated from the absolute threshold cycle (C_t) values of the qPCR analysis.
Summary/Conclusions: Previous studies performing *KIT* D816V mutation analysis of PB in ISM have reported highly different diagnostic yields. The reported differences may be explained by several factors. However, our direct comparison of mRNA-based and gDNA-based analysis indicate that the difference in mRNA-based and gDNA-based analysis is a major determinant of diagnostic yield in PB. Previous inconsistencies are therefore likely to be primarily explained by this parameter. We conclude that gDNA-based *KIT* D816V mutation analysis should always be preferred over mRNA-based in PB in ISM. In addition, mRNA-based *KIT* D816V mutation analysis may have added value in BM aspirates if gDNA-based analysis produces a borderline or weakly positive result, or test unexpectedly negative.

P670

3167 NEWLY DIAGNOSED MPN PATIENTS, A REPORT FROM THE SWEDISH MPN REGISTRY

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Background: Newly diagnosed patients with Philadelphia chromosome negative myeloproliferative neoplasms (MPN) are included in the Swedish MPN Registry since 2008.
Aims: The aim was to report incidence, blood values, earlier vascular complications and planned treatment in newly diagnosed MPN patients.
Methods: From January 2008 through December 2014 a total of 3167 were registered. The diagnosing clinics report age, gender, blood values, earlier vascular complications and planned treatment. Registration of cancers is mandatory. About 95% of MPN were reported to the registry at the time of analysis.
Results: Polycythemia vera (PV) was diagnosed in 1105, essential thrombocythemia (ET) in 1285, myelofibrosis (MF) in 440 and MPN unclassified 337 patients. The annual incidence per 100,000 inhabitants for PV was 1.7, for ET 1.9, for MF 0.66 and for MPN unclassified 0.53. The median age at diagnosis was lowest for ET, 68 years, for PV was 69 years, for MF and MPN unclassified 70 years. In PV and MPN unclassified the proportion of men and women were equal, in ET there was a female predominance (58%), whereas 55% of the MF patients were male. The blood values at diagnosis are presented in Table 1.

Table 1.

	Gender female/male	Age±years	Hemoglobing/dL	Hematocrit %	WBC x10^9/L	Platelets x10^9/L	EPOU/L	JAK2 V617F/wt	Vascular complicationyes/no
PV	550/555	68±13	17.2±2.2	53±7	12.7±9.8	569±285	3.1±3.9	988/22	407/708
ET	745/540	65±16	13.8±1.5	42±5	9.7±5.3	859±337	9±20	708/400	446/825
MF	198/242	69±13	11.3±2.1	38±7	12.0±10.3	490±394	59±112	206/158	115/325
MPN uc	165/172	69±15	12.8±2.8	40±8	20.1±32.5	583±415	30±103	178/103	124/228

The prevalence of vascular complications prior to MPN diagnosis was highest for the PV, ET and MPN unclassified, 37%, 35% and 34% respectively. The MF patients had significantly lower prevalence, 26% (p<0.001 compared with PV and ET, p=0.018 compared with MPN unclassified). The time from a previous vascular event to diagnosis was not reported in the registry. The majority of complications were thromboembolic; 32% of PV patients, 30% of ET patients, 28% of MPN unclassified patients and 20% of MF patients. The corresponding frequencies for hemorrhagic events were 5%, 5%, 8% and 7%, respectively. The most frequent vascular events were of cerebral, cardiac and deep vein thrombosis/pulmonary embolism. ET patients with reported vascular complications had a significantly lower hemoglobin and hematocrit (p=0.002 and p=0.005, respectively) and higher WBC count (p=0.031), whereas the platelet concentration was comparable in patients with or without recorded complication. JAK2 V617F mutation was present in 71% of ET patients with vascular complications prior to diagnosis, compared to 60% of the patients without complications (p<0.001). Also, the PV patients with earlier reported vascular events had a significantly higher mean age, lower hemoglobin and hematocrit at diagnosis compared to patients without vascular complications (p<0.001, respectively). Myelosuppressive treatment was planned in 66.6% of the ET patients compared to about 50% of the patients with PV, MF and MPN unclassified (p<0.001, respectively). Phlebotomy treatment was planned in 82% of the PV patients. Transfusion was planned to be given to 16% and EPO to 13% of the MF patients. ASA prophylaxis was recorded for about 60% of the PV and ET patients, compared to about 30% of the MF and MPN unclassified patients.
Summary/Conclusions: ET and PV were the most common MPN entities, 40% and 35% respectively. JAK2 V617F mutation was present in 98% of PV patients and in 57-64% in the other MPN etities. Reported vascular complications prior to diagnosis were common, especially in PV, ET and MPN unclassified. The majority of events were thromboembolic.

P671

PREDICTIVE ROLE OF CIRCULATING LEUCOCYTE-PLATELET AGGREGATES FOR THROMBOEMBOLIC COMPLICATIONS IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A PROSPECTIVE STUDY

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Background: Thrombosis represents the main complication in Philadelphia negative myeloproliferative neoplasms (Ph$^-$MPN). It is supposed that inappropriate activation of leukocytes and platelets with their mutual interaction represents an important mechanism of thrombosis tendency in these diseases. The role of circulating leukocyte-platelet (Le-Plt) aggregates as risk factor for thrombosis in Ph$^-$MPN has been investigated so far in retrospective studies which included significant proportion of patients (pts) on antiplatelet or cytoreductive therapy.
Aims: The aim of our prospective study was to investigate the predictive value of circulating neutrophyle-platelet (Neu-Plt) and monocyte-platelet (Mo-Plt) aggregates as well as soluble selectins for occurence of thromboembolic events in pts with Ph$^-$MPN.
Methods: The study included 95 consecutive pts with *de novo* Ph$^-$MPN (39 polycythemia vera, 27 essential thrombocythemia, and 29 primary myelofibrosis), diagnosed according to WHO criteria. All analysis were performed after diagnosis, and before the start of antiplatelet or cytoreductive therapy. Flow cytometric analysis of Le-Plt aggregates was performed on whole blood samples anticoagulated with EDTA/CTAD. Le-Plt aggregates were estimated as a fraction (%) of CD42b$^+$CD61$^+$ neutrophyles and monocytes. The plasma levels of E-, L-, and P-selectins were determined by enzyme immunoassay. All arterial and venous thrombotic events, except microcirculatory disturbances, were recorded during mean follow-up period of about 3.25 years after diagnosis.
Results: During the follow-up thromboembolic complications occurred in 12.6% Ph$^-$MPN pts (arterial in 9.4%, venous in 3.2%), with mean time to thromboembolic event of 39 months. The overall incidence rate of main thrombotic events in whole group of Ph$^-$MPN during the follow-up was 4.36 per 100 pts-years. The levels of Neu-Plt (26.7% *vs* 22.4%) and Mo-Plt (17.8% *vs* 12.3%) aggregates did not differ significantly between groups of pts with and without thrombosis. Using multivariate COX proportional hazard regression model, it was proved that Mo-Plt aggregates represent independent predictive factor for thrombosis development (HR=1.561, 95% CI: 1.007-2.420, p=0.046). Frequency of HTA was significantly higher in group of Ph$^-$MPN pts with thrombosis compared to those without thrombosis (p<0.05), and multivariate COX proportional hazard regression model confirmed that interaction between Mo-Plt aggregates and HTA may be additive (HR=1.975, 95%CI: 1.215-3.212, p=0.006). The level of soluble P-selectin was significantly higher in group of Ph$^-$MPN pts with thrombosis than in group of pts without thrombosis (346.89 ng/ml *vs* 286.39 ng/ml, p=0.034), but has not been proved as a predictive risk factor for occurrence of thrombosis (COX proportional hazard regression model). The levels of E- and L-selectin did not differ significantly between Ph$^-$MPN groups, according to the presence of thrombosis.
Summary/Conclusions: This is the first prospective study investigating predictive role of Le-Plt interactions on occurence of thromboembolic complication in Ph$^-$MPN. Among several investigated parameters, only increased level of Mo-Plt aggregates was shown as independent predictive risk factor for development of thromboembolic complications. Concomitant presence of HTA further strengthens predictive role of Mo-Plt aggregates. Since the reduction of thrombotic risk is a primary goal of therapy in Ph$^-$MPN, special attention should be paid to the treatment of HTA.

P672

PREDICTORS FOR RESPONSE TO RUXOLITINIB IN REAL-LIFE: AN OBSERVATIONAL INDEPENDENT STUDY ON 266 PATIENTS WITH MYELOFIBROSIS

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Background: Response to ruxolitinib (RUX), the only JAK1/2 inhibitor commercially available for the treatment of Myelofibrosis (MF) may vary among patients (pts) and is largely unpredictable at therapy start. Due to the lack of predictors of response, pts' selection is based on clinical needs.
Aims: To evaluate the impact of pre-treatment clinical/laboratory factors, as well as RUX dose, on response to RUX in a cohort of "real-life" MF pts.
Methods: A multicenter observational study on WHO-defined MF was conducted in 11 Italian Hematology Centers. Data were extracted from an electronic database that included retrospective data on pts treated before January 2015. Response to RUX was evaluated according to IWG-MRT criteria.
Results: Between June 2011 and June 2015, 266 pts with PMF (140 pts,52.6%), or PET-MF (51,19.2%) or PPV-MF (75,28.2%) were treated with RUX in participating Centers. At diagnosis, baseline characteristics were (median): age, 64.2 y (range, 26-86); ≥65 y, 45.5%; male, 58%; hemoglobin (Hb), 11.5 g/dL (5-17.7); Hb <10 g/dL, 27.4%; PLT, 356×10^9/L (30-1110); PLT <100×10^9/L, 5.2%; spleen enlargement, 87.5% (spleen length ≥10cm: 40.6%); constitutional symptoms, 48.8%. Molecular analysis was performed on 215 pts (81%) and was positive in 90% (*JAK2*V617F), 7% (*CALR*), 1% (*MPL*W515K/L); 2% (triple negative). Karyotype was abnormal in 43 (30%) out of 145 evaluable pts (unfavorable: 9%). Median follow-up from MF diagnosis was 4 yr (0.5-29.6) and median RUX exposure was 22 mos (3-54). Overall, 92 out of 232 (39.7%) pts with spleen ≥5cm achieved a spleen response at any time during RUX therapy (Table 1).

Table 1. Patients characteristics at RUX start.

Characteristics	Spleen response (n.92)	No spleen response (n.140)	p
Male sex, no. (%)	59 (64.1%)	80 (57.1%)	*0.28*
PMF, no (%)	48 (52.2%)	72 (51.4%)	*0.91*
JAK2 V617F mutation, no (%)	74 (93.7%)	95 (88%)	*0.59*
Median age, years (range)	67 (26-86)	68 (26,3-87)	*0.30*
Median hemoglobin, g/dl (range)	12 (5,9-17,3)	10.1 (4-17,3)	***0.0007***
Median leukocyte, x10^9/l, (range)	12,3 (1,2-106,9)	9,2 (1,6-57,5)	***0.0068***
Median platelet, x10^9/l (range)	322 (15-1657)	211 (4-2513)	***0.0006***
Constitutional symptoms, no (%)	44 (47.8%)	72 (51.4%)	*0.42*
Spleen ≥ 10cm, no (%)	46 (50%)	96 (68.6%)	***0.005***
Palpable hepatomegaly, no (%)	24 (26.1%)	32 (27.4%)	*0.87*
Unfavorable karyotype, no (%)	5 (10.9%)	6 (7.3)	*0.49*
Grade 3 marrow fibrosis, no (%)	10 (13%)	27 (27%)	***0.02***
Platelet <100	6 (6.5%)	15 (10.7%)	*0.27*
Platelet >200	63 (68.5%)	76 (54.3%)	***0.03***
Age >65 yrs, no (%)	54 (58.7%)	91 (65%)	*0.33*
Transfusion dependence, no (%)	16 (20%)	41 (33.1%)	***0.04***
Previous cytoreductive therapy, no (%)	59 (64.1%)	106 (75.7%)	*0.09*
Time from MF diagnosis to RUX start (range)	1 (0.3-27.5)	2.2 (0.3-30.1)	***0.001***

At 3 and 6 mos, the response was achieved by 28.1% and 31% of evaluable pts, respectively. In univariate analysis, pre-treatment factors negatively correlating with spleen response were: transfusion dependence, platelet count ≤200x10^9/l, spleen palpable ≥10 cm below costal margin, grade 3 marrow fibrosis and interval between MF diagnosis and RUX start ≥2y. Also, the rate of spleen response significantly correlated with RUX starting dose, with 18.5%, 33.8% and 47.7% of responses observed in pts treated with 5mg BID, 15mg BID and 20mg BID, respectively (p=0.008). Also, spleen response correlated with the average RUX dose in the first 12 wks, with pts treated with doses ≥10 mg BID having better response rates (44.4% *vs* 25.4% in pts treated with ≤10 mg BID, p=0.01). Three variables remained significant in multivariate regression logistic analysis: large splenomegaly (HR:2.01, 95%CI:1.1-3.1;p=0.015), time between MF diagnosis ≥2y (HR:1.8, 95%CI:1.0-3.2;p=0.037) and median RUX dose ≤10mg BID in the first 12 wks (HR:2.29, 95%CI:1.1-4.3;p=0.02).Overall, 178 pts out of 217 symptomatic patients (82%) had a symptom response. Factors associated with worse responses were: grade 3 marrow fibrosis (p=0.035), transfusion dependency (p=0.001) and RUX titrated dose ≤10 mg BID (p=0.014). In multivariate analysis, symptom response rate correlated only with RUX titrated dose (HR:2.4, 95%CI:1.1-4.9,p=0.016). Neither spleen or symptoms response significantly correlated with survival.
Summary/Conclusions: In the real-life setting, IWG-MRT-defined spleen and symptoms response rates were observed in 40% and 70% of pts, respectively. Among pre-treatment features, large splenomegaly and a delay in treatment start ≥2 yrs from diagnosis identified pts with lower spleen response rates. Additionally, median titrated doses ≤10mg BID significantly correlated with poorer spleen and symptoms responses. Overall, these data point out the role of an early treatment in order to achieve better therapeutic results. Also, since drug-induced anemia was not significantly associated with RUX doses, the study supports the importance to start and maintain over time the maximum tolerated RUX dose.

P673

MYELOPROLIFERATIVE NEOPLASIA ASSOCIATED WITH SPLANCHNIC VEIN THROMBOSIS IS CORRELATED WITH DISTINCT CLINICAL FEATURES AND LOW JAK2 V617F ALLELE BURDEN

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Background: Splanchnic vein thrombosis (SVT) is strongly associated with myeloproliferative neoplasms (MPN) such as Polycythemia Vera (PV), Essential Thrombocythemia (ET), Myelofibrosis (MF). This association was established when routine molecular analysis of JAK2 V6176F mutation was introduced as a diagnostic assessment for MPN. Up to 50% of Budd-Chiari syndrome and 30% of extraepathic thrombosis are associated with the presence of JAK2 V617F mutation. However, 30% of these patients do not meet the WHO criteria for the diagnosis of PV, ET or MF.
Aims: The study aims to compare and contrast the pathophysiological and genotypic features of JAK2 V617F positive SVT patients with those meeting the standard diagnostic criteria for MPN.
Methods: We conducted a retrospective single centre study of 43 patients with JAK2 V617F positive SVT at the Queen Elizabeth Hospital NHS Trust to document the clinical and haematological features of these patients at presentation, their long-term outcome and their JAK2 mutational status and allele burden by quantitative digital droplet PCR.
Results: SVT occurred before MPN was diagnosed according to WHO criteria in 28 out of 43 patients, and in 3 patients a thrombotic event at a different site occurred before onset of MPN. Within this cohort 18 patients were females and 13 were males. The mean age at diagnosis of SVT/ thrombotic event was 35 years (range 22-67). During follow-up 10 patients developed PV (3 female, 7 males), 7 patients developed ET (3 females, 4 males), 9 patients developed U-MPN (7 females, 2 males), 5 patients did not develop a recognisable MPN at their last follow-up. The median hemoglobin at SVT diagnosis was 14.05 g/dL in females and 14.85 g/dL in males, the median WBC was 9.1x10^9/L, the median platelet count at SVT diagnosis was 266x10^9/L. These values would not have met the WHO diagnostic criteria for ET, PV or MF at diagnosis. In this study we considered JAK2 mutational burden in the range of 0-25% as low, 26-74% as intermediate and >75% as high (AM Vannucchi *et al.*). In our cohort, the median JAK2 mutational burden was in the lower range with a median allele burden of 17% (range 0.6-81%). The median JAK2 mutational burden in the patients diagnosed with SVT without developing MPN at their last follow up had a median allele burden of 14% (range 9.9-22%), which is lower than the mutational burden found in PV and PMF (26-50%) and similar to ET (0-22%).
Summary/Conclusions: JAK2 V617+ SVT constitute an early but distinct MPN subtype presenting with young age, unremarkable haematolgical parameters and low JAK2 V617F allele burden.

P674

BONE MARROW MICROVESSEL DENSITY AND PLASMA ANGIOGENIC FACTORS IN BCR-ABL1 NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: A STUDY OF 90 PATIENTS WITH CLINICOPATHOLOGICAL AND MOLECULAR CORRELATIONS

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Background: Increased angiogenesis in BCR-ABL1 negative myeloproliferative neoplasms (MPNs) has been recognized but its connection with clinical and molecular markers needs to be defined.
Aims: The aim of our study was to (1) asses bone marrow (BM) angiogenesis measured by microvessel density (MVD) using two different monoclonal antibodies CD34 and CD105; (2) analyze correlation of MVD with plasma angiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and Interelukin-8 (IL-8); (3) examine the possible association of MVD with clinical, laboratory, morphological and molecular markers.
Methods: We examined serum and BM specimens from 90 patients with MPNs (30 polycythemia vera (PV), 30 primary myelofibrosis (PMF), 30 essential thrombocythemia (ET)) at the time of diagnosis. Ten age-matched patients without hematological disorder were used as controls. MVD was analysed by immunohistochemistry "hotspot" method while serum angiogenic factors VEGF, bFGF and IL-8 were tested by Elisa immunoassay. Mutation analysis of JAK2-V617F, CALR exon 9 was analyzed by DNA sequencing and allelic PCR.
Results: MVD was significantly increased (CD34=37.6±17.6; CD105=30.8±15.3) in whole MPN cohort compared to controls (CD34=19.8±12; CD105=11.7±24.4). MVD was highest in PMF (CD34=44.5±20.3; CD105=38.4±16.9) followed PV (CD34=43.2±15.8; CD105=31.7±13.4) then ET (CD34=26.6±8.9; CD105=24.7±11). Correlation between MVD and plasma angiogenic factors was found in all cases. MVD was significantly increased in patients with JAK2-V617F mutation and correlated with JAK2 mutant allele burden (CD34-MVD: ρ=0.491, p<0.001; and CD105-MVD: ρ=0.276, p=0.02) but regarding CALR mutation no significant MVD differences were detected in whole cohort. MVD analyzed by both antibodies correlated with leukocyte count, serum lactate dehydrogenase, hepatomegaly, and splenomegaly. BM fibrosis was significantly associated with CD34-MVD (p=0.002), CD105-MVD (p=0.008), IL-8 (p=0.001) and JAK2 mutant allele burden (p=0.013). JAK2 homozygote status had positive predictive value (100%) for BM fibrosis. Patients with prefibrotic PMF (CD34-MVD=39.1±13.4, CD105-MVD=37.1±14.4) had significantly higher MVD then patients with ET (CD34-MVD=26.6±8.9; CD105-MVD=24.7±11) (p<0.001).
Summary/Conclusions: This study highlights the strong correlation of MVD with plasma angiogenic factors, JAK2 mutant allele burden and BM fibrosis in MPNs. We identified specific angiogenic factors, which correlated with BM fibrosis. According to our results, we could recommend MVD to be additional histopathological marker to distinguish ET from prefibrotic PMF.

P675

ANAGRELIDE CONTROLLED RELEASE (GALE-401) SAFETY PROFILE CONSISTENTLY WELL TOLERATED IN MYELOPROLIFERATIVE NEOPLASMS PATIENTS AND HEALTHY VOLUNTEERS

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Background: The most frequently reported AEs leading to treatment discontinuation with anagrelide (Agrylin®, Shire) were headache, diarrhea, edema, palpitations, and abdominal pain. Of the 942 subjects treated with anagrelide for a mean duration of approximately 65 wks, 161 (17%) were discontinued due to AEs or abnormal laboratory test results (PI, Agrylin). These effects may be attributable to anagrelide's inhibitory effects on human PDE3. Modification of the PK profile of anagrelide to reduce peak plasma levels while maintaining therapeutic blood levels may offer a means to retain platelet lowering activity while reducing potential side-effects. In the Phase 1 HV study, BIO-ANA101, 2 such novel controlled release formulations of anagrelide (CR and IR/CR) were compared with the EU marketed formulation (Xagrid®, Shire) in a single dose cross-over study at a dose of 0.5 mg. The CR formulation demonstrated C_{max}/$AUC_{0\text{-}inf}$ values that were 23%/72% of Xagrid's, respectively leading to the development program of anagrelide CR (GALE-401).
Aims: Characterize the safety profile of GALE-401 in HV and MPN subjects.
Methods: To date, 98 HV and 18 MPN subjects have been enrolled among 5 Phase 1 clinical trials (BIO-ANA101, 102, 103, 104, 105) and a Phase 2 single arm, open label pilot study (NCT02125318) of GALE-401, respectively. All studies were conducted in accordance with ICH Guidelines and GCP principles. Safety events were compared across HV *vs* MPN subjects treated with GALE-401 compared to historical Agrylin published data.
Results: In the HV studies, single and multiple doses of GALE-401 were safe and well tolerated and there were no clinically relevant changes in vital signs, ECGs, and safety laboratory parameters other than a reduction in platelet counts. The most frequent TEAEs reported included headache, pain in extremities or back, palpitations and gastrointestinal disturbances. In particular, in BIO-ANA105, with 20 subjects randomized to receive either GALE-401 or Agrylin (0.5 mg BID for 7d) after which they were washed out for a minimum of 21d and received the alternative drug product for an equal period, the overall AEs considered by the Investigator to be related to treatment were observed [GALE-401 (7 events/6 subjects) *vs* Agrylin (12 events/8 subjects)]. With the exception of GI disorders (15%, GALE-401 *vs* 10.5%, Agrylin), the incidence of TEAEs across SOCs was higher during Agrylin treatment compared to GALE-401 treatment. In the Phase 2 MPN study, subjects treated with GALE-401 exhibited fewer of the more common AEs associated with Agrylin (cardiac; general; gastrointestinal; respiratory, thoracic, and mediastinal; skin and subcutaneous tissue; nervous system) or equivalent (musculoskeletal and connective tissue) AEs associated with Agrylin. Some of the less common AEs of Agrylin were comparatively more frequent for GALE-401 (vascular; hepatobiliary; blood and lymphatic). Additionally, fewer moderate to severe (Grade 3/4) AEs and fewer AEs per patient (2.3 *vs* 3.3) were observed with GALE-401 *vs* Agrylin, respectively. Further, based on treatment discontinuation due to an AE, 5 subjects who were previously intolerant to Agrylin (because of AEs) have continued their treatment with GALE-401. Overall, 3 of these 5 subjects were on study for a longer duration [mean time on study, 106d, (47–196d)] compared to their experience with Agrylin prior to enrollment into the Phase 2 study (~7d). Two subjects (40%) have remained in the study and have been able to continue their treatment with GALE-401 for 15 and 22m, respectively. Given this information, GALE-401 seems to confer an improved overall safety profile and potentially offers patients improved tolerability compared to the licensed product.
Summary/Conclusions: Across HV and MPN subjects, GALE-401 consistently demonstrated a well-tolerated safety profile. Moreover, in a small subset of subjects enrolled in the Phase 2 MPN pilot study, some previously treated Agrylin intolerant subjects, demonstrated a continued prolonged clinical benefit with GALE-401. A randomized trial comparing Agrylin *vs* GALE-401 is needed, alternatively or together with a trial evaluating Agrylin intolerant subjects is warranted.

Non-Hodgkin & Hodgkin lymphoma - Biology

P676

PROGNOSTIC IMPACT OF INTEGRATED GENOMIC PROFILING IN ADULT T-CELL LEUKEMIA/LYMPHOMA

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Background: Adult T-cell leukemia/lymphoma (ATL) is an aggressive peripheral T-cell neoplasm associated with human T-cell leukemia virus type-1 retrovirus. ATL includes a heterogeneous group of patients with regard to pathological and clinical features as well as prognosis, suggesting the presence of underlying molecular pathogenesis that can account for such heterogeneity among patients. Recently, through an integrated molecular analysis of a large cohort of ATL cases, we delineated the complete registry of genetic alterations in ATL.
Aims: We sought to analyze possible associations between these genetic/epigenetic lesions and clinical/pathological phenotypes in a large set of ATL patients, especially focusing on the impact of mutations and copy number alterations (CNAs) on clinical outcome.
Methods: We investigated a total of 361 ATL samples, including acute (n=192), lymphoma (n=66), chronic (n=89), and smoldering (n=14) subtypes, for recurrent mutations and CNAs, and assessed their impact on disease phenotypes and prognosis.
Results: Aggressive (acute and lymphoma) subtypes were characterized by a higher number of mutations and focal amplifications/deletions, hyperploid status, and CIMP phenotype, compared with indolent (chronic and smoldering) tumors. Two mutations (*TP53* and *IRF4*) and eight focal deletions involving 1p13 (*CD58*), 6p21 (*HLA-B*), 9p21 (*CDKN2A)*, 10p11 (*CCDC7*), 13q32 (*GPR183*), 16q23 (*WWOX*), 17p13 (*TP53*), and 19q13 (*CEBPA*), were more common in aggressive ATL than in indolent ATL. In contrast, showing a similar mutational distribution to those found in large granular lymphocytic leukemia, *STAT3* mutations were characteristic of indolent diseases. Next, we examined the impact of mutations and CNAs on prognosis among 215 ATL cases, for which survival data were available. Multivariate analysis revealed that disease subtype (aggressive *vs* indolent) was the most significant predictor of clinical outcome in ATL. Subsequent multivariate analysis showed that within the patients with aggressive ATL, older age (≥70 years), *CCR4* mutations, and 9p24 amplification were independently associated with an inferior outcome. Based on the number of the risk factors, patients with aggressive ATL were classified into three categories showing marked difference in 3-year overall survival (OS) (P <0.001): those with no (OS, 32%), one (18%), and two or more risk factors (0%). Among patients with indolent ATL, we found *IRF4* and *TP53* mutations, 9p24 amplification, and deletions in 9p21 and 10p11 were significantly associated with worse prognosis. Interestingly, these alterations, except for 9p24 amplification, were more frequently found in aggressive ATL. More importantly, based on these risk factors, the patients with indolent ATL can be classified into two major categories showing marked difference in clinical outcomes: patients with no risk factors (OS, 89%) and those with one or more risk factors (OS, 21%) (P <0.001, HR=16.8, 95% CI:5.4-52.5), suggesting that patients with indolent ATL harboring a genetic feature of the aggressive subtypes might represent a genetically and biologically distinct subset, which should be better treated as having an aggressive disease.
Summary/Conclusions: Through comprehensive genetic profiling, we demonstrated that the known subtypes of ATL can be further classified into molecularly distinct subsets characterized by discrete sets of genetic alterations and substantially different prognosis. Our results suggest that genetic profiling can improve the prediction of prognosis in ATL patients and better guide therapy.

P677

HNRNP K: A NOVEL ONCOGENE THAT DRIVES C-MYC-DEPENDENT LYMPHOMAS AS A POTENTIAL THERAPY TARGET

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Background: hnRNP K is a versatile molecule that regulates numerous critical cellular process, such as transcription and translation. Recently, we demonstrated that alterations in hnRNP K expression can drive hematological malignancies. Additionally, using reverse phase protein array (RPPA) analyses, we discovered that hnRNP K overexpression correlates with increased c-Myc levels and activation of putative c-Myc targets in patients with hematological malignancies. Likewise, RT-PCR analysis revealed that *HNRNPK* is overexpressed in specific subsets of lymphoma patients. These findings could explain an alternative mechanism by which c-Myc is overexpressed in lymphoma patients without c-Myc alteration.
Aims: The aim of this work is understand the oncogenic potential of *hnRNP K* and evaluate its role in driving c-Myc-dependent lymphomas that do not harbor MYC translocations.
Methods: To directly examine the impact of hnRNP K overexpression on lymphomagenesis, we generated a transgenic mouse model that specifically overexpresses hnRNP K in the B-cell compartment (*Eμ-Hnrnpk*). Phenotypes were characterized by cytokine analyses, CBC, and flow cytometry of serum, blood, and bone marrow. Livers, lymph nodes, spleens, and thymi were measured and molecularly investigated through qRT-PCR, immunohistochemistry, and western blot analyses. Molecular mechanisms were validated using ChIP- and RIP-assays. Malignant lymphoma cells from *Eμ-Hnrnpk* mice were examined for sensitivity to bromodomain inhibitors *in vitro* and *in vivo* assays.
Results: *Eμ-Hnrnpk* mice overexpressed hnRNP K in the B-cell compartment in multiple transgenic lines resulting in a highly penetrant lymphoma phenotype marked by a significant reduction in survival, enlargement of hematopoietic tissues, and dissemination into distant organs (Figure 1A-B). *Eμ-Hnrnpk* mice also display a significant increase in immature lymphoblasts that were transplantable and reproduced the aggressive lymphomas observed in the donor mice (Figure 1C). Together, these results indicate that hnRNP K is an oncogene when overexpressed. Molecularly, we observed a direct correlation between hnRNP K overexpression and elevated c-Myc levels in tissues from transgenic mouse (Figure 1D), as well as in transfected cell lines. RIP analysis demonstrated a directly interaction and causal relationship between hnRNP K overexpression and translation of the *Myc* transcript. In addition to the hnRNP K-mediated activation of the c-Myc pathway, pro-lymphoid cytokines such as IL-9 and CXCL-1 were also elevated in the sera of *Eμ-hnRNP K* mice, resulting in the overexpression/activation of the JAK-STAT pathway. c-Myc is currently an "undruggable target." However, the discovery of small molecules that inhibit the function of Bromodomain and extra terminal (BET) family members (*e.g.* JQ1) have shown efficacy in disrupting c-Myc activities. Using JQ1 and BRD4-Protac (Arvinas®) in *in vitro* and *in vivo* studies, we demonstrate that lymphoma cells from *Eμ-Hnrnpk* mice have a significant reduction of viability *in vitro* with both compounds. Furthermore, engraftment studies using NSG mice showed that JQ1 treatment reduced the percent of engrafted cells compared to mice treated with vehicle alone.

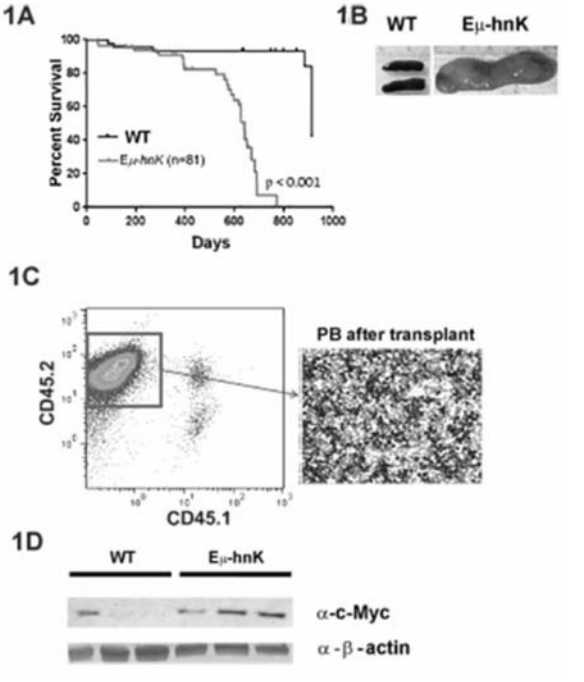

Figure 1.

Summary/Conclusions: We demonstrate for the first time that hnRNP K is a bona fide oncogene with the capacity to drive c-Myc dependent lymphomas. Given our observations that hnRNP K is overexpressed in patients lymphoma, these results suggest that hnRNP K represents a potential biomarker to select patients for target therapies that inhibit c-Myc in the absence of *MYC* translocations or gene amplifications.

P678

GENOMIC PROFILING REVEALS SPATIAL HETEROGENEITY IN FOLLICULAR LYMPHOMA: IMPLICATIONS FOR PRECISION MEDICINE

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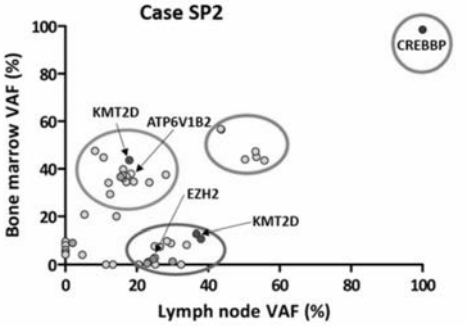
Background: Follicular lymphoma (FL) is an incurable B-cell malignancy characterised by advanced disease and a heterogeneous clinical course. Recent temporal analyses of the genetic changes in these tumours suggest that each disease episode evolves from a putative lymphoma-propagating population. These studies have relied on profiling 'single' FL biopsies over several time points, however, multi-site sampling in solid cancers has demonstrated profound intra-tumour heterogeneity. Whether such patterns are seen in FL has not been examined, with their presence likely to complicate precision medicine based initiatives of these lymphomas in the future.
Aims: To measure the extent of genetic diversity in FL by profiling spatially-separated biopsies.
Methods: Eighteen tumour samples from several tissue sources (lymph node, LN; bone marrow, BM; skin, spleen, ascites or pleural effusion) were collected from 7 FL patients (SP1-7) representing 9 distinct episodes of disease (diagnosis (5); progression (2), transformation (2)). FL B-cells and normal T-cells were enriched by fluorescence activated cell sorting (FACS) in the majority of cases. Whole exome sequencing (WES) was performed on tumour and germ-line DNA from all patients (mean coverage 142x). Somatic variants, acquired uniparental disomy (aUPD), copy number alterations (CNAs) were defined using established bioinformatics tools. Variant allele frequencies (VAFs) were used to define the clonal architecture. Validation of variants and CNAs were completed by targeted deep sequencing using the Fluidigm-Miseq platform (>1000x) and multiplex ligation-dependent probe amplification (MLPA) respectively.
Results: Amongst the 18 tumours, we identified a mean of 63 non-synonymous somatic variants (range 34-128). Together with *BCL2-IGH* translocations, mutations in *CREBBP* (7/7) and *KMT2D* (6/7) were shared and clonally represented in all spatially-related samples supporting their roles as early "drivers" of FL. Outside of these 3 aberrations, there was widespread intra-tumour heterogeneity with discordance of variants across spatially-separated sites ranging from 3% - 47%. While these site-specific or private mutations included genes recurrently mutated in FL (*TNFRSF14*, *RRAGC*, *TNFAIP3*), there was variegation in VAFs at predominantly subclonal levels, alluding to their acquisition at different evolutionary time points. In SP2, we observed differential (sub) clonal selection with the p.Y641S *EZH2* mutation preferentially expanded in the LN compared to the corresponding BM, whereas the reverse was the case with a p.R400Q *ATP6V1B2* mutation (Figure 1). This dichotomy in mutation preference suggests that the local microenvironment may impact on the fitness of a particular mutation. CNA analyses mirrored mutational patterns with both shared 16p aUPD (encompassing the locus of *CREBBP*) in 5 of 7 cases but also site-specific alterations observed in 4 of 7 cases. Overall, the extent of spatial heterogeneity was highest at histological transformation with >30% of mutations discordant across biopsies and included mutations in *TNFAIP3* (SP3) and *CCND3* (SP4).



Diagonal plots comparing the VAFs of the mutations in the lymph node (LN) and bone marrow (BM) in SP2. Green circles indicate variants that are clonal in both LN and BM compartments. Orange circle indicates variants that are clonal in the BM but subclonal in the LN. Blue circle indicates variants with higher allelic burden in the LN than in the BM.

Figure 1.

Summary/Conclusions: We demonstrate spatial genomic heterogeneity is prevalent in FL and is more pronounced at transformation. The existence and diversity of site-specific aberrations suggests that a single biopsy can capture some but not all of the clonal complexities in a patient's lymphoma at a given time-point. These spatial variations need to be considered in biomarker-led clinical studies.

P679

SOMATIC MUTATION PROFILING FOR PROGNOSIS AND TREATMENT STRATIFICATION OF DIFFUSE LARGE B-CELL LYMPHOMA IN THE REMODL-B TRIAL

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Background: Recent studies on mutation discoveries have identified an ever growing long list of potential pathogenic mutations in diffuse large B-cell lymphoma (DLBCL). There are also remarkable variations in the spectrum and frequency of somatic mutations identified among different studies with only a few genes showing mutations in >10% of cases. It is imperative to validate the incidence of the recurrent mutations and investigate their inter-relationship and potential correlation with lymphoma molecular subtype in a large cohort of well documented DLBCL, and explore their potential value in prognosis and treatment stratification. The REMoDL-B trial utilised GEP (gene expression profiling) to stratify patients with newly diagnosed DLBCL (n=1132) for the addition of bortezomib to R-CHOP, based on the hypothesis that this agent may selectively improve the outcome of ABC-DLBCL, thus providing an excellent opportunity for validation of potential genetic biomarkers.
Aims: 1) to investigate the incidence of recurrent mutations, their inter-relationship and distribution according to COO molecular subtype in the REMoDL-B trial; 2) to access the potential value of these genetic changes, together with other clinicopathological and molecular parameters, in disease prognosis and treatment stratification.
Methods: In the first phase of the study, mutation screening was performed for 19 genes involving the NF-kB pathway (*CD79A, CD79B*, *CARD11*, *TNFRSF11A*, *MYD88, TNFAIP3*, *TRAF3*), plasma cell differentiation (*PRDM1*), apoptosis regulation (*TP53*, *FAS*, *DDX3X*), antigen presentation and recognition (*B2M*, *CD58*), and histone modifiers (*CREBBP*, *EP300*, *EZH2*, *MLL2, MEF2B*, *KDM2B*). Mutation in these genes was screened in duplicate by Fluidigm multiplex-PCR and Illumina sequencing using DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) diagnostic biopsies (Wang *et al.* JMD 2015). Mutation data was correlated with COO molecular subtype, which was determined by Illumina WG-DASL^{TM} GEP using RNA samples from FFPE diagnostic biopsies (Care *et al.,* PLOS ONE 2013).
Results: To date, mutation data was obtained in 240 cases of DLBCL including 121 cases of GCB-DLBCL, 72 cases of ABC-DLBCL and 47 cases of unclassified. Among the 19 genes investigated, 5 genes showed a significant difference in their mutation frequencies between ABC and GCB-DLBCL, with *CD79B* (13% in ABC, 4% in GCB, p=0.04) and *MYD88* (32% in ABC, 5% in GCB, p=7x10^{-7}) mutations significantly enriched in ABC-DLBCL, while *EZH2* (27% in GCB, 0% in ABC, p=1x10^{-6}), *MLL2* (16% in GCB, 6% in ABC, p=0.04) and *CBP* (24% in GCB, 3% in ABC, p=7x10^{-5}) mutations significantly enriched in GCB-DLBCL. In general, there was a tendency of mutual exclusion among gene mutations in the NF-kB pathway, apoptosis regulation, and to a lesser extent in histone modifiers. By grouping genes according to their molecular function, mutations in the NF-kB pathway (51% in ABC, 29% in GCB, p=0.002) were also significantly enriched in ABC-DLBCL, while mutations in histone modifiers (57% in GCB, 21% in ABC, p=1x10^{-6}) were significantly enriched in GCB-DLBCL. Interestingly, there was a significant association in the mutations between the NF-kB pathway and plasma cell differentiation programme (p=0.02).
Summary/Conclusions: The data collected so far confirm the association between mutations in individual genes or molecular pathways, and DLBCL molecular subtype. Ongoing investigations to expand both the number of cases and the list of the genes for mutation analysis are in progress.

P680

ADHESION MOLECULE JAM-C: A POTENTIAL TARGET FOR MANTLE CELL LYMPHOMA THERAPEUTICS

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Background: Junctional adhesion molecule C (JAM-C), a member of the JAM

family is expressed by endothelial cells, platelets, NK cells, B lymphocytes and some B-cell lymphoma subtypes, in particular mantle cell lymphoma (MCL). MCL constitutes 3-10% of all malignant non-Hodgkin B cell lymphomas and the median survival of patients remains only 4-5 years, despite recent drug developments. We have shown previously, that blocking JAM-C using polyclonal antibodies inhibited B cell homing to lymphoid organs and decreased lymphoma engraftment in a NOD/SCID mouse model.
Aims: Homing of malignant B-cells to bone marrow and secondary lymphoid organs is of critical importance for lymphoma spread and growth. The aim of the current work was to develop and to test monoclonal antibodies with JAM-C blocking activities for potential therapeutic use in the clinics.
Methods: Monoclonal antibodies (mAb's) were generated by classic hybridoma technology and tested for binding to JAM-C. mAb's with highest affinity were selected and tested *in vitro* and in our NOD/SCID mouse model. For *in vitro* studies, B-cells from peripheral blood of healthy donors and of lymphoma patients were activated with interleukins and CD40L, with or without mAb's, then phosphorylation of intracellular kinases, proliferation, cell cycle status and JAM-C expression were assessed. For *in vivo* studies, primary B cells and Jeko-1 cells (MCL cell line) were injected i.v. into NOD/SCID mice and homing and engraftment of cells to lymphoid organs were studied in mice treated with mAb's compared to non-treated animals.
Results: From six different mAb's generated, one mAb H225, significantly reduced proliferation of normal and malignant JAM-C pos B-cells *in vitro* by 30 and 35%, respectively. This reduction was found to be paralleled by a decrease in the phosphorylation of ERK1/2 by 20%, whereas the phosphorylation of other signaling kinases remained unchanged (Akt, JNK, p38 and STAT5). In mice injected with primary B cells incubated with mAb H225, homing to bone marrow, spleen and lymph nodes was significantly decreased by 50%, 60% and 40%, respectively. Treatment of mice with H225 for two weeks starting several days after injection of Jeko-1 cells, resulted in drastically reduced lymphoma cell numbers in spleen, liver, bone marrow and lymph nodes (decrease of 100%, 99%, 95% and 100%, respectively), compared to control mice. Lymphoma cells recovered from treated mice showed decreased proliferation kinetics (34% decrease) compared to cells from non-treated animals.
Summary/Conclusions: Our results show that mAb H225 effectively blocks homing of lymphoma B cells to supportive lymphoid organs and decreases lymphoma engraftment in bone marrow, spleen, and lymph nodes. Additionally, we show for the first time an inhibitory effect of JAM-C blocking on B cell proliferation. Targeting JAM-C could therefore constitute a new therapeutic strategy for the treatment of B cell lymphomas expressing JAM-C, in particular MCL.

P681

ATM GENE MUTATIONS REPRESENT A HALLMARK OF MANTLE CELL LYMPHOMA BUT DO NOT IMPACT PATIENTS' SURVIVAL

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Background: Mantle cell lymphoma (MCL) represents 5-10% of non-Hodgkin's lymphomas and is characterized by aggressive clinical course with median survival reaching only 3-5 years. Mutations in the *TP53* tumor suppressor gene detected in approximately 20% of patients negatively influence overall survival (OS). In addition, ATM kinase, the upstream p53 activator, was described to be mutated in up to 56% of MCL patients; however, the data about clinical impact of this aberration is still limited.
Aims: To explore the *ATM* mutations frequency in our MCL patients' cohort and to assess their impact on OS.
Methods: Samples from predominantly (85%) untreated patients containing at least 5% of malignant cells according to flow cytometry were selected for NGS analysis. Amplicon-based targeted sequencing using an in-house methodology was done on MiSeq instrument (Illumina). Whole coding regions and splicing sites of *ATM* and *TP53* genes were analyzed (*ATM*: exons 4-65, *TP53*: exons 2-11). Final data was processed by CLC Genomic Workbench software. The median coverage was 4212 reads and a cut-off for the variant allele frequency (VAF) was set at 1%. Functional impact of detected variant was assessed using dbSNP, COSMIC, and HGMD mutation databases. All detected presumably pathogenic alterations were verified either by Sanger sequencing (mutations above 20% VAF) or by repeated NGS analysis (mutations under 20% VAF). The 11q and 17p loci deletions were analyzed using MLPA technology in samples containing >30% of malignant cells.
Results: Samples from 78 MCL patients were analyzed using the targeted NGS. *ATM* mutations were identified in 41/78 patients (53%). In total, 57 mutations were disclosed with 12 patients carrying two mutations and 2 patients even three *ATM* mutations. Truncating variants represented 42% of all mutations. The analysis of germline status performed in 16 patients with available DNA from remission period showed the germline origin in one case (mutation c.3939_3940del). The MLPA analysis was performed in 33/78 patients and showed *ATM* locus deletion in 16 cases (48%). A majority of *ATM*-mutated patients (70%) carried accompanying allelic deletion, while all remaining *ATM*-affected patients manifested two or three *ATM* mutations; presumably on opposite alleles. Only two patients carried isolated 11q deletion. To reliably assess a clinical impact of *ATM* mutations, the known impact of *TP53* gene aberration must have been set aside; therefore the *TP53* gene status was analyzed as well. *TP53* mutations were identified in 18/78 patients (23%). Besides that, four patients carried isolated 17p deletion. In line with presumably common ATM/p53 activity in one biological pathway, concurrent mutations in both genes were rare occurring in 3 patients. The OS analysis then unambiguously showed a negative impact of *TP53* defects but not of *ATM* mutations (P=0.02 and P=0.74, respectively) with the median OS being 81 months in *ATM*-mutated patients, 49 months in *TP53*-defective group and "not reached" in *ATM*/*TP53* wt patients.
Summary/Conclusions: Although *ATM* mutations represent the second most frequent genetic abnormality in MCL besides cyclin D1 translocation t(11;14), their impact on survival seems absent. This is in a striking contrast to *TP53* defects, which significantly deteriorate overall survival of MCL patients.
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P682

MODE OF ACTION OF DIFFERENT PI3K-INHIBITORS IN MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL), is a distinct lymphoma subtype with an aggressive clinical course and a median survival of 3-5 years. New emerging strategies include especially inhibitors of the B-cell receptor pathway (BTK, PI3K, Akt, mTOR) which is constitutively activated in MCL and plays a critical role in tumor growth and survival.
Aims: In the present study we investigated different PI3K-Inhibitors (Copanlisib, Pictilisib, AS-605240, duvelisib, A66, idelalisib and TGX-221) targeting different isoforms (PI3Kα/δ, PI3Kγ/α, PI3Kγ, PI3Kγ/δ, PI3Kα, PI3Kδ and PI3Kß) in MCL.
Methods: MCL cell lines (Z-138, Mino-1, Granta-519, Jeko-1, Rec-1, Maver-1) were exposed to different PI3K-Inhibitors (Copanlisib, Pictilisib, AS-605240, IPI-145, A66, idelalisib and TGX-221; 0,3125-5μM) with or without murine feeder layer (M210B4). The effect of drugs was evaluated by cell count (trypan-blue staining), cell metabolism (WST-assay or ONE-Glo^{TM} Luciferase assay for luciferized MCL cell lines), cell cycle (FACS) and apoptosis (Annexin V PE/7-AAD staining). Subsequently, combinations with other inhibitors of the PI3K/mTOR pathway were analysed. Proteome Profiler phospho-kinase antibody array as well as Western blot analyses were performed after exposure to the various PI3K inhibitors and correlated to the sensitivity of cell lines. Finally, the effects of drug combination were confirmed in primary patient samples.
Results: The most efficient PI3K inhibitor (tested at 5μM) was Copanlisib (PI3Kα/δ) with a median reduction of cell proliferation of 77% (57.5%>89.4%) followed by the dual isoform inhibitor Pictilisib (PI3Kγ/α) reaching a median reduction of cell proliferation rate of 60% (47%>72.5%). For the single PI3K isoform inhibitors AS-605240 (PI3Kγ) was the most efficient reaching a median reduction of cell proliferation of 26% (8.2%>49.7%). Comparison of single isoform inhibition to combined inhibition of the different isoforms in MCL cell lines confirmed the superiority of the combined inhibition of all four isoforms to inhibition of three by 7,2%, to two by 19,2% and to single by 33,7%. Interestingly triple combinations including targeting of the α-and γ-isoforms were almost as efficient as inhibition of all 4 isoforms. In addition reduction of cell proliferation of dual-PI3K-Inhibitors (Pictilisib and Copanlisib) were higher (60% and 77%) than combinations of single isoform inhibitors (AS-605240 and A66: 34%; A66 and idelalisib: 36%; respectively). In primary patient samples copanlisib revealed the highest cytotoxicity followed by duvelisib, idelalisib, TGX221 and A66 (80.9%; 62.5%; 48.7%; 50% and 40.5%, respectively, 5μM). The differential impact of single and multiple PI3K isoform inhibition on phospho kinases suggests that concurrent phosphorylation inhibition of p53, Akt, p70S6 Kinase, RSK1/2/3, STAT3, c-Jun, eNOS, WNK1, Pyk2 and PLCγ-1 is responsible for increased biological activity to multiple PI3K-isoform inhibition. The observation that inhibitors of different isoforms of PI3K result in different protein phosphorylation patterns supports the hypothesis that the PI3K isoforms act on different downstream signaling pathways. Additional data on the influence of microenvironment on the different PI3K-Isoform inhibitors will be presented.
Summary/Conclusions: Based on the comprehensive analysis of PI3K inhibition, inhibition of multiple isoforms of PI3K appears to be most efficient in MCL. Thereby the superiority of combined PI3K-isoform inhibition *vs* selective single isoform inhibition leads to the assumption that each PI3K-isoform regulates different downstream pathways. In addition, the modulating effect of the murine feeder layer confirms that the microenvironment plays a critical role for the mode of action of inhibitors of the B-cell receptor pathway.

P683

TP53 AND KMT2D MUTATIONS PREDICT PFS IN MCL PATIENTS TREATED WITH HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION: RESULTS FROM THE MCL0208 PHASE III TRIAL FROM FONDAZIONE ITALIANA LINFOMI (FIL)

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Background: Recent studies have described the landscape of recurrently mutated genes in mantle cell lymphoma (MCL). However, with the exception of TP53, little is known about the clinical relevance of these mutations.
Aims: Thus, we performed deep sequencing analysis of a MCL gene panel in the prospective series of patients enrolled in the FIL-MCL0208 phase III trial (EudraCT-Number: 2009-012807-25, high-dose immunochemotherapy followed by autologous transplantation for untreated, advanced stage <65 years MCL patients).
Methods: The mutational study included a subgroup of patients with availability of tumor DNA. A specifically designed, targeted resequencing gene panel, including coding exons and splice sites (genes: ATM, BIRC3, CCND1, KMT2D, TP53, TRAF2, WHSC1, NOTCH1) was analyzed in tumor DNA from baseline bone marrow CD19+ purified MCL cells and, for comparative purposes to filter out polymorphisms, in the paired normal genomic DNA (available in 55% of cases) using a TruSeq Custom Amplicon target enrichment system followed by deep next generation sequencing (Illumina, median depth of coverage 2356x). Variants represented in >10% of the alleles were called with VarScan2 with the somatic function when the paired germline DNA was available. For patients lacking germline DNA, a bioinformatic pipeline including a number of stringent filters was applied to protect against the misclassification of polymorphisms as somatic variants. Primary endpoint of the analysis was progression free survival (PFS) and clinical data were retrieved at the time of the first, planned, interim analysis (as of May, 2015).
Results: Out of the 300 enrolled patients, 174 were evaluable for the mutational study (median age: 57 years, range 35-66; males 75%). The MIPI was intermediate or high-risk in 39% of patients, the Ki67 ≥30% in 44%, and blastoid histology occurred in 9%. Patients not included in the study, due to unavailable tumor DNA (n=124) showed superimposable clinical features, except from a lower stage IV rate (79% *vs* 91%, p=0.003), as expected. At the first planned interim analysis, median follow-up of alive patients was 26 months. At 2-years, 79% of patients were progression free and 91% alive (Cortelazzo *et al.* EHA 2015). Overall, at least one mutation was detected in 114/174 cases (66%), including mutations of ATM in 43% of cases, WHSC1 in 16%, CCND1 in 13%, KMT2D in 12%, TP53 in 8%, NOTCH1 in 8%, BIRC3 in 5% and TRAF2 in 1%. By univariate analysis, mutations of TP53 (2y PFS 42% *vs* 83%; p<0.0001) and KMT2D (2-years PFS 69% *vs* 81%; p=0.008) associated with a significantly shorter PFS. By multivariate analysis, mutations of TP53 (HR: 5.3) and KMT2D (HR: 2) associated with an increase of the hazard of progression. By recursive partitioning analysis, three MCL subgroups were hierarchically classified (Figure 1).

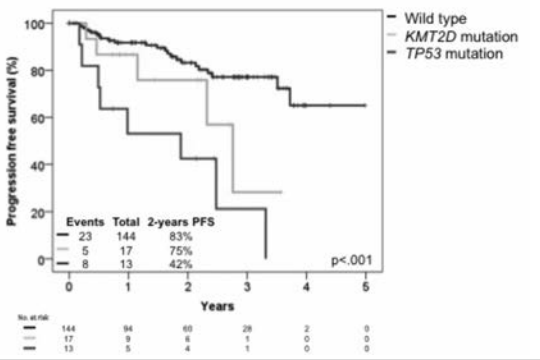

Figure 1.

The high-risk category (TP53 +/- KMT2D mutation, 7% of patients) showed a 2y PFS of 42%, the intermediate risk (KMT2D mutation without TP53 mutation, 10%) showed a 2y PFS of 75%, while the low-risk (no TP53, no KMT2D mutations, 83%) had a 2y PFS of 83%. The low number of events so far recorded prevented any analysis on overall survival.
Summary/Conclusions: Though limited by the short follow-up, our data show that: i) the combination of TP53 and KMT2D mutations predicts a shorter PFS in younger MCL patients receiving high-dose therapy; ii) intensive chemotherapy does not overcome the negative prognostic impact of TP53 mutations; and iii) KMT2D mutations may represent a novel genetic biomarker in MCL.

P684

DECIPHERING THE IMPACT OF IDELALISIB ON FOLLICULAR LYMPHOMA AND ITS IMMUNE MICROENVIRONMENT

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Background: The PI3K pathway is known to contribute to B-cell non-Hodgkin lymphoma (NHL) cell survival by regulation of cell cycle, apoptosis, proliferation, differentiation, homing and retention. The PI3K p110 subunit constitutes the catalytic domain of class I PI3Ks and exists as four isoforms. p110-δ isoform is primarily expressed in cells of hematopoietic origin and constitutes a key component of the BCR pathway. Idelalisib (IDELA/GS-1101/CAL-101) is a first-in-class PI3Kδ selective inhibitor that is approved for the treatment of relapsed chronic lymphocytic leukemia, follicular B-cell non-Hodgkin lymphoma (FL), and small lymphocytic lymphoma. The mechanisms of IDELA sensitivity and resistance remain to be fully elucidated.
Aims: Characterization of the cellular and molecular effects of IDELA in an *in vitro* model utilizing a primary FL coculture system with follicular dendritic cells (FDC) or M2-polarized macrophages (M2).
Methods: FL primary cells from lymph node (LN) biopsies were co-cultured (24-72h)with the FDC cell line HK or M2 generated from M-CSF activated monocytes from peripheral blood of normal donors. Viability of FL cells was assessed by flow cytometry (AnnexinV/7-AAD). B cells were isolated using CD20 magnetic beads, RNA extracted and subjected to Gene Expression Profiling (GEP). Significant pathways were identified by Gene Set Enrichment Analysis (GSEA).
Results: In the absence of accompanying cells, IDELA (100-500nM) induced a moderate cytotoxic effect in FL cells (n=10). FDC or M2 co-cultures increased FL cell viability up to 2-fold. IDELA was also able to reduce this pro-survival effect of FDC or M2, highlighting its impact on lymphoma-microenvironment interactions (Figure 1A). To characterize the molecular mechanisms of these observations, FL primary cells (n=5) were cultured either alone,with FDC or M2, and treated with 500nM IDELA for 48h. GSEA indicated that both FDC and M2 induced the transcription of genes related to B-cell receptor (BCR) and CD40 signaling pathways, together with Germinal Center program and BLIMP-targets. IDELA significantly (FDR<0.05) reduced the engagement of these transcriptional pathways. Additionally, FDC promoted the transcriptional activation in FL cells of gene sets related to angiogenesis, trans-endothelial migration, extracellular matrix (ECM) adhesion and integrin signaling. IDELA was able to counteract this gene upregulation in a subset of patients and preliminary functional experiments validated that IDELA abrogates ECM adhesion (Figure 1B) and VEGF-A secretion (Figure 1C). However, IDELA was inefficient at blocking the secretion of the macrophage attractant CCL-2, the matrix metalloproteinase MMP-2 and the pro-inflammatory cytokines IL-6 and IL-8. In FL-M2 co-culture, GSEA showed that M2 activated the transcriptional activation in FL cells of gene sets that were not sensitive to IDELA treatment, such as Toll-like receptor (TLR) and CCR5 signaling pathways. Moreover, the matrix metalloproteinase MMP-9 was highly elevated in FL-M2 co-culture and was not modulated by IDELA exposure.

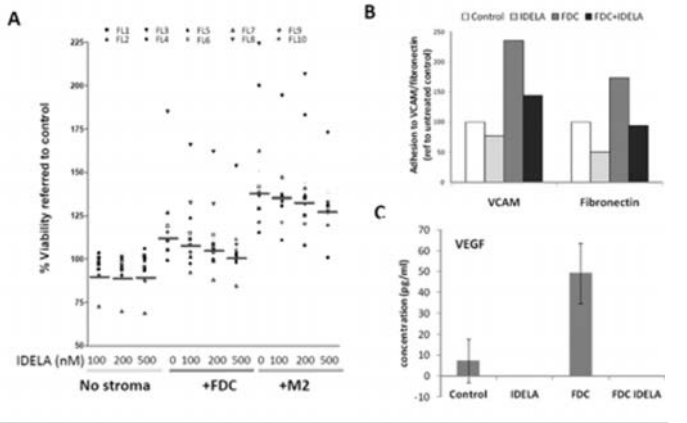

Figure 1.

Summary/Conclusions: We have characterized the impact of IDELA on FL cells and their crosstalk with two immune populations (FDC and M2 macrophages) present in the LN which are associated with poor prognosis. These results suggest that although IDELA targets several important B-cell pathways (BCR, CD40, angiogenesis, trans-endothelial migration, ECM adhesion), other pathways related to pro-inflammatory immune response and cell disseminationare not affected.

P685

MIR-34A PREVENTS LUNG INJURY IN BLEOMYCIN INDUCED PULMONARY FIBROSIS

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Background: Bleomycin is a critical component in the treatment of Hodgkins disease however its major side effect, namely lung fibrosis prevents its use in certain subgroups, especially elderly patients. Prevention of this life threatening side effect could potentially increase the cure rate in this disease. A prominent feature of fibrotic lungs is the development of inflammation leading to accumulation of abnormal myofibroblast populations resistant to death receptor-induced apoptosis. We have previously shown that this resistance is mediated by the expression of cellular FLICE-Inhibitory Protein (c-FLIP). In this work we study the regulation of c-FLIP expression by miR-34a.
Aims: To assess the critical role of miR-34a on FLIP expression during in-vivo bleomycin-induced fibrosis.
Methods: Lung fibrosis was induced using a well-established model of Bleomycin- and control saline-treated C57BL/6 and miR-34 knock-out (KO) mice. Fibrosis was measured by histological H&E semi morphological index (SMI) score, bronco-alveolar-lavage (BAL) cytological ratio and by Collagen abundance using Sircoll-collagen kit. miR-34KO and C57BL/6 myofibroblasts were isolated from day 0/14/28 and day 56 of the model. qPCR and Western blot assays were used to analyze level of miR-34A and FLIP. Furthermore, isolated myofibroblasts were transfected with miR-34 overexpression lentiviral-vector *vs* scrambled vector and levels of FLIP were measured. Apoptosis was induced in miR-34 over-expressed myofibroblasts co-cultured with T-cells and the apoptosis ratio was measured by caspase-3 cleavage and annexin-v staining.
Results: In-silico DIANA prediction tools found FLIP among the top 10th putative genes with an 85% probability to be targeted by miR-34a. in-vitro we detected an inverse correlation between RNA levels of miR-34a and FLIP in myofibroblasts isolated from Bleomycin- and saline-treated mice; saline-treated Lung-myofibroblasts isolated from day 14 express high levels of miR-34a and low levels of FLIP while Bleomycin-treated mice lung-myofibroblasts isolated from day 14 (when fibrosis severity is at its highest) show low levels of miR-34a expression and high levels of FLIP (FLIP: 0.98±0.3 *vs* 3.5±1.2 ; miR-34a: 1.03±0.1 *vs* 0.48±0.07, respectively. p<0.05). The same pattern as Bleomycin-treated myofibroblasts is seen in miR-34 KO untreated myofibroblast; low levels of miR-34a and high levels of FLIP. Restoration of MiR-34a into miR-34KO naive myofibroblasts reduced FLIP levels back to a normal state as opposed to scrambled control counterparts. Moreover, miR-34a over-expression regained myofibroblast susceptibility to apoptosis when cocultured with T-cells. In-vivo, both Bleomycin treated C57BL/6 and miR-34KO groups show high levels of FLIP and severe fibrosis on day 14 compared to healthy saline treated groups. However, while C57BL/6 Bleomycin treated mice recover from fibrosis on day 28, the miR-34KO Bleomycin treated mice continue to express high FLIP levels and persistent fibrosis.
Summary/Conclusions: miR-34a is an important regulator of FLIP expression and is effective in attenuation of Bleomycin lung fibrosis progression. Maintaining high levels of miR-34a in Hodgkins disease patients treated with Bleomycin may prevent lung fibrosis.

Indolent Non-Hodgkin lymphoma - Long-term and outcome

P686

INTERIM ANALYSIS OF POST MARKETING SURVEILLANCE OF YTTRIUM-90 IBRITUMOMAB TIUXETAN IN JAPANESE PATIENTS WITH RELAPSED OR REFRACTORY INDOLENT B-CELL NON-HODGKIN LYMPHOMA OR MANTLE CELL LYMPHOMA

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Background: Yttrium-90 ibritumomab tiuxetan (^{90}Y-IT) is a radiolabeled anti-CD20 monoclonal antibody approved in Japan since 2008 for use in relapsed or refractory (R/R) indolent B-cell non-Hodgkin lymphoma (NHL) or mantle cell lymphoma (MCL). Here we present data from its real world use in Japanese patients (pts) after marketing authorization.
Aims: To evaluate safety and efficacy of ^{90}Y-IT in Japanese pts with R/R B-cell NHL or MCL in usual clinical settings.
Methods: This is a prospective study of consecutive clinical cases collected from 63 institutions across Japan. Pts were enrolled between Aug, 2008 and May, 2010. The data cut-off was Aug 27, 2015. Adverse events were assessed for intent-to-treat population. Efficacy was analyzed during standard observation period according to IWC 1999 and followed annually for outcomes and secondary malignancy.
Results: Safety was evaluated for 413 pts enrolled. Median age was 61 (range 31-87) with 77.2% follicular lymphoma and 12.1% MCL according to WHO classification. 97.3% of pts were performance status 0 - 1 with Ann Arbor staging I, II, III and IV ranging 10.9%, 22.5%, 35.4% and 29.8% respectively. Median number of previous treatments were 3 (range 1 - 22). Of the 409 pts who received ^{90}Y-IT, 315 received 14.8 MBq/kg (0.4 mCi/kg) and 89 received 11.1 MBq/kg (0.3 mCi/kg). Adverse drug reactions (ADRs) were reported in 373 pts (90.3%). There was no difference observed in the incidence rate of ADRs across patients demographics. The most common ADRs were hematotoxicities (88.6%): thrombocytopenia (75.5%), leukopenia (57.6%) and neutropenia (35.6%). Infection was reported in 45 cases (10.9%), with most common being herpes zoster 10 (2.4%), nasopharyngitis 6 (1.5%) and bronchitis 5 (1.2%). Secondary malignancy was reported in 13 pts, including 9 myelodysplastic syndrome and 2 acute myeloid leukemia. Of 409 pts, 59 were excluded from the efficacy analysis data set due to unavailable best response, off-label use, or ^{90}Y-IT not being administered. The overall response rate (ORR) in 354 evaluable pts was 76.8% [95% CI: 72.1 to 81.1%] and complete response (CR) rate was 47.7% [95% CI: 42.4 to 53.1%]. ORR and CR for those receiving 2 or less prior regimens were 86.0% [95% CI: 79.6 to 91.0%] and 56.1% [95% CI: 47.9 to 64.0%] respectively, while ORR and CR for those receiving more than 2 prior regimens were 69.3% [95% CI: 62.2 to 75.8%: p=0.0003] and 40.7% [95% CI: 33.7 to 48.1%: p=0.005] respectively.
Summary/Conclusions: The interim analysis of this surveillance confirms ^{90}Y-IT is a tolerable and efficacious treatment option for pts with R/R B-cell NHL or MCL in Japan, demonstrating good benefit-risk balance consistent with the currently available international and Japanese data. (NCT01448928)

P687

PATIENTS WITH FOLLICULAR LYMPHOMA (FL) SHOWING A DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) COMPONENT HAVE AN INTERMEDIATE OUTCOME BETWEEN FL AND DLBCL

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Background: Whereas histologic transformation to DLBCL during the follow-up of FL determines a poor outcome, it is not clear the prognostic impact of the presence of a DLBCL component (FL/DLBCL) in the diagnostic biopsy of patients with FL.
Aims: to analyze the clinical characteristics, treatment, response, progression free survival (PFS) and overall survival (OS) of patients diagnosed with FL/DLBCL in comparison with FL or DLBCL patients.
Methods: 877 patients diagnosed either FL or DLBCL between 2002 and 2015 in a single institution were included in the study. The distribution was as follow: FL, 369 cases (161 M/208 F; median age, 59 years), DLBCL, 508 cases (274 M/234 F; median age, 65 years). The main clinical, biological features and outcome were analyzed. All biopsies of FL/DLBCL patients were reviewed in order to quantify the DLBCL component. *NOTCH1* mutations were assessed in 195 cases.
Results: 41 patients showed a DLBCL component ranging from 5% to 95% (median: 50%). Patients with FL/DLBCL showed more frequently than DLBCL

cases, ambulatory performance status, absence of B symptoms, primary nodal origin, advanced stage and low risk IPI with these features being closer to those of FL patients (p<0.01 in all cases). On the contrary, FL/DLBCL patients had an intermediate position between FL and DLBCL cases regarding bone marrow infiltration, anemia and serum LDH levels. All FL/DLBCL patients were treated as aggressive lymphoma (R-CHOP). Response and outcome of the patients according to the histology are listed in the Table 1. Of note, the proportion of primary refractoriness in FL/DLBCL patients was significantly higher than in FL and similar than that of DLBCL patients. In Figure 1, PFS and OS of the 3 groups are plotted. No significant differences were seen according to the degree of DLBCL component. In a multivariable analysis with FL patients (including FL/DLBCL, B2 microglobulin and FLIPI), FLIPI demonstrated to be significant for OS (p<0.001). *NOTCH1* were more frequently mutated in FL/DLBCL (11%) in comparison with FL (2%) or DLBCL patients (1.5%) (p=0.06).

Table 1. Response and outcome of the patients according to the histology.

Characteristics	FL (n=328) n (%)	FL/DLBCL(n=41) n (%)	DLBCL(n=508) n (%)
Response to therapy			
CR	216; 75	26; 65	343; 69
PR	58 (20)	6 (15)	40 (8)
Failure	14 (5)	8 (20)*	116 (23)
Relapse/progression	100 (31)	15 (37)	224 (44)
PFS-5 years (95% CI)	65% (59-71)	56% (40-72)	50% (45-55)
OS-5 years (95% CI)	85% (81-89)	73% (57-89)	63% (59-67)

** p<0.05 with FL.*

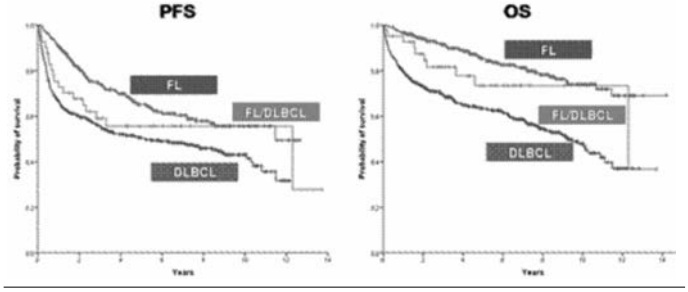

Figure 1.

Summary/Conclusions: FL patients with a component of DLBCL showed an intermediate outcome between FL and DLBCL. These patients may represent a different group of FL that warrants further biological studies.

P688

DISTINCT INCREASING TREND AND BIRTH-COHORT EFFECT OF FOLLICULAR LYMPHOMA INCIDENCE IN TAIWAN: EPIDEMIOLOGICAL ANALYSES AND IMPLICATIONS

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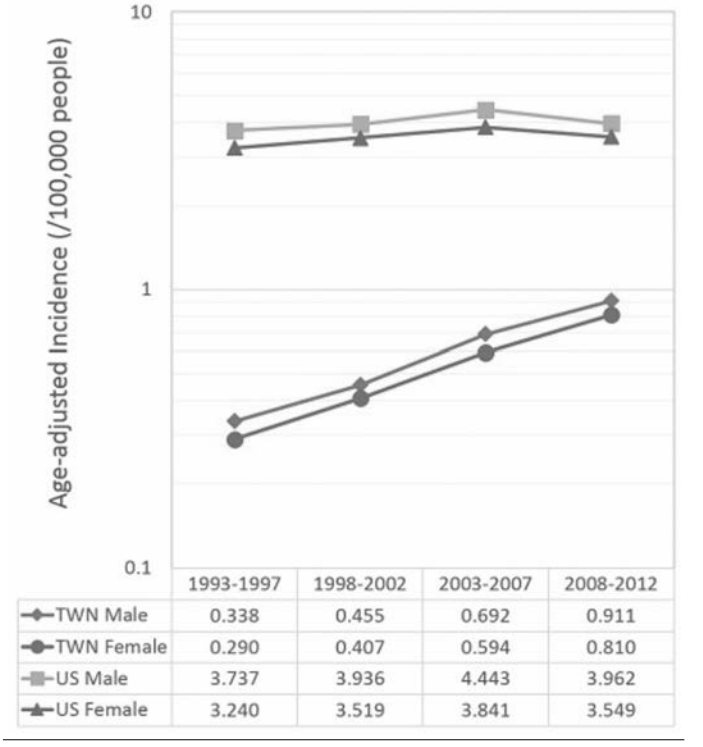
Background: Follicular lymphoma (FL) is much less prevalent in Asia than in the West. However, the recent reports have shown that the relative rate of FL among all lymphoma subtypes is increasing in Taiwan as well as in other Asian populations. Since lifestyle and environmental factors have been proposed to be associated with the risk of FL, it is of interests whether the Westernized lifestyle and environmental alternations that are increasingly affecting Taiwanese may have an important impact on this change.
Aims: The study is aimed at drawing the epidemiological pictures of FL in both Taiwan and the US and dissecting the individual factors contributing to the epidemiological differences between these two areas. The comparison of the epidemiological characteristics of FL between Taiwan and the West may help clarify the factors relevant to the development of this disease.
Methods: The relevant epidemiological data of FL during 1993-2012 for Taiwanese were obtained from the Taiwan National Cancer Registry Database, and the corresponding data of Caucasian Americans, the Surveillance, Epidemiology, and End Results (SEER) Program Research Data. Age-specific incidence rates of FL were plotted by calendar year at diagnosis and by birth-cohort for both populations. The individual effects of time-period and birth-cohort on the incidence trends were evaluated with the age-period-cohort analysis. Patients' outcomes, represented by 5-year relative survivals (RS) between 1990 and 2009, were calculated for outcome comparisons.
Results: The age-standardized incidence rate of FL for the Taiwanese was continuously increasing during the 20-year period while that for Caucasian Americans remained steady (Figure 1). The age-specific incidence rates for Taiwanese were much lower than those for Caucasian Americans for all age group. The estimations of the average annual percentage changes (AAPC) of incidences were significantly positive for all age groups in Taiwan, indicating a consistently increasing incidence trend in all age groups, whereas the AAPC for Caucasian Americans were small and without a consistent pattern. Comparisons of the age-specific incidences in representative birth cohorts demonstrated that for Caucasian Americans, there were almost no differences in the incidences between earlier and later birth cohorts; but for the Taiwanese, the incidence was higher for every later birth cohort in every given age group. With the age-period-cohort modeling approach to dissect the individual effects contributing to this distinct incidence trend, a much stronger birth-cohort effect was identified for the Taiwanese but not for Caucasian Americans. In regard of the outcomes, the 5-year RS in Taiwan and the US were both improving. However, patients' RS in Taiwan during this period were steadily 10~15% inferior to that in the US in both younger and older patients.



	1993-1997	1998-2002	2003-2007	2008-2012
TWN Male	0.338	0.455	0.692	0.911
TWN Female	0.290	0.407	0.594	0.810
US Male	3.737	3.936	4.443	3.962
US Female	3.240	3.519	3.841	3.549

Figure 1.

Summary/Conclusions: Despite the much lower incidence, there is a distinct increasing trend of FL in Taiwan; underlying this increasing trend is a strong birth-cohort effect, suggesting that changes in life style may be an important factor for the difference in incidence trend between the East and the West and providing an evidence of associations between environmental factors and the development of FL. The improved RS in both areas implies that therapeutic advances are changing the clinical course of FL, but the stable RS gap between Taiwanese and American patients indicates that the genetic background is affecting FL patients' outcomes.

P689

LONG-TERM OUTCOMES OF 131I-RITUXIMAB RADIOIMMUNOTHERAPY IN FOLLICULAR NON-HODGKIN LYMPHOMA: TEN YEAR UPDATE ON TOXICITY, TIME-TO-NEXT-TREATMENT AND SURVIVIAL OF THE PHASE II INITIAL STUDY

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Background: Follicular lymphoma (FL) is the second most common non-hodgkin lymphoma (NHL) in Western countries. The decision to treat is generally based on the disease grade and presence of GELF criteria. Standard rituximab chemotherapy regimens improved progression free survival (PFS) but have not translated into an improvement in overall survival (OS). Conventional therapy has a risk of myelotoxicity including myelodysplastic syndrome (MDS). Our phase II INITIAL clinical trial was the first to report significant efficacy of first-line ^{131}I-rituximab outpatient therapy in advanced symptomatic FL. To allay concerns regarding long-term toxicity we report the incidence and nature of both short and long-term myelotoxicity over 10 years of follow up. An update

of the TTNT and long-term survival outcomes of our INITIAL experience of front-line ^{131}I-RIT for follicular lymphoma is also reported.
Aims: We report the toxicity, time-to-next-treatment (TTNT) and survival outcomes after a decade of follow-up of the original phase II INITIAL study patient cohort treated with Iodine-131-rituximab radioimmunotherapy (^{131}I-RIT).
Methods: We reviewed the 68 patients in the original prospective phase II INITIAL study commenced April 2006. Standard eligibility criteria were applied; baseline entry myeloid function required platelets >70x10^9/L, neutrophils >1x10^9/L and hemoglobin >100g/L. All patients met GELF criteria for treatment. Therapy comprised 375mg/m^2 rituximab at weekly intervals for 4 weeks. Standard maintenance rituximab was then given. Individual dosimetry was performed using tracer 250Mbq ^{131}I-rituximab whole body SPECT/CT. A prescribed radiation absorbed dose to the whole body was fixed at 0.75Gy and each patient received an individual administered activity for therapy based upon this dose. Patients were monitored with weekly blood counts during the first 8 weeks and reviewed at regular intervals thereafter.
Results: No patient was lost to follow up. All patients were treated at the fixed radiation dose of 0.75Gy to whole body. Of 68 patients, 5 were excluded from toxicity analysis due to insufficient data. All patients were included in the OS and TTNT assessments. Median age at enrollment was 60 years (37 females and 31 males) with median follow-up of 70 months. No patient required hospital admission for management of bleeding, sepsis or any other complication. Myelotoxicity of grade 3/4 (CTCAE) during the first 8 weeks of therapy occurred in 23 patients (36%); 6 with grade 3/4 thrombocytopenia, 14 with grade 3/4 neutropenia and 3 patients with concurrent grade 3/4 thrombocytopenia and neutropenia. Grade 3/4 myelotoxicity manifested as a nadir of platelets at week 4 and of neutrophils at week 6. Resolution was essentially complete by week 8. No significant hemoglobin reduction was seen. On multivariate analysis bone marrow involvement was independently associated with development of acute thrombocytopenia (p=<0.01). No significant long-term myelotoxicity was observed. There was no instance of MDS or acute leukemia. Deauville criteria were fulfilled for complete response (CR) based on ^{18}F-FDG PET/CT at 3 months follow up in 56 patients (82%). Mean TTNT was 97 months (95 months for FLIPI 0/1, 104 months for FLIPI 2 and 83 months for FLIPI >3). Mean OS was 107 months (94 months for FLIPI 0/1, 109 months FLIPI 2 and 99 months for FLIPI >3). There was no statistically significant difference between groups for TTNT or OS. Achievement of CR at 3-month follow-up ^{18}F-FDG PET/CT was predictive of long-term survival (relative risk=0.36 in contrast to 2.8 for patients not achieving CR) (Figure 1).

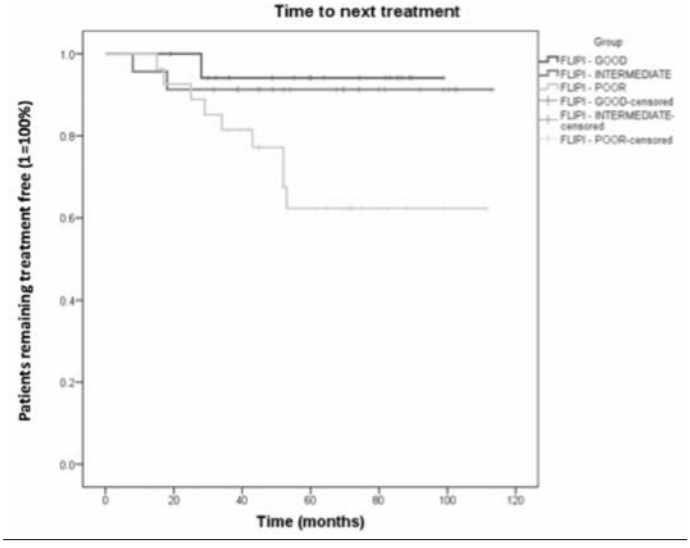

Figure 1.

Summary/Conclusions: First-line^{131}I-RIT for advanced FL is safe and effective. Long-term follow-up of ^{131}I-RIT of advanced FL demonstrates durable response without toxicity. Myelosuppression is modest and self-limited. Deauville criteria at 3-month ^{18}F-FDG PET/CT are predictive of TTNT and OS.

P690

LONG TERM OUTCOMES IN PRIMARY OCULAR ADNEXAL MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMAS (POAML): A LARGE SINGLE INSTITUTE COHORT STUDY

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Background: POAML are rare malignant proliferations affecting the orbit, lacrimal gland, eyelids and conjunctiva, accounting for 35%– 80% of the Primary Ocular Adnexal Lymphoma (POAL) cases. We present the outcomes of the largest single institute study on 182 POAML patients followed for a medium of 63.5 (range 1-387) months.
Aims: To report natural history and identify clinical parameters associated with POAML relapses/progression.
Methods: A retrospective study of 182 patients with POAML diagnosed or treated between January 1984 and December 2015, based on the tumor registry at the University of Miami Sylvester Comprehensive Cancer Center. Information on demographics, medical history, anatomic location, International Prognostic Index (IPI), laboratory/imaging data, stage, treatment and response, details on relapse and management, progression free survival (PFS), time to progression (TTP) and overall survival (OS) was extracted. Kaplan-Meier survival curves were constructed and analyzed by the log rank test with JMP® statistical discovery software.
Results: Of the analyzed 182 patients with POAML, 145 (79.6%) presented in Ann-Arbor stage I and 30 (16.4%) in stage II-IV disease. Median age at diagnosis was 72 years (range, 7-92 years). Patients with lacrimal POAML tended to present at a younger age (median 49.5 years) compared to those with orbital (median 65 years) (p=0.002) and conjunctival POAML (median 62 years) (p=0.05). Overall, 147 of 174 (84.4%) treated patients achieved a complete response (CR) after first-line therapy. In 98 patients with stage I disease who received initial radiation therapy (RT), CR was more commonly observed in patients treated with radiation dose ≥30.6 Gy compared to patients receiving lower RT doses (p=0.04). Similarly, there was also statistically significant difference between these groups in 5 and 10-year PFS (p<0.0001), but no difference in OS. Overall, the long-term local control was achieved in 14 (70%) patients treated with RT <30.6 Gy and 69 (88%) patients treated with RT ≥30.6 Gy (p=0.04). In patients treated with RT for stage I disease 5(4.5%) developed CNS relapses with significantly higher relapses (3/5) in the group receiving <30.6 Gy (p=0.04). Among 174 patients with available follow up data, 36(20.6%) relapsed/progressed and in 147 who achieved a CR after first-line treatment, 5-year and 10-year relapse rates were 23.7% and 43.3%, respectively. Transformation to diffuse large B-cell lymphoma was diagnosed in 7 (4%) and accounted for 7/36 (19.4%) progressions/relapses, leading to death in 2 patients. Median OS for all patients was 250 months (95% CI: 222-upper limit not reached), median PFS was 134 months (95% CI: 87-198) and the median time to progression was 198 months (95% CI:109-upper limit not reached). Kaplan-Meier estimates for the FFP for all 174 patients at 1, 5, and 10 years after completion of initial treatment were 92.7% (95% CI: 87.6%-95.8%), 73.8% (95% CI: 65.8%-80.4%), and 60% (95% CI: 49%-70%), respectively. On univariate analysis, age >60 years, radiation dose, bilateral ocular involvement at presentation and advanced stage were significantly associated with shorter PFS (p=0.006, p=0.0001, p=0.002 and p=0.0001 respectively) Multivariate analysis showed that age >60 years (HR=2.44) and RT<30.6 Gy (HR=4.17) were the only factors associated with shorter PFS (p=0.01 and p=0.0003, respectively).
Summary/Conclusions: Patients with POAML have CNS relapses in 2.8% and transform to DLBCL in 4% of cases. RT at doses ≥30.6 Gy are associated with better local control; fewer local, CNS and distant relapses and longer PFS. Early stage POAML do well with localized RT for stage I disease but demonstrate long-term constant risk for relapse.

P691

EARLY TRANSFORMATION IN LOW-GRADE LYMPHOMAS AND ITS ADVERSE PROGNOSTIC SIGNIFICANCE ON SURVIVAL

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Background: Transformed lymphoma (TL) is a very heterogeneous condition that usually represents the final stage of low grade non-Hodgkin's lymphomas (LG-NHL) progression. It is commonly associated with poor survival. Previously, we reported the prognostic impact of the Revised International Prognostic Index (R-IPI) and National Comprehensive Cancer Network-IPI scores at histologic transformation in survival (Tsao *et al.,* ASH 2013 and Griffin *et al.,* ASH 2014). Here we investigate the impact of early transformation in TL.
Aims: To determine if early transformation of LG-NHL has a prognostic impact in high-risk TL.
Methods: Patients with a biopsy proven diagnosis of TL from LG-NHL between January 2001 and December 2014 that were treated at Moffitt Cancer Center were identified using the Total Cancer Care Database. High risk TL was defined based on an R-IPI over 3 points and NCCN-IPI over 3 points. Early transformation was empirically defined as the evidence of aggressive transformation within 24 months (TT24) from LG-NHL diagnosis. Overall survival (OS) was calculated from the date of transformation, estimated by the Kaplan-Meier method (K-M), and compared using the Log-rank test. Predictors of survival were assessed using Cox proportional hazards model, and multivariate analy-

ses of significant factors were assessed by backward step elimination for variables with a p<0.05.
Results: A total of 149 patients with transformed DLBCL were identified. The median age at transformation was 58 years (range 21–89), the M/F ratio was 1.4. The most common type of LG-NHL was follicular lymphoma (75.8%). At TL, B-symptoms were present in 30%, stage III/IV in 64.4%, elevated LDH in 55.2%, serum albumin (SA) less or equal than 3,7 g/dL in 28% of patients. Forty-five percent and 44.6% had IPI score ≥3 and NCCN-IPI score ≥3, respectively. The most common treatment regimen at time of transformation was R-CHOP (61%) then by R-ICE (12.7%). The median OS was 6.4 years (95% CI, 5.48-7.49). The 5 and 10-years OS were 54% and 33% respectively. Patients with NCCN-IPI and R-IPI ≥3 were associated with poor survival (median OS of 4.2 years, p=0.024; and 3.76 years, p<0.001, respectively). SA ≤3.7 g/dL was predictive for inferior OS (3.5 y *vs* 8.6 y, p=0.04). TT24 was associated with poor outcomes (OS 3.3 *vs* 6.1 y, p=0.008) (Figure 1). Multivariate analysis showed that TT24 (HR 4.13, p=0.042), NCCN-IPI >3 (HR 3.16, p=0.075) and IPI ≥3 (HR 3.47, p=0.063)were statistically significant for shorter OS. The impact of TT24 was particularly significant in high risk TL (NCCN-IPI >3 and/or IPI ≥3) with very poor outcomes (OS 1.7 *vs* 4.4 y, p=0.038 and 1.3 *vs* 4.4 y, p <0.001; respectively).

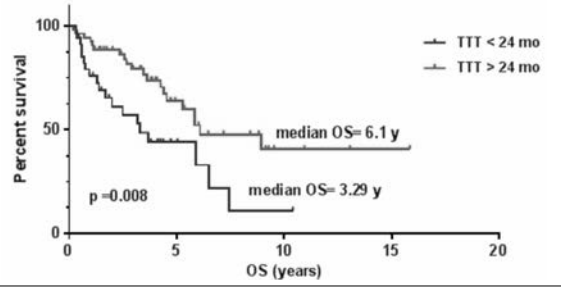

Figure 1. OS by early transformation (TTT24).

Summary/Conclusions: Early time of transformation (TT24) to a more aggressive lymphoma is predictive of shorter OS in patients with TL, especially in cases of high risk TL, defined by the R-IPI and/or NCCN-IPI scores. New strategies in prospective studies based on TT24 are warranted for patients with TL.

P692

OFATUMOMAB FOR THE TREATMENT OF EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF THE MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA): THE O-MA1 STUDY
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Background: To date there is no defined treatment standard for Helicobacter pylori (HP)-eradication refractory, extragastric or advanced mucosa-associated lymphoid tissue lymphoma (MALT lymphoma). Immuno-/chemotherapy combinations have been successfully tested in this setting, however, due to the indolent course of this disease less toxic approaches are warranted. Rituximab-monotherapy has been tested for MALT lymphoma and has shown symptomatic activity but a relatively low rate of durable remissions. Based on data generated in other B-cell malignancies second-generation compound ofatumumab (OFA) appears to be an attractive agent for the treatment of MALT lymphoma.
Aims: The primary endpoint of this trial was to evaluate the capacity and safety of OFA to induce objective responses in patients with HP-eradication refractory or extragastric MALT lymphoma
Methods: The O-MA1 study was a single-center phase II study. In case of HP-positive MALT lymphoma a minimum of 12 months follow-up after successful HP-eradication was required. OFA monotherapy was given at four weekly doses (1000mg i.v., weeks 1,2,3,4) followed by four doses at two-month intervals, starting at week 8. Restaging was performed at weeks 12, 24 and at end of treatment. To date, the planned number of 16 patients has been included and the study is closed to recruitment.
Results: The median age at treatment start was 69 years with an absolute range of 38 to 85 years. Five of 16 patients (31%) had primary gastric MALT lymphoma while the remaining 11 (69%) presented with extragastric manifestations including lymphoma of the ocular adnexa (n=6), the lung (n=1), the bladder (n=1), the liver (n=1), the breast (n=1) and bilateral lymphoma of the parotid glands (n=1). In terms of staging 12 patients (75%) had localized lymphoma *i.e.* stage I or II according to Ann Arbor, and four patients (25%) disseminated disease *i.e.* stage IV. All patients had an ECOG performance status below two, but a total of nine patients (56%) had more than four significant comorbidities. IPI-Score was 0 - 1 in 12 patients (75%) and 2 - 3 in four patients (25%). A total of eight patients (50%) had been pretreated including five patients (31%) with prior immuno- or chemotherapy. Median time from last treatment to the first dose of OFA in pretreated patients was 8.9 months (range; 4.5-59). At the time of this analysis all patients had at least one restaging and were thus evaluable for response. Twelve of 16 patients showed an objective response for an overall response rate of 75%. The complete remission rate was 50% (8/16), while 25% had a partial remission (4/16) and 25% disease stabilization (4/16) as best response. However, one patient with gastric lymphoma and complete remission at first and second restaging had a focal gastric relapse at final assessment. This patient is currently asymptomatic and no salvage treatment has been indicated so far. The median time to best response was 3.3 months (range; 2.5-10). Tolerability of treatment was excellent with no related adverse events greater CTCAE grade I except infusion reaction grade I/II in 13/16 patients (81%) during the first OFA-application, which could be easily handled with steroids or antihistamines and did usually not reoccur in the following. However, one patient was taken off-study due to unrelated prolonged hospitalization for erysipelas but was still evaluable for response as this was after the fourth application of OFA. No relevant grade III or IV hematologic adverse events have occurred. To date at a median follow-up time of 16.2 months (range; 3.9-28.2) 14 of 16 patients have finished treatment with no further relapse or progress, while two patients are on treatment. More mature data will be presented at the meeting.
Summary/Conclusions: This is the very first study to evaluate OFA as a chemotherapy-free approach for the treatment of MALT lymphoma. With 16/16 planned patients recruited we report a response rate of 75% and a disease stabilization rate of 94%. Toxicities were negligible even in elderly patients with significant comorbidities.

P693

BENDAMUSTINE PLUS RITUXIMAB *VERSUS* R-CHOP AS FIRST-LINE TREATMENT FOR PATIENTS WITH INDOLENT NON-HODGKIN'S LYMPHOMA: EVIDENCE FROM A MULTICENTER, RETROSPECTIVE STUDY
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Background: The optimal first line treatment for advanced low-grade non-Hodgkin lymphomas (LG-NHL) is still highly debated. Recently, the StiL and the BRIGHT trials showed that the combination of rituximab and bendamustine (R-B) is non-inferior to rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with a better toxicity profile.
Aims: Utilizing a retrospective analysis, we compared the efficacy and safety of both regimens in clinical practice.
Methods: From November 1995 to January 2014, 263 LG-NHL patients treated with either R-B or R-CHOP were retrospectively assessed in 7 European cancer centers.
Results: Ninety patients were treated with R-B and 173 with R-CHOP. Overall response rate was 94% and 92% for the R-B and the R-CHOP group, respectively. The percentage of complete response was similar for both groups (63% *vs* 66% with R-B and R-CHOP, respectively; p=0.8). R-B was better tolerated and less toxic than R-CHOP. The median follow-up was 6.8 and 5.9 years for the R-CHOP and the R-B group, respectively. Overall, no difference in progression free survival (PFS) (108 months *vs* 110 months; p=0.1) was observed in the R-B group compared to the R-CHOP cohort. However, R-B significantly prolonged PFS in FL patients (152 and 132 months in the R-B and R-CHOP group, respectively; p=0.05).
Summary/Conclusions: We confirm that the R-B regimen administered in patients with LG-NHL is an effective and less toxic therapeutic option than R-CHOP in clinical practice. In addition, we suggest R-B as the treatment of choice for FL patients due to the long-term disease control achieved.

P694

FINAL RESULTS OF IHC ANALYSIS AND PROGNOSTIC FACTORS IN POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS AFTER SOLID ORGAN TRANSPLANTATION
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Background: Post Transplant Lymphoproliferative Disorder (PTLD) represents one of the most fatal complications following solid organ transplants (SOT). A multicenter analysis in 2010 reported a 3yr overall survival (OS) of 62%. In 2014 a single institutional study reported an improved median OS of 8 years

in the rituximab era. Clinical studies have attempted to subdivide post transplant diffuse large B cell lymphomas (PT-DLBCL) into immunophenotypic subgroups with most reviews demonstrating a higher incidence of activated B-cell (ABC) subtype. Because of the rarity of PTLD, insight into disease development and progression is currently limited

Aims: Our preliminary analysis of data on 52 patients (pts) was presented at the American Society of Hematology meeting in 2015 (Abstract 1443). We hereby present our final analysis on a larger cohort of 86 pts with PTLD treated at the University of Colorado over a 25-year period.

Methods: This retrospective analysis focused on 86 adult SOT pts who were treated for PTLD at our institution between Jan 1989 to April 2015.

Results: 86 pts were evaluated. The median age at transplantation was 49.5 yrs (5-74 yrs). Median time from SOT to PTLD was 37 months (1.4-499).The most common transplanted organ included kidney (40%), liver (38%), lung (13%) and heart (9%). Grafted organ was involved with lymphoma in 25% pts. 62% were male, 36% had Stage III-IV, 60% were EBV positive, 16% had bone marrow involvement, 19% had CNS involvement and 30% had extranodal Disease. 31% pts had early PTLD (<12mnths of SOT) and 69% pts had late PTLD(>12mnths of SOT). 77% pts with early PTLD and 49% of pts with late PTLD were EBV positive. Of the 86 patients, 75 pts (87%) had monomorphic histology. Amongst monomorphic PTLD 48 pts (64%) had DLBCL, 9% had Burkitt Lymphoma, 12% had CNS lymphoma, 1% had plasmablastic lymphoma, 1% had NK/T cell lymphoma, 3% had hodgkins lymphoma and 5% had plasma cell neoplasm. 88% pts were CD20 positive, 67% had elevated LDH and 50% had an IPI >1. 85% were on >1 immunosuppressive agent (ImSx) with a median ImSx treatment (Rx) duration of 3 years. Interestingly 80% of pts with CNS lymphoma were on mycophenolate mofetil ImSx. 86% of pts with Burkitt lymphoma had undergone liver transplantation. All Pts with CNS Lymphoma were recipients of kidney transplantation. Initial Rx included reduction in ImSx in 80% pts, rituximab monotherapy in 27% pts, rituximab +chemotherapy in 31% pts and chemotherapy alone in 11%pts. 63 of 86 patients were assessable for response. 90% achieved a complete remission after first line therapy while 17% were refractory to front line therapy. 9 out of 63 pts(14%) pts relapsed. IHC staining utilizing the Hans Algorithm was applied on 32 of 48 samples with DLBCL. ABC subtype was identified in 75% (24/32 samples) and GCB (germinal center B-cell) subtype in 25% (8/32) samples. Median overall survival was 133 months (11yrs). On univariate analysis ABC DLBCL subtype (HR 0.28, 95% CI 0.085-0.970, p=0.03) was associated with improved survival. Graft organ involvement with PTLD and CNS disease showed a trend to poor survival. EBV positivity, Polymorphic PTLD and Early PTLD showed a trend toward improved survival. Rituximab containing therapy (Monotherapy or with Chemotherapy) was better than non-Rituximab therapy (HR 0.6 P=0.3). Rituximab plus Chemotherapy had worse outcomes than Rituximab monotherapy (HR 2.06) (Figure 1).

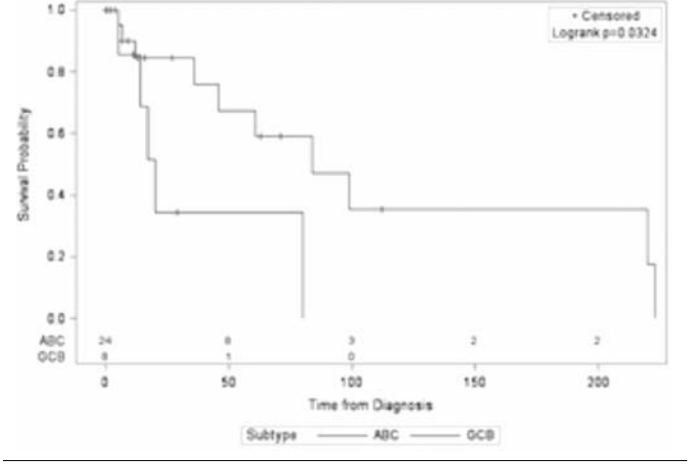

Figure 1. Product-limit survival estimates. With number of subjects at risk.

Summary/Conclusions: In this consecutive series of PTLD after SOT from a single institution over a 25 year period we noted a 3yr OS of 81% and a 5yr OS of 76%. ABC subtype was associated with improved survival whereas graft organ involvement and CNS disease was associated with a trend to poor outcomes. Rituximab based therapy was associated with improved outcomes. In one of the first ever-reported data in PT-DLBCL cases we were able to demonstrate a higher incidence and an improved survival with ABC DLBCL subtype compared to GC DLBCL. The reason for this is not fully understood and merits further evaluation in larger pt cohorts.

Aggressive Non-Hodgkin lymphoma - Clinical

P695

IMPROVED OUTCOMES WITH HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANT IN PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA WITH PRESERVATION OF COGNITIVE FUNCTION

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Background: Outcome for patients with primary central nervous system lymphoma (PCNSL) have improved with the use of high-dose (HD) methotrexate-based (MXT) regimens but relapses are frequent. To prevent relapse, consolidation of first response is desirable, the main options being whole-brain radiotherapy (WBRT) or high-dose chemotherapy (HDC) and autologous stem cell transplant (ASCT). Our centre has favoured the latter given the high incidence of neurotoxicity with WBRT.

Aims: To retrospectively review the outcomes for patients with PCNSL treated with HDC and ASCT and report analysis of prospective neurocognitive assessments.

Methods: 63 patients were diagnosed with PCNSL between 2008 and 2015. The median age at diagnosis was 60 years (range 27-80; 37 male). 21 patients went on to receive HDT-ASCT with BCNU-thiotepa conditioning. A proportion of these patients also had prospective neurocognitive assessment. 5 cognitive domains were assessed: intellectual, memory, language, perception and executive functions. All employed tests have published, validated normative data. Raw test scores were converted into age adjusted standard scores and compared with published normative values.

Results: 49/63 patients (78%), ≤70 years and hence suitable for HDC-ASCT, were treated with HD-MXT based regimens. 23 (47%) had a response *i.e.* complete response (CR) or partial response (PR) to first-line treatment and 14/23 proceeded to HDC-ASCT. 15/26 patients who did not have a response to HD-MTX went on to receive second line treatment (6 radiotherapy, 7 chemotherapy) and, of these, 7 achieved a response (CR/PR) of whom 4 proceeded to HDC-ASCT. 9/23 patients that initially responded to HD-MXT but did not proceed to HDC-ASCT all relapsed. 5 received further treatment (2 chemotherapy and 3 radiotherapy) and of these, 3 proceeded to HDC-ASCT in second response. Overall, 21 patients received HDC-ASCT, median age 54 years (range 26-68; 15 male). Histology was DLBCL in 19, mantle cell lymphoma in 1 and ALK+ anaplastic large cell lymphoma in 1. For all 63 patients, the median follow-up is 8.3 months (0-92 months) and 36 patients (57%) have died, all except one from lymphoma. The median first remission duration is 10 months (1-71) and median overall survival (OS) 8.7 months. For the 21 patients receiving HDC-ASCT, the median follow-up is 26 months (11-92) and the median progressive free survival and OS has not been reached (Figure 1). 6/21 (29%) have relapsed; 2 with systemic lymphoma. 5 have died; 4 from lymphoma and 1 treatment-related (hepatitis E infection). 9 patients had neurocognitive assessments at baseline (pre- ASCT) and 1 year post ASCT (6 of whom had undergone baseline assessment) and 7 patients at 2 years post ASCT (3 of whom had undergone both baseline assessment and 1 year post ASCT). Intellectual, language and perceptual functions were preserved over the 2 year period. However, some patients displayed impairment in memory and executive function at baseline. While no deterioration in executive functions was demonstrated over the 2 year period, there was a suggestion of mild decline in aspects of verbal recall memory at 1 year post ASCT (paired t-tests of mean score on list learning at 1 year follow-up significantly below baseline mean score, p=0.021).

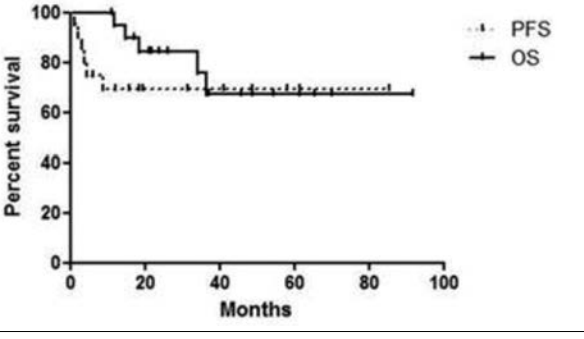

Figure 1.

Summary/Conclusions: In this unselected group of patients, the outcome for PCNSL remains poor. However, for those patients that respond to induction regimens and are fit enough to receive HDC-ASCT, prolonged disease control can be achieved with relative preservation of neurocognitive function.

P696

LENALIDOMIDE IN RELAPSED OR REFRACTORY DIFFUSE LARGE B CELL LYMPHOMA: IS IT A VALID TREATMENT OPTION?

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Background: Diffuse large B cell lymphoma (DLBCL) is the most frequent aggressive non-Hodgkin lymphoma (NHL) in the western country. Despite its typical morphology, DLBCL is characterized by molecular heterogeneity, which can be classified by gene expression profiling (GEP) in two main subgroups based on the cell of origin, namely germinal center B cell (GCB) and activated B-cell. However, GEP is not routinely performed, which is why immunohistochemistry (IHC) is commonly used for designating these two subtypes. Because of the addiction to different oncogenic driver pathways, each subtype differs in response to drugs, leading to a distinct prognosis. In particular, the cure rate of ABC is only 40% after standard immunochemotherapy, hence novel therapeutic approaches are urgently needed. Prospective randomized studies regarding the use of lenalidomide in relapsed/refractory (R/R) DLBCL have demonstrated efficacy and feasibility of this immunomodulatory agent.
Aims: Up to now, data evaluating this drug in clinical practice are lacking. Therefore, we assessed its toxicity and efficacy in the largest R/R DLBCL real-life cohort carried out up to now.
Methods: From January 2006 to January 2015, 123 consecutive patients affected by R/R DLBCL who underwent lenalidomide monotherapy were retrospectively assessed in 3 European cancer centers.
Results: All patients received a starting dose of either 15 mg/day or 25 mg/day of lenalidomide. Response to treatment differed significantly between the two IHC subgroups: patients with non-GCB DLBCL achieved a complete remission (CR) in 32% and a partial remission (PR) in 33% compared to 0% and 3% in the GCB group (p<0.0001 and p=0.001). Toxicity was limited and reversible. The median follow-up was 4.5 years (range 2-108 months). The median duration of response was 4 months (range 1-10 months) and 15 months (5-23 months) in GCB and non-GCB DLBCL, respectively (p<0.001). Patients receiving the 25 mg daily of lenalidomide had an overall superior outcome compared to those who underwent the 15 mg regimen, namely 21% achieved a CR and 23% a PR compared to 0% and 8% (p=0.007 and 0.05). Also median duration of response varied significantly between both dosing groups (10 *vs* 4 months; p=0.03). Overall, the median progression free survival was 34 months (range 2-108 months), ranging between 37 and 30 months according to the non-GCB and GCB DLBCL group (p<0.0001) and between 24 and 34 months (p=0.002) according to the higher or lower lenalidomide dosing. However, overall survival was similar between the different subgroups (38 *vs* 41 months in non-GCB and GCB DLBCL, p=0.2; 38 *vs* 42 months in 15 and 25 mg/die, p=0.4).
Summary/Conclusions: In this real-life setting, we demonstrated that lenalidomide is a valid treatment option for R/R DLBCL with only limited and reversible toxicity. As expected, lenalidomide is more efficient in patients with non-GCB DLBCL, but responses were also observed in the other subgroup, which is why it can be considered also for GCB DLBCL. If clinically feasible, the 25mg dosing should be preferred.

P697

NONPEGHYLATED LIPOSOMAL DOXORUBICIN AS A COMPONENT OF R-CHOP IS EFFECTIVE AND SAFE IN THE TREATMENT OF A CARDIAC HIGH-RISK AND ELDERLY LYMPHOMA PATIENTS

L Rigacci[1,*], F Lancia[1], L Flenghi[2], F Angrilli[3], U Vitolo[4], ML Vigliotti[5], V Tarantino[6], A Ferrari[7], M Spina[8], G Benelli[1], S Bolis[9], G Gaidano[10], C Cox[11], PR Scalzulli[12], AM Mamusa[13], R Mazza[14], PL Zinzani[15], S Ferrari[16], G Gini[17], A Mulè[18], M Ladetto[19], N Di Renzo[20], F Zaja[21], V Zilioli[22], P Musto[23], V Zoli[24], D Marino[25], A Fabbri[26], A Guarini[27], A Carpaneto[28], F Ballerini[29], M Gotti[30], M Petrini[31], A Di Rocco[11], A Bosi[1]

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Background: Doxorubicin is the most effective single agent in the treatment of non Hodgkin's lymphoma (NHL). Its use is limited for the cardiac toxicity particular in elderly patients (pts) and in pts with history of cardiac disease. Liposomal doxorubicin has been proven to reduce cordiotoxicity.
Aims: The aim of this retrospective study was to evaluate in real life in a high-risk population the use of nonpeghylated liposomal doxorubicin (NPLD) in term of efficacy and cardiac events.
Methods: 965 consecutive pts treated with R-COMP (doxorubicin was substituited with NPLD, Myocet) were collected from 32 Italian Hematologic Centers. After the evaluation of pts' records 16 were excluded, so the final analysis was conducted on 937 pts. Median age was 73 (range 26-92), the reasons for use of NPLD were: age (449 pts), cardiac disease (340 pts), uncontrolled hypertension (123 pts), other reasons (55 pts). According to clinicians evaluation 48% of pts would not have used standard doxorubicin for different situations (age, cardiomiopathy, previous use of doxorubicin, uncontrolled hypertension). 115 pts showed at diagnosis a LVFS <50% (in 108 pts this data was not reported). 5083 cycles were infused and 841 pts (90%) performed 4 or more cycles according to the therapeutical program. 462 pts (49%) were over 74 years, 66% of pts had stage III or IV, 95% were DLBCL.
Results: Overall 678 pts (72%) obtained a complete remission (CR) and 116 experienced a relapse. CR rate was not significantly different between the 52% of pts which could be able to use standard doxorubicin (76%) and the 48% of pts which would not have used doxorubicin (70%). During treatment 98 pts (11%) presented a cardiac toxic event in particular: 29 heart failure, 29 severe cardiac arrhythmia, 15 myocardial infarction, 4 atrial fibrillation, 3 cardiac arrest and 18 other mild cardiac complications. No statistical differences was reported between pts with normal FE *VS* or less tha 50% according to this cardiac toxic events. After a median period of observation of 32 months, 651 pts are alive and the overall survival (OS) was 72%. OS was significantly superior in pts considered eventually elegible to standard doxorubicin in comparison with other not elegible 79% *vs* 70% (p .001). 282 pts were death and 4 were lost to follow-up. The causes of death: 181 due to lymphoma progression, 37 due to complications other than cardiac, 15 due to cardiac complications, 12 due to second neoplasm and in 41 the cause was not known. After a median observaton period of 23 months PFS was 58%. Either in univariate or in multivariate OS and PFS were not significantly affected by age or cardiac disease.
Summary/Conclusions: In conclusion NPLD introduced in R-CHOP is effective and safe in a cardiac high-risk population and in a negatively selected group of pts. Moreover the use of this NPLD permitted that about half of our population have had the opportunity to receive the best available treatment.

P698

METHYLATION OF CELL-FREE CIRCULATING DNA IN PLASMA PREDICTS POOR OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: The prognostic value of aberrant DNA methylation of cell-free circulating DNA in plasma has not previously been evaluated in diffuse large B-cell lymphoma (DLBCL).
Aims: The aim of this study was to investigate if aberrant promoter DNA methylation can be detected in plasma from DLBCL patients and to evaluate this as a prognostic marker. Furthermore, we wanted to follow possible changes in methylation levels during treatment.
Methods: Seventy-four patients were enrolled in the study, of which 59 received rituximab and CHOP-like chemotherapy. Plasma samples were collected from all patients at the time of diagnosis and from 14 healthy individuals used as controls. In addition, plasma samples were collected during and after treatment for surviving patients. In total, 158 plasma samples were analyzed for DNA methylation in the promoter regions of *DAPK* (*DAPK1*), *DBC1*, *MIR34A*, and *MIR34B/C* using pyrosequencing.
Results: Aberrant methylation levels at the time of diagnosis were detected in 19, 16, 8, and 10% of the DLBCL plasma samples for *DAPK1*, *DBC1*, *MIR34A* and *MIR34B/C*, respectively. *DAPK1* methylation levels were significantly correlated with *DBC1* and *MIR34B/C* methylation levels (P<0.001). For the entire cohort five-year overall survival (OS) rates were significantly lower in the groups carrying aberrant *DAPK1* (P=0.004) and *DBC1* methylation (P=0.044), respectively. Multivariate analysis identified *DAPK1* as an independent prognostic factor for OS with a hazard ratio of 5.1 (95% CI: 1.9-13.4, P=0.001). Patients with *DAPK1* methylated cell-free circulating DNA at time of diagnosis, who became long term survivors, lost the aberrant methylation almost immediately after treatment initiation. Conversely, patients that regained or maintained aberrant *DAPK1* methylation died soon thereafter.
Summary/Conclusions: Aberrant promoter methylation of cell-free circulating DNA can be detected in plasma from DLBCL patients, and *DAPK1* methylation is an independent prognostic marker that may also be used to assess treatment response.

P699

REAL-WORLD EXPERIENCE OF IBRUTINIB IN >700 PATIENTS WITH MANTLE-CELL LYMPHOMA: DATA FROM A GLOBAL NAMED PATIENT PROGRAM

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Background: A global named patient program (NPP) was opened in numerous countries worldwide in order to allow access to ibrutinib for eligible patients with relapsed/refractory mantle-cell lymphoma (MCL) prior to approval in those countries. This program provides real-world data on estimated outcomes with ibrutinib across a large, global MCL population.
Aims: To use real-world data from the ibrutinib MCL NPP to investigate whether treatment benefits reported in randomized clinical trials are reflected in outcomes observed in clinical practice.
Methods: In an analysis of ibrutinib ordering/reordering, we estimated patient time on treatment, in order to provide a conservative approximation of progression-free survival (PFS) using Kaplan-Meier analysis and Cox proportional hazard regression. Reordering data were censored at the date of last ibrutinib supply or resupply (ibrutinib was resupplied every 1-3 months depending on stage of the NPP). Patients transferring to commercial ibrutinib after approval were censored at the time of NPP closure in their country.
Results: In total, 715 patients with MCL from 26 countries enrolled in the NPP were included in this analysis; median age was 70 years, and 76.1% were male. After 12 months, 52.3% (95% CI, 43.5-60.4%) of the global population remained on treatment. This estimate is similar to the 12-month time on treatment (57.6% [95% CI, 48.9-65.3%]) and PFS (58.0% [95% CI, 49.3-65.7%]) rates observed in the phase 3 RAY (MCL3001) study of ibrutinib for relapsed/refractory MCL (inclusion criteria were similar for the MCL NPP and RAY). Moreover, Kaplan-Meier curves for time on treatment for the global MCL NPP population and the RAYstudy population were not statistically different (Figure 1; MCL NPP *versus* RAY: HR, 1.14 [95% CI, 0.83-1.54]). Limited baseline demographic information collected at NPP enrollment allowed an exploration of time on treatment via multivariate analysis (Figure 2).

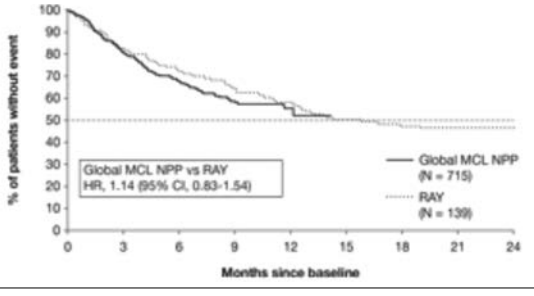

Figure 1. Time on treatment for Global MCL NPP population versus RAY.

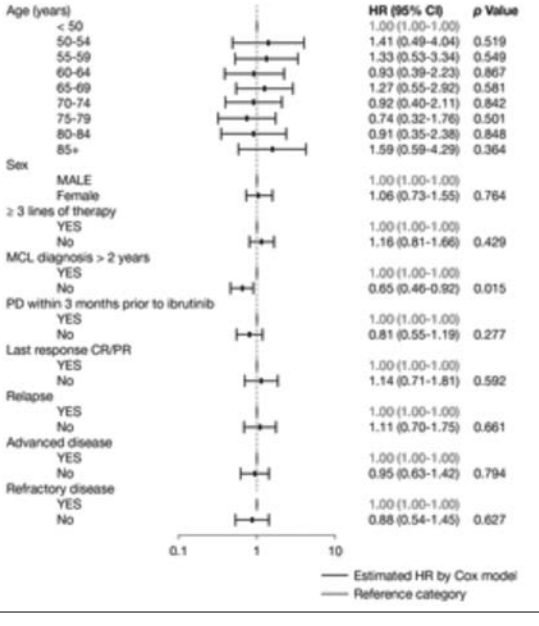

Figure 2. Multivariate analysis of time on treatment.

Timing of MCL diagnosis was the only independently significant variable in the multivariate analysis, with time on treatment being longer in patients diagnosed with MCL within the last 2 years. Of note, in this analysis, neither refractory disease (defined as no response to prior therapy [ie, stable disease or progression]), advanced disease (involvement of the bone marrow, extranodal sites, or both), relapsed disease, nor prior response with the previous therapy additionally impacted time on treatment. In total, 168 patients (23.5%) discontinued treatment during the observation period, the most common reasons being death (10.8%), disease progression (7.3%), and AEs (1.3%).
Summary/Conclusions: Although NPP data are based on physician declarations and are unmonitored, this analysis provides a real-world estimate of time on treatment, which can be considered a conservative proxy for PFS. The estimates, determined from a large, global MCL population, were similar to those for the RAY study, which suggests that results observed in MCL clinical trials with ibrutinib are reproducible in clinical practice.

P700

A SPECIFIC SIGNATURE IN LYMPH NODE BIOPSIES OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PREDICTS CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT

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Background: The infiltration of immunoprivileged sites, including the central nervous system, either at diagnosis or at relapse, confers an ominous outcome to the patients with DLBCL. A specific gene expression profiling (GEP) has been identified for primary CNS lymphomas (pCNSL). Similarly, GEP studies could help in selecting DLBCL patients with high risk for CNS infiltration.
Aims: To identify a GEP "CNS signature" in the lymph nodes of patients with DLBCL that can predict CNS involvement.
Methods: GEP analysis (Affymetrix HG-U219) was performed in 11 cases of DLBCL patients with CNS infiltration (3 cases at diagnosis and 8 during the follow up). The control group consisted of 27 DLBCL, not otherwise specified, without extranodal involvement, that showed no CNS infiltration during follow up. No case of transformed or immunodeficiency -related DLBCL was included in the control group. Lymph node samples at diagnosis were used in both groups. Differential gene expression between the two subgroups was analyzed using the Limma package (bioconductor.org, R version 3.2.0), and biological pathways assignment was performed by Gene set enrichment analysis (GSEA) using custom gene sets related to B cell biology, canonical pathways (C2CP) and transcription factor (C3TFT) public databases.
Results: Our analysis (log fold change >1,5 and P<0.05) identified 25 upregulated genes in the CNS group (Figure 1), including SRRM2, SRSF5 and TRA2A (involved in splicing), EVL (cell motility), MKI67 and TRIB2 (associated with brain metastasis in other cancers) and BCL6 (associated with pCNSL); and 11 downregulated genes, including LSP1 (implicated in transendothelial migration of leukocytes). Pathway analysis with GSEA (False discovery rate (FDR) <0.05 and Normalized enrichment score (NES) >1.8) revealed that the CNS group showed an overrepresentation of signaling pathways related to: 1) extracellular matrix molecules, migration (FAK) and adhesion-related pathways (integrins), 2) angiogenesis, 3) cell proliferation (E2F) and 4) NOTCH (Table 1). Immunohistochemistry analyses to validate these findings are ongoing.

Table 1.

Pathway name	Number of genes	NES	FDR q-val
ECM-migration adhesión patwhays			
NABA_BASEMENT_MEMBRANES	40	2.5	<0.001
PID_INTEGRIN1_PATHWAY	66	2.21	<0.001
KEGG_ECM_RECEPTOR_INTERACTION	83	2.18	<0.001
KEGG_FOCAL_ADHESION	197	2.15	<0.001
NABA_COLLAGENS	43	2.08	0.001
PID_AVB3_INTEGRIN_PATHWAY	75	2.07	0.001
PID_FAK_PATHWAY	59	1.93	0.006
Angiogenesis			
REACTOME_SIGNALING_BY_PDGF	118	1.89	0.008
PID_LYMPH_ANGIOGENESIS_PATHWAY	25	1.89	0.008
PID_VEGFR1_2_PATHWAY	69	1.87	0.01
Cell cycle			
V$E2F_Q4	230	1.97	<0.001
NOTCH pathway			
REACTOME_SIGNALING_BY_NOTCH	100	1.96	0.004
REACTOME_PRE_NOTCH_EXPRESSION_AND_PROCESSING	42	1.98	0.004

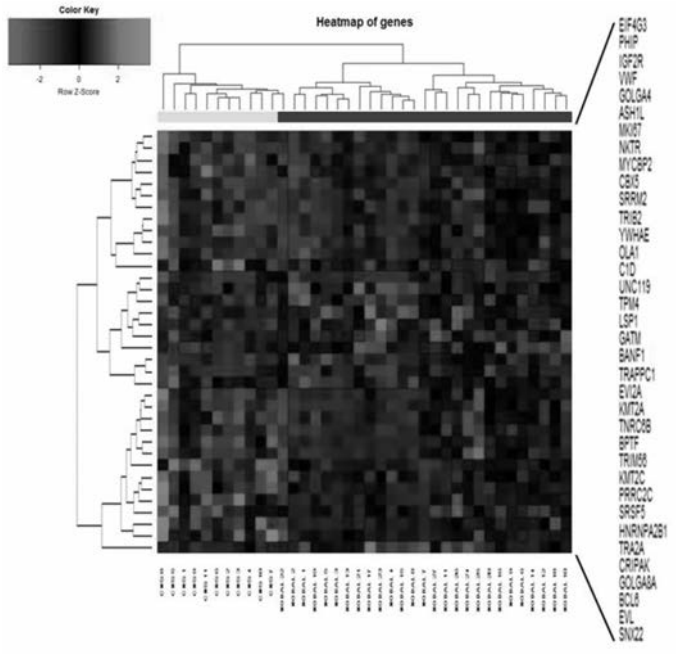

Figure 1.

Summary/Conclusions: The CNS signature identified in our study at diagnosis could represent a specific feature of DLBCL patients with high risk for CNS involvement. If confirmed, these findings could better guide initial treatment of these patients to improve their outcome.

P701

DIFFUSE LARGE B-CELL LYMPHOMA IN THE RITUXIMAB ERA: REAL LIFE ANALYSIS OF PATIENTS WITH REFRACTORY DISEASE OR RELAPSE

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Background: The addition of Rituximab as part of first line treatment for diffuse large B- cell lymphoma (DLBCL) has improved outcome. However, in the case of refractory disease or relapse, high dose chemotherapy with autologous stem cell transplant (ASCT) is regarded the only curative option.
Aims: To analyze treatment and outcome for patients with DLBCL with primary refractory or relapsed disease in the Rituximab Era.
Methods: Patients diagnosed with *de novo* DLBCL in the period 2000-2013 were identified in the Danish National Lymphoma Registry. Patients not responding to first line treatment or who subsequent developed relapse were included. Patients with primary CNS lymphoma were excluded. Survival analysis was performed according to the Kaplan Meier method and compared by log-rank tests. Survival time was measured from date of relapse to death or censored at the time of analysis.
Results: A total of 5203 patients were diagnosed with DLBCL in the study period. Median age was 68 years, and 56% were male. In 4842 patients a first line treatment was started. 3295 patients were treated with Rituximab containing treatment. 2852 (86%) achieved partial remission or better after first line therapy, however 443 (14%) had primary refractory disease, and 446 (14%) patients developed relapse (CNS involvement n=90, other lymphoma subtype n=68). The median overall survival was 8.8 years. Of the 731 patients with either primary refractory or relapsed disease, 330 received a second line treatment; 242 received salvage therapy, of these 89 patients completed ASCT. After 2 years, 46 patients receiving ASCT were still alive. Intention to treat analyses reveals significantly better outcome for patients with relapsed diseased treated with salvage strategy compared to those treated with palliative strategy (p=0.03). However, no difference was seen for the patients with primary refractory disease (p=0.3), Figure 1. As expected, patients with primary refractory disease not receiving ASCT had a dismal survival compared to patients with later relapse (p=0.003), but no significant difference was found in the group receiving ASCT. For the 1547 patients not treated with Rituximab, 1143 (73%) achieved partial remission or better, however 404 (27%) had primary refractory disease, and 344 (22%) developed relapse (CNS involvement n=37, other lymphoma subtype n=32). The median overall survival was 2.3 years. Of the 681 patients with primary refractory or relapsed disease, a total of 150 patients received second line treatment, 113 patients received salvage therapy and of them 37 patients also received ASCT. After 2 years, 28 patients receiving ASCT were still alive. Of the patients not receiving ASCT, 41 patients were still alive after 2 years. In both groups, patients with primary refractory disease had dismal survival compared to patients with later relapse (p=0.007 *vs* p=0.03).

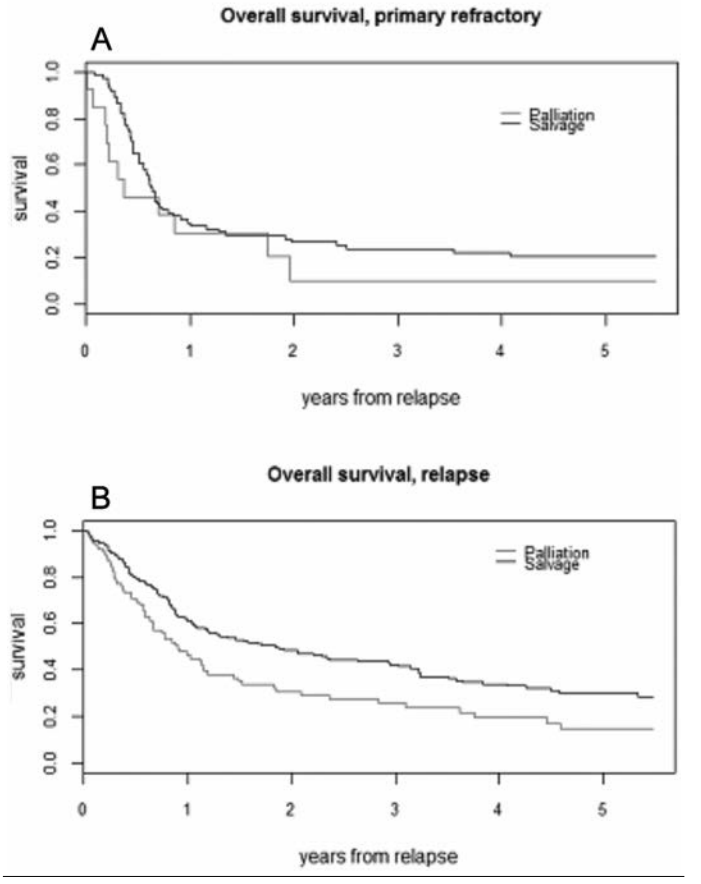

Figure 1. Survival for patients with primary refractory disease (A) and relapsed disease (B) based on intention to treat analyses.

Summary/Conclusions: The addition of Rituximab has significantly improved outcome after first line treatment for DLBCL, and the incidence of relapse has decreased. In the relapse setting, salvage with ASCT has been the standard second line treatment for DLBCL for over 20 years, but only 38% of the patients treated with salvage strategy did actually receive ASCT. Intention to treat analyses show, that patients intended to receive ASCT have significantly better outcome, but patients not being eligible for ASCT actually had a 2 year survival of 31%. This shows that patients with relapse of DLBCL do have a chance to survive although not being eligible for ASCT.

P702

CENTRAL NERVOUS SYSTEM INTERNATIONAL PROGNOSTIC INDEX IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: The central nervous system (CNS) is a sanctuary for lymphoma cells. Once CNS involvement in lymphoma occurs, it is almost fatal. CNS involvement is thought to be a rare but important complication in the lymphoma's clinical course. We previously demonstrated that the involvement of the breast, adrenal gland, and/or bone was an independent risk factor for CNS involvement based on clinical data in 1,221 patients with diffuse large B-cell lymphoma (DLBCL) treated with R-CHOP (Cancer Sci 2012). Afterward, several groups proposed a new prognostic model consisting of 6 factors, including the 5 risk factors in the International Prognostic Index, and involvement of kidney/adrenal gland, predicting the risk of secondary CNS involvement (Schmitz N, *et al.* 2013, Savage KJ, *et al.* 2014, and El-Galaly, *et al.* 2015).
Aims: To validate the usefulness of this prognostic index using our previously fixed data in DLBCL patients who did not receive any CNS prophylaxis.
Methods: Clinical data of untreated, *de novo* DLBCL patients were collected in 2010 from 47 institutions in Japan. Included patients had been treated with R-CHOP therapy as the primary treatment between 2003 and 2006. Clinical data collected were the presence or absence of 26 extranodal sites including the kidney and adrenal gland, along with factors including age, sex, clinical stage, serum lactate dehydrogenase (LDH) level, performance status (PS), bulky mass, and B symptoms. Patients who received any CNS prophylaxis were excluded. Patients with primary CNS involvement or with distinct forms of DLBCL, such as intravascular lymphoma, primary effusion lymphoma, and primary mediastinal large Bcell lymphoma were also excluded.
Results: A total of 1,220 CD20-positive DLBCL patients with complete information related to risk factors were available (data from 1 patient was withdrawn due to an error in one of the items mentioned). Patient characteristics were as follows: 62% were aged >60 years, 55% had elevated LDH, 46% had advanced stage disease, 21% had PS >1, 20% had >1 extranodal sites, and 4% (n=51) had kidney/adrenal involvement. With a median follow-up of 48 months in living patients, 82 (6.7%) cases of CNS involvements were observed. More than half of the CNS involvement was the parenchymal type (53.7%), followed by the leptomeningeal type (31.7%) and both (14.6%). Systemic (except for CNS) lymphoma status at CNS involvement was as follows: 38 (46%) patients were in the first complete remission (CR), 8 (10%) patients were in the second or more CR, and 36 (44%) patients were in non-CR. The median time interval between the initiation of therapy and CNS involvement was 9 months. In the 51 patients with kidney/adrenal involvement, 9 (18%) developed CNS involvement. According to sum of the above-mentioned 6 factors, patients were categorized into 3 CNS-risk categories of 0-1 (n=483, 40%), 2-3 (n=504, 41%), and 4-6 (n=233, 19%), which represented low-, intermediate-, and high-risk groups, respectively. The cumulative incidence of CNS involvement at 2 years was 1.4% for low-risk, 5.9% for intermediate-risk, and 12.9% for high-risk patients, respectively (Figure 1).

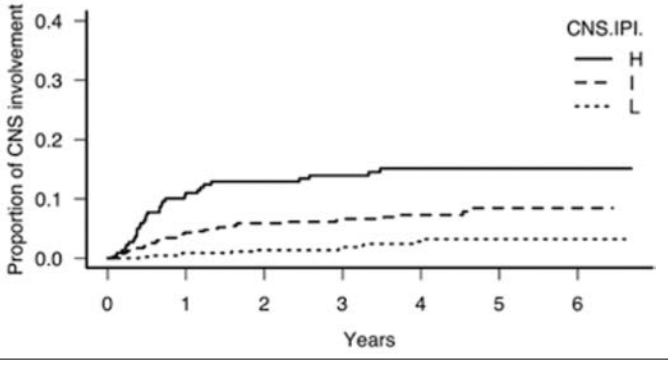

Figure 1. Cumulative risk of CNS involvement according to CNS-IPI.

Summary/Conclusions: The prognostic model for CNS involvement (CNS-IPI) was useful in a DLBCL patient cohort treated with R-CHOP and without any CNS prophylaxis.

P703

BETTER PROGNOSTICATIONS WITH INTERIM PET/CT RESPONSES BASED ON METABOLIC TUMOR VOLUME COMPARED TO VISUAL AND SUV-BASED ASSESSMENTS IN THE PATIENTS WITH DLBCL WITH EXTRANODAL INVOLVEMENTS

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Background: [18]F-FDG PET is currently used in diffuse large B-cell lymphoma (DLBCL) for staging and evaluation of therapeutic efficacy at various time points. Nevertheless, the predictive value of interim PET/CT (iPET/CT) has not been consistent throughout the studies. Particularly, iPET/CT assessment in patients with multifocal, non-contiguous involvement at extranodal (EN) sites may result in a false determination of prognosis due to tracer uptake of inflammatory or physiologic anatomic sites.
Aims: The purpose of this study is to investigate the predictive accuracy of iPET/CT response based on visual, quantitative SUV-based and MTV-based assessment in patients with DLBCL and EN involvements.
Methods: iPET/CT responses for 163 patients with newly diagnosed DLBCL and EN involvements were investigated retrospectively. iPET/CT responses were based on the visual, SUV-based and MTV-based assessments. The assessment of PET/CT was performed at the time of diagnosis, at the third or fourth cycles and at the completion of R-CHOP. For visual assessment, the five-point scale (5-PS) based on the Deauville criteria was used and graded as negative (grade 1-3) or positive (grade 4-5) by comparison with initial PET/CT scan. Second, the quantitative analysis of [18]F-FDG uptake changes was based on the percentage of SUVmax reduction (ΔSUVmax) and the rates of reduction in the metabolic tumor volume (ΔMTV2.5) between initial and interim PET/CT scans. The cutoff point of ΔSUVmax was 65.7% and that of ΔMTV2.5 was 99.3% based on previous reports.
Results: Median age was 61 years (range 18-83 years) and 88 patients (54.0%) in advanced disease (III/IV). Patients were classified according to the IPI risk with 95 patients (58.3%) being classified as low or low-intermediate and 68 patients (41.7%) as high-intermediate or high risk. Number of extranodal site(s) were 1 site in 102 patients (62.6%), 2 sites in 39 (23.9), 3 sites in 18 (11.0), and 4 sites in 4 (2.5%). iPET/CT responses based on visual analysis were classified into grade 1-3 in 99 patients (60.7%) and grade 4-5 in 64 (39.3%), and based on SUV-based, classified into higher the cutoff of ΔSUVmax (>65.7%) in 140 patients (85.9%) and lower (<65.7%) in 23 patients (14.1%), respectively. Among the patients with MTV2.5 data (n=110), higher ΔMTV2.5 (>99.3%) was observed in 81 patients (73.6%) and lower ΔMTV2.5 (<99.3%) in 29 (26.4%). On visual assessment, iPET/CT-positive patients had no difference of relapse rates (28.3±5.4%) compared to those of iPET/CT-negative patients (23.1±4.5%) (p=0.419). Among the patients with 5-PS grade 4-5, higher the optimal cutoff of ΔSUVmax (>67.5%) and ΔMTV2.5 (>67.5%) were achieved in 46 patients (71.9%) and 18 (22.2%). The 5-year overall survival (OS) rates and progression free survival (PFS) rates were 75.6±3.8% *vs* 60.6±11.7% (p=0.056), and 77.9±3.7% *vs* 55.9±12.1% (p=0.007) depending on the cutoff of ΔSUVmax, respectively. Based on the cutoff of ΔMTV2.5, the 5-year OS rates were 77.1±4.8% and 61.9±9.1% (p=0.046), and the 5-year PFS rates were 80.2±4.6% and 64.4±9.0% (p=0.020). In the multivariate analysis, age at diagnosis, ECOG PS 2-4, EN involvement >1 site, and lower ΔMTV2.5 (<99.3%) were unfavorable factors for predicting PFS and OS.
Summary/Conclusions: The MTV2.5-based assessments in iPET/CT could have significant potential as a prognostic predictor of PFS and OS than visual assessment or SUVmax reduction in patients with EN involvements. However, the visual assessments have the limitations to predict long-term outcomes with high false positive rates at EN involvements. The quantitative SUV-based assessments in iPET/CT were significant prognostic predictor of PFS and OS, especially in the patients with 1 EN site involvement. However, the visual assessments have the limitations to predict long-term outcomes with high false positive rates at EN involvements.

P704

DETECTION OF BONE MARROW INVOLVEMENT OF DLBCL - COMPARISON OF PET-CT AND BONE MARROW BIOPSY

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Background: In malignant lymphoma, bone marrow involvement is considered as clinical stage IV, which adversely affects International Prognostic Index. Detecting bone marrow lesion is therefore important in staging of malignant lymphoma. Bone marrow biopsy (BMB) has been a classical method to detect bone marrow infiltration. Recently, positron emission tomography combined with computed tomography (PET-CT) became a routine tool in staging of malignant lymphoma. Especially, in diffuse large B cell lymphoma (DLBCL), PET-CT shows high sensitivity of detecting viable nodal and extra-nodal lesions.
Aims: The aim of this retrospective study is to evaluate the diagnostic accuracy of PET-CT in detecting bone marrow infiltration in patients with newly diagnosed DLBCL.

Methods: We collected data from all patients who were newly diagnosed with DLBCL from January 2005 to October 2015 at Yokohama City University Hospital, and Yokohama City University Medical Center. Patients with DLBCL who underwent both PET-CT and BMB prior to the initiation of treatments were finally included in the analysis. Bone marrow biopsy: Results of unilateral BMB of posterior iliac crest were collected from written reports. BMB specimens were evaluated by hemato-pathologists of each institution. The presence of lymphoma cells in the bone marrow was based on morphological and immunohistochemical findings. PET-CT analysis: Written reports were used to collect PET-CT data. Interpretation of images was made by radiologists of each institution where PET-CT scans were performed. Bone marrow involvement in PET-CT was defined as greater intensity of FDG uptake in the bone marrow than those in liver. This study was approved by the Internal Review Board of Yokohama City University Graduate School of Medicine.
Results: In total, 430 patients were newly diagnosed with DLBCL from January 2005 to October 2015. Of 430 patients, 254 underwent both PET-CT and BMB before treatment who were evaluated in the further analysis. Median age at diagnosis was 68 years (range: 21-93). Four patients were diagnosed with intravascular lymphoma, and 4 with primary mediastinal large B cell lymphoma. All other patients were diagnosed with DLBCL, not otherwise specified (NOS). Bone marrow FDG uptake was elevated according to the defined criteria in 23 patients (9%), while the infiltration of lymphoma cells in the bone marrow was detected by BMB in 26 patients (10%). Of 23 patients with elevated PET uptake in the bone marrow, 6 (32%) patients were diagnosed as negative for bone marrow infiltration by BMB. Among the 6 patients who were positive for PET-CT and negative for BMB, the pattern of FDG uptake in the bone marrow was focal in 3, diffuse in 1, and unknown in 2 (Figure 1). The patient with diffusely increased bone marrow FDG uptake had chronic myelogenous leukemia prior to diagnosis of DLBCL. Among the 26 patients positive for BMB, bone marrow FDG uptake was increased in 17 (65%). The remaining 9 BMB positive patients were negative for PET-CT. Of 17 patients positive for both BMB and PET-CT, the pattern of FDG uptake in the bone marrow was diffuse in 8, focal in 5, and unknown in 4.

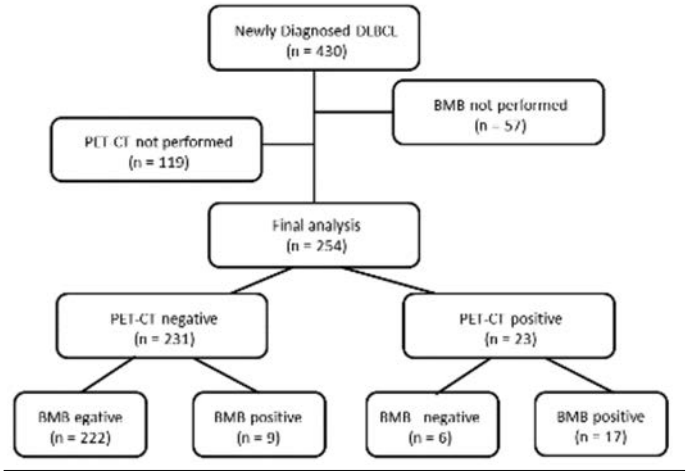

Figure 1. Study flow diagram, and comparison between PET-CT and bone marrow biopsy in detection of bone marrow involvement.

Summary/Conclusions: In conclusion, our study revealed that significant number of patients showed discrepancy between the results of PET-CT and BMB in detecting bone marrow involvement of lymphoma cell. Although PET-CT is highly sensitive tool for detecting viable lymphoma cells and is commonly used for staging in routine practice, our data indicated that PET-CT still cannot replace BMB for identifying lymphoma cells in the bone marrow.

LB705

PILLAR-2: PHASE 3 STUDY OF ADJUVANT EVEROLIMUS *VERSUS* PLACEBO IN PATIENTS WITH POOR-RISK DIFFUSE LARGE B-CELL LYMPHOMA WHO ACHIEVED COMPLETE REMISSION WITH RITUXIMAB-COMBINED CHEMOTHERAPY

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Background: The high-risk of relapse in patients (pts) with diffuse large B-cell lymphoma (DLBCL) who achieved a complete response (CR) to first-line rituximab-based chemotherapy (R-chemo) highlights the need for effective treatments to improve outcomes.
Aims: The phase 3 PILLAR-2 (NCT00790036) study aimed to reduce DLBCL relapse after 1 year of adjuvant everolimus (EVE) treatment to poor-risk patients (International Prognostic Index [IPI] ≥3) who had achieved a CR with R-chemo.
Methods: Pts with histologically confirmed stage III/IV poor-risk (IPI ≥3) DLBLC who had a PET/CT-confirmed CR to first-line R-chemo were randomized 1:1 to EVE 10 mg/day or placebo (PBO) for 1 year or until disease relapse, unacceptable toxicity, or death. The primary endpoint was disease-free survival (DFS) by local assessment using revised IWRC. Secondary endpoints were overall survival (OS), lymphoma-specific survival (LSS), and safety.
Results: Enrolled pts (N=742) were randomly assigned to EVE (n=372) or PBO (n=370). Among randomized pts, 48% in the EVE arm and 67% in the PBO arm completed study treatment per protocol. The main reasons for discontinuation were AEs (EVE, 30%; PBO, 12%) and relapsed disease (EVE, 7%; PBO, 13%). Median follow-up was 50.4 months (range, 24.0–76.9 months). Overall, 47% of pts were ≥65 years, 50% were men, and 42% had an IPI of 4+5. In the primary analysis DFS was not significantly different between EVE and PBO (Log-rank p=0.276). The 2-year DFS rates (95% CI) were 78% (73-82%) with EVE and 77% (72-81%) with PBO. The 2-year OS rates were 91% with EVE and 88% with PBO (Hazard Ratio [HR], 0.75; 95% CI: 0.51-1.10). Results of exploratory subgroup analyses showed a trend favoring EVE over PBO for both DFS and OS in pts with IPI 4+5, in males, and in pts <65 years of age (Table 1). There was also a trend favoring EVE over PBO for LSS (Table 1). Common grade 3/4 AEs with >3% difference for EVE *vs* PBO included neutropenia, stomatitis, CD4 lymphocytes decreased, lymphopenia and anemia. During study treatment, 5 pts in the EVE arm and 2 pts in the PBO arm died.

Table 1.

	Number of patients EVE/PBO	EVE vs PBO HR (95% CI)
DFS*		
Overall (N = 742)	372/370	0.92 (0.69–1.22)†
IPI 4+5 (n = 313)	148/165	0.65 (0.42–1.01)
Male (n = 372)	168/204	0.68 (0.45–1.05)
Age <65 years (n = 393)	192/201	0.79 (0.52-1.21)
OS*		
Overall (N = 742)	372/370	0.75 (0.51–1.10)
IPI 4+5 (n = 313)	148/165	0.63 (0.37–1.07)
Male (n = 372)	168/204	0.55 (0.32–0.94)
Age <65 years (n = 393)	192/201	0.62 (0.34–1.13)
LSS* (N = 742)	372/370	0.64 (0.39–1.04)

* Median duration not estimable. †Log-Rank p = 0.276.

Summary/Conclusions: Adjuvant EVE for 1 year did not improve DFS in poor-risk pts with DLBCL who achieved a CR after R-chemo. Trends favoring adjuvant EVE for DFS and OS in selected pt subgroups and for LSS in the overall population suggest that EVE may provide anti-lymphoma activity in poor-risk DLBCL that warrants further investigation.

Stem cell transplantation - Clinical 3

P705

EFFICACY OF BONE MARROW DERIVED MSC FOR STEROID REFRACTORY ACUTE GVHD IS RELATED TO THE AGE OF THE MSC DONOR

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Background: Acute graft *versus* host disease (aGVHD) remains a life-threatening complication and substantially reduces efficacy of allo-HSCT. In particular, the outcome of patients with severe steroid-refractory aGVHD continues to be poor. Administration of Mesenchymal Stromal Cells (MSC) has been reported by others and us as an interesting treatment option. However, variability in products and the inability to link surrogate markers of the product to clinical efficacy could prove to be a major threat in daily clinical practice.
Aims: Evaluate the impact of MSC donors as well as donor properties such as age on the outcomes clinical response and overall survival.
Methods: We evaluated the impact of individual donors as well as donor properties such as age on clinical response in a cohort of 102 patients with grade II-IV steroid refractory aGVHD treated with bone marrow derived MSC from third party non-HLA matched donors. Primary outcome measures were one year overall survival (OS) and response of GVHD. Cox proportial hazards models, competing risk analyses (Gray's test) and Kaplan Meier estimates were used for analyzing response and OS respectively.
Results: 102 patients received in total 299 MSC infusions derived from 10 different BM donors. Median number of infusions was 3 (range 1-4). 75,5% of patients received all MSC infusions from the same donor, 20,6% with MSC from 2 donors and 3,9% with MSC from 3 different donors. Two donors were used to treat 28,4% and 43,1% of patients respectively. When testing impact on one year OS of an individual product no differences between patients treated with either the 2 main contributing donors or the patients treated with the 'other' MSC donors could be observed. However, when donor age was used to cluster cohorts (donor age <and >10 years) differences were observed (Figure 1) with a survival benefit for patients treated with young donors. Competing risk analysis also revealed a significant benefit for patients only treated with young donors. Furthermore in multivariate analysis, MSC donor age remained predictive for OS (HR 2,00, p-value 0,025).

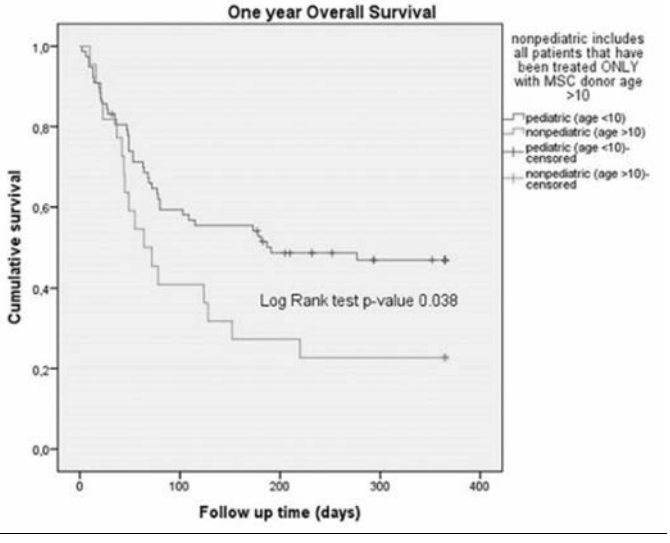

Figure 1. One year overall survival estimation by means of Kaplan Meier depending on age of MSC donor used.

Summary/Conclusions: In our cohort of 102 patients with steroid refractory acute GVHD that were treated with MSC we observed differences in both achieving complete resolution of GVHD symptoms as in OS. Patients only treated with MSC derived from very young bone marrow donors (<10 years old) had significantly better resolution of GVHD symptoms as well as an improved OS. These findings may have implications for both future as well as currently ongoing clinical trials with MSC.

P706

CHRONIC GRAFT-*VERSUS*-HOST-DISEASE AND B-CELL RECONSTITUTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN

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Background: Hematopoetic stem cell transplantation (HSCT) is an established therapy for many pediatric hematological diseases. Besides relapse, chronic GvHD (cGvHD) is the most important determinant of posttransplant morbidity and mortality. Although the precise mechanisms underlying cGvHD are not completely understood so far, convincing evidence suggests an important contribution of B-cells in cGvHD pathophysiology. Multiple studies in adult cGvHD patients have described altered patterns of B-cell reconstitution.
Aims: Since data on B-cell reconstitution and cGvHD in children, who generally show different kinetics of immune reconstitution than adults, are lacking so far, we wanted to investigate this relationship in a purely pediatric cohort of alloHSCT recipients.
Methods: First, in a retrospective cohort of 104 pediatric alloHSCT recipients transplanted between 2005 and 2013, we analyzed 2151 flow cytometric immune profiles. To identify differences in lymphocyte distribution in children with and without GvHD over time we applied hierarchial linear analysis. Second, in a prospective cohort of 74 children, we investigated more closely the distribution of B-cell subsets, cytokine production in B- and T-cells, serum cytokine levels including BAFF, autoantibody production and apoptosis resistance of peripheral B-cells.
Results: In the first retrospective cohort, median age was 8.9±7.0 years. Incidence of cGvHD was 15%, median time to onset was 132±198days. In a univariate analysis, risk factors for cGvHD were: a history of previous aGvHD, radiation-based conditioning and a donor-host sex mismatch. As expected, relapse rate was significantly lower in cGvHD patients. Hierarchial linear analysis showed that children later experiencing cGvHD had elevated T-cell frequencies during the first 180 days (63±17 *vs* 45±23% $CD3^+$ in children with *vs* without cGvHD, resp.). In contrast, B-cells were significantly lower in cGvHD children once cGvHD had occurred. Interestingly, reconstitution of naïve T-cells was delayed but not abrogated in children with cGvHD, reaching a mean of 44±17% of naïve T-cells in the CD4+ compartment after 2 years postHSCT (compared to 58±15% in children without GvHD). A more detailed analysis of B-cell subsets in the prospective cohort revealed that the lower number of B-cells in cGvHD children were due to a defect in naïve B-cell regeneration. Memory and $CD24^{++}CD38^{++}$ transitional B cells were expanded. We detected autoantibodies (mainly ANAs) in 88% of cGvhD children, compared to 13% in children without cGvHD. In approx. 50% of cGvHD children these autoantibodies became negative after 2 years postHSCT. Apoptosis resistance is a mechanism also operational in pediatric cGvHD, as B-cells from cGvHD children showed reduced rates of apoptosis after 48h in culture, a phenomenon that could be induced by exogenous BAFF in a dose dependent way. In contrast to what has been reported in adults, serum BAFF levels were not elevated continuously during cGvHD, but progressively declined over time. A detailed analysis of bone marrow B-cell precursor subsets has been performed, which is currently under biometrical evaluation.
Summary/Conclusions: Our data represent the first large and comprehensive data set on B-cell reconstitution and cGvHD in a purely pediatric cohort. We confirm patterns of B-cell dysbalance which have been described in adult cGvHD patients before. However, other features such as the preserved thymic reconstitution despite cGvHD and the rapid decline of BAFF and autoantibodies are in contrast to previous reports. Thus, the higher regenerative capacity of children seems to have a significant impact on the disturbed B-cell homeostasis in cGvHD.

P707

A NOVEL QUANTITATIVE PCR APPROACH TARGETING INSERTION/DELETION POLYMORPHISMS (INDEL-PCR) FOR CHIMERISM QUANTIFICATION: FINALLY HIGH SENSITIVITY AND QUANTIFICATION CAPACITY TOGETHER

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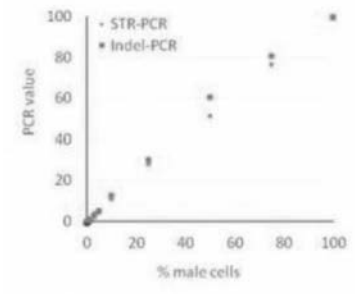
Background: Post-hematopoietic stem cell transplantation (SCT) chimerism monitoring is important to assess engraftment, anticipate relapse and provide information on the development of graft *versus* host disease, facilitating therapeutic intervention.
Aims: The aim of this study was to test the technical efficacy and clinical utility of a novel quantitative PCR approach targeting insertion/deletion polymorphisms (indel-PCR).
Methods: This study included 157 samples (81 bone marrow, 60 peripheral blood (PB), 11 T-cells, 2 myeloid cells, 2 CD34-cells, 1 NK-cells purified using immunomagnetic technology) of 24 patients who underwent SCT for haemato-

logical malignancies. Additionally, 2 sets of 11 artificial mixtures were created using PB leukocytes of two healthy subjects (a male and a female) with known percentages of male leukocytes (putative recipient): 100, 75, 50, 25, 10, 5, 3, 1, 0.1, 0.01, 0. Chimerism analysis was performed by the gold-standard STR-PCR (AmpFISTR SGM Plus®, Life Technologies, USA) and by indel-PCR (Mentype® DIPscreen, Mentype® DIPquant, Biotype, Germany). Concordance between both methods was calculated with SPSS using intraclass correlation coefficient.

Results: The number of informative loci identified with indel-PCR (>3/patient) was higher than with STR-PCR for all patients. Concordance between both methods for the 157 patient samples and 11 artificial mixtures was "very good" (intraclass correlation coefficient=0.96). Of note, analysis of artificial mixtures provides evidence of significantly (≥2 logs) higher sensitivity by indel-PCR (0.01%) than by STR-PCR (1%, Figure 1). Moreover, indel-PCR shows unprecedented quantification capacity (Figure 1). Out of the 168 samples analyzed, 32 were positive and 15 negative by both methods, while 121 were positive only by indel-PCR (95% with <1% recipient). Clinical outcome and chimerism dynamics of 24 patients are described in Figure 1, data of 4 of them were censored (engraftment failure, disease progression, insufficient number of samples). All the samples presented complete donor chimerism by STR-PCR. Of the 9 patients who showed stable or decreasing percentage of recipient cells, only one (11%) presented extramedulary relapse. Of the 11 patients who presented increasing percentage of recipient cells in one determination, relapse occurred in 5 patients (45%, 3/6 patients who did not have further determinations, 2/4 who showed stable or decreasing recipient cell percentage, and 0/1 patient who presented increasing recipient cells in subsequent determinations).



% male cells	STR-PCR	Indel-PCR
100	100	100
75	76.85	80.9
50	51.75	60.9
25	28.51	30.6
10	11.25	13
5	5.25	5.5
3	4.20	2.9
1	1.82	0.8
0.1	0	0.1
0.01	0	0.02
0	0	0

Figure 1. Analysis or artificial mixtures.

Summary/Conclusions: This novel indel-PCR is a simple and accurate technique that, in comparison with the current gold standard STR-PCR, shows very good concordance and provides higher rates of informative loci per patient, as well as unprecedented combined sensitivity and quantification capacity. Such features allow minutely monitoring chimerism dynamics and might improve clinical management of transplanted patients, specially predicting relapse in those patients who do not have a molecular marker available for disease follow up.

P708

A NEW APPROACH OF DUAL-SCT WITH UNMANIPULATED HAPLO-IDENTICAL GRAFT AND UNRELATED CORD BLOOD IN PATIENTS WITH HEMATOLOGICAL DISORDERS

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Background: Dual-SCT with T-cell depleted haplo-identical graft and cord blood (CB) has developed in recent years, which revealed a promising outcome. In China, graft manipulation is seldom performed, so we proposed an alternative dual-SCT strategy with unmanipulated haplo-identical graft co-infusing with unrelated CB unit, and published an encouraging outcome based on preliminary results.

Aims: Here we reported the recent updates of this prospective study.

Methods: This is an on-going prospective study approved by local Ethics Committee. The main inclusion and exclusion criteria are: (1) definitely diagnosed as hematological disorders; (2) with an indication of allo-SCT; (3) without an available matched related or unrelated donor. Myeloablative conditioning of either modified Bu/Cy regimen or modified TBI/Cy regimen was applied. Selection of donor, conditioning, GVHD prophylaxis and supporting care follows the principles described previously. In order to identify the superiority of dual-SCT, data of patients receiving haplo-identical donor transplantation in the same time frame were analyzed as parallel control in this report.

Results: From January 2011 through December 2014, 219 patients were recruited in this study including patients with AML (29%), ALL (38%), MDS (10%), SAA (10%) and other diseases. The median age was 26 (15-60) years old. The median counts of MNC was 10.5×10^8/Kg in haplo-identical graft and 1.83×10^7/Kg in cord blood unit, respectively. Only 4 patients achieved stable engraftment of CB unit. Univariate analysis in the study cohort suggested that lower MNC count (less than 12×10^8/Kg) in haplo-identical graft was related to a significantly lower Grade II-IV aGVHD incidence (P=0.041), lower TRM (P=0.008) and better survival (P=0.007). Comparing with 5/6 or 6/6 matched CB unit, 4/6 matched CB unit resulted in higher TRM with a statistical significance (P=0.023), and lower OS with marginal significance (P=0.071). Parallel control group contains 176 patients with roughly comparable baseline characteristics. In the comparison analysis, 2-year TRM was 14.9±3.0% *versus* 29.4±5.4% for dual-SCT and haplo-SCT (P=0.003), and 2-year relapse incidence was 14.8±3.5% *versus* 23.3%±4.4% (P=0.011), respectively. Finally, better 2-year OS (81.5±3.1% *versus* 64.4±4.2%, P<0.001) and PFS (79.1±3.0% *versus* 58.9±4.1%, P<0.001) were observed in dual-SCT group compared to haplo-SCT. No difference of GVHD incidence was identified between two groups (Figure 1).

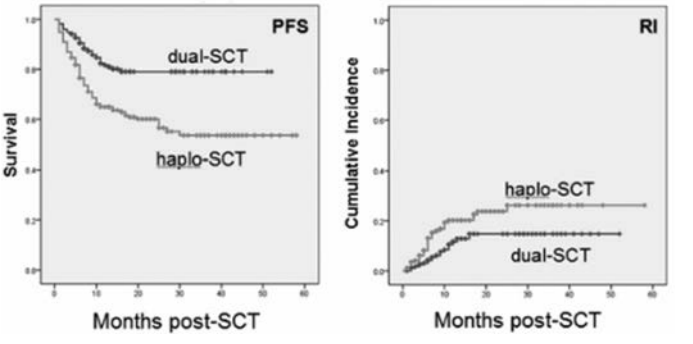

Figure 1.

Summary/Conclusions: Our report showed promising outcomes of dual-SCT with unmanipulated haplo-identical graft and unrelated CB unit. Lower MNC count in haplo-identical graft and 5-6/6 matched CB unit were related to a better prognosis after dual-SCT. Efficacy of this approach merits further confirmation by longer follow-up, and moreover, by well designed randomized clinical trials. Besides, probable mechanisms and interactions between stem cells needs to be explored.

P709

RECIPIENT CCR5 GENETIC VARIATION PREDICTS TRANSPLANT OUTCOMES AFTER HLA-MATCHED UNRELATED DONOR BONE MARROW TRANSPLANTATION

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Background: The chemokine receptor CCR5, mainly expressed on macrophages, dendritic cells, memory T cells, and the endothelium, plays roles in the adaptive immune response by enhancing the production of antigen-specific T cells through directing $CD8^+$ T cells to sites of $CD4^+$ T cells and dendritic interactions. Its genetic variant (rs1800023, -2086 A>G), which resides in the promoter region, has been reported to be associated with susceptibility to various infectious diseases, including HIV infection.

Aims: To investigate the impact of CCR5 genetic variation on transplant outcomes of HLA-matched unrelated donor bone marrow transplantation (BMT).

Methods: CCR5 genotyping was performed on 333 patients with hematologic malignancies who underwent BMT through the Japan Marrow Donor Program between January 2006 and December 2009 and their HLA-matched unrelated donors, and its association with the transplant outcomes was retrospectively examined.

Results: The genotype frequencies of A/A, A/G, and G/G were 34%, 49% and 17% in the recipients and 35%, 50% and 15% in the donors (*P*=0.93), respectively. The recipient A/A genotype was associated with a significantly better overall survival (OS) than the recipient A/G or G/G genotype (69% *vs* 43%, P=0.04; Figure 1A). A multivariate analysis showed that the recipient A/A genotype has a significantly advantage regarding the OS (hazard ratio [HR], 0.53; 95% confidence interval [CI], 0.30-0.93; P=0.03) and the relapse rate (HR,

0.45; 95% confidence interval [CI], 0.21-0.97; P=0.04). These effects were not seen according to the donor CCR5 genotype.
Summary/Conclusions: These results suggest an association between the recipient CCR5 genotype and the OS and relapse rate after unrelated BMT. Genotyping for the CCR5 rs1800023 polymorphism could therefore be useful in predicting the prognoses and risk-adapted management of transplanted patients and may offer novel therapeutic insights to induce the graft-*versus*-tumor effect effectively.

P710

PRESUMABLY FALSE POSITIVE MINIMAL RESIDUAL DISEASE (MRD) QPCR RESULTS AFTER ALLOGENEIC SCT UNCOVERED BY NEXT GENERATION SEQUENCING (NGS)

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Background: MRD detection using qPCR for IG/TR rearrangements has been succesfully used in pediatric acute lymphoblastic leukemia (ALL) patients already for two decades and risk-adapted therapy based on its results has proven to be highly beneficial. Also MRD after allogeneic stem cell transplantation (SCT) in relapsed pediatric ALL has been shown to predict subsequent relapse and post-transplant MRD values have been used for guiding therapeutic interventions. We have previously shown that MRD detection by qPCR for clonal IG/TR rearrangements can yield false positive results in patients after SCT caused by massive regeneration of B-cells (Fronkova, Bone Marrow Transplantation, 2008), despite following strict EuroMRD guidelines for defining the MRD positivity (van der Velden, Leukemia, 2007). From our previous work (Kotrova, Blood, 2015), we know that NGS and qPCR have comparable sensitivity for MRD detection. We hypothesized that NGS of IG/TR rearrangements can distinguish between false and real positivities and can also identify the source of false positive results.
Aims: To detect MRD by NGS in our pediatric BCP-ALL cases after allogeneic SCT, who had at least one low-positive (not quantifiable) qPCR MRD result and remained in complete remission. No therapy intensification was implemented in these patients, except for one of them, who received DLI.
Methods: Sequencing libraries were prepared from 500ng of bone marrow DNA via a two-round PCR: in the first round rearranged IGH or TRG genes were amplified using universal primers. In the second round products from the first round were flanked by sequencing adaptors and sample-specific barcodes. Libraries were sequenced on Ion Torrent PGM sequencer using the Hi-Q chemistry. For detection of clonal leukemia-specific IG/TR rearrangements we used our in-house bioinformatic algorithm. We sequenced 30 qPCR positive (not quantifiable) post-transplant samples from 18 patients (16 patients carrying 24 IGH and 2 patients carrying 3 TRG qPCR targets) who remained in complete remission after SCT with a median follow-up of 10.4 years (range 1.8-15.5). One low-positive post-transplant sample from patient who later developed CNS relapse was added as positive control. The median coverage was 500,000 reads/sample.
Results: Besides the positive control patient, only 2 of 30 (7%) follow-up samples were found to be MRD-positive, defined as the presence of leukemia-specific sequence(s) as identified at diagnosis in the follow-up samples. One of the positive samples belonged to patient with the shortest follow-up and unclear outcome so far (1.8 years). To find the cause of false positive qPCR results we searched for matches of the clone-specific qPCR primers sequences from samples which were qPCR positive and NGS negative in all resulting fastq files. For all primers, similar sequences (with maximally 2 mismatches) were found in fastq files from different patients, thus likely explaining false positivities by qPCR analysis.
Summary/Conclusions: Our results indicate that in this specific situation of massive regeneration post-transplant qPCR can detect false positive results, despite high degree of standardization and strict rules for defining positivity.
Next generation sequencing of IG/TR rearrangements can solve this problem, because it provides the sequence of the entire CDR3 region of all rearrangements in the sample, and therefore allows specific identification of the leukemia-specific sequence.
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P711

PRE-EMPTIVE THERAPY WITH IFN-A-2B FOR ACUTE LEUKEMIA PATIENTS WITH HIGH RISK OF RELAPSING TENDENCY POST ALLO-HSCT

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Background: Relapse still remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%.Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL.
Aims: In order to investigate the role of IFN-α in the field of prevention and treatment of acute leukemia relapse post allo-HSCT.
Methods: We dynamically monitored the minimal residual disease of 986 AL patients who underwent allo-HSCT from January 1, 2006 to March 31, 2014 in our hospital. There were 98 patients who presented increasing tendency of MRD and were enrolled in this study and preemptive treatment were performed once a relapse tendency was detected. pre-emptive therapy was given as long as one of the following conditions were met: 1. 3–5% bone marrow blasts; 2. MRD $\geq 1\times10^{-3}$; 3. Positive specific fusion gene detection or WT-1 gene copy number >200 copies/10,000 abl copies; 4. DC ≤90% detected by short tandem repeat (STR) technology; or 5. More than two listed conditions were met.Among them, 31 patients received IFN-α-2b pre-emptive therapy, and 67 patients received non-IFN-α-2b therapy such as: withdraw immunosupressant, traditional DLI or DC-CIK immunotherapy.
Results: There were no significant differences in disease characteristics between two groups. For the 31 patients who received IFN-α-2b pre-emptive therapy (IFN group), the median time of IFN-α treatment was 60 days (range: 5–720 days), Twenty five patients had response to the treatment without progressing to hematological relapse (response rate 80.6%); 3 patients had stable disease (SD) after IFN treatment; 3 patients had no response and eventually progressed to hematological relapse. Regarding 67 patients who received non-IFN-α-2b therapy(non IFN group), 22 patients had response to the treatment (RR 32.8%), 45 patients failed to the treatment and progressed to hematological relapse at a median time of 35 (range: 6–940) days, There was significant difference of RR between two group(P=0.000). 31 patients of IFN group tolerate well and no patient terminated therapy due to side effects. During the treatment of IFN, 18 patients(58.1%) developed GVHD: 6 patients (19.4%) with aGVHD and 14 (45.2%) with limited cGVHD. The median follow-up time was 21 (4.5-78.5) months. 22 of 31 cases of IFN group maintained disease-free survival. The 5-year overall survival rate (OS) and the leukemia-free survival rate (LFS) of IFN group were 47.0%±13.9% and 38.7%±13.1% respectively. However, the 5-yr OS and LFS of non IFN group were 14.5%±10.7% and12.5%±9.4% respectively. The difference were significantly (P=0.000,P=0.002 respectively). Patients with GVHD had significantly better response than patients without GVHD (88.9% *vs* 53.8%, P=0.043, P <0.05).
Summary/Conclusions: IFN-α-2b pre-emptive therapy can effectively prevent high-risk patients with relapsing tendencies for disease progression post allo-HSCT. Further large-scale investigation is warranted.

P712

A STUDY OF RHTPO IN COMBINATION WITH CHEMOTHERAPY AND G-CSF FOR PERIPHERAL BLOOD PROGENITOR CELL MOBILIZATION IN PATIENTS WITH RELAPSED NON-HODGKIN LYMPHOMA

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Background: High dose chemotherapy followed by autologous stem cell transplant (ASCT) is the standard treatment for relapsed non–Hodgkin lymphoma patients who are still chemotherapy–sensitive. Unfortunately, collection of an adequate number of hematopoietic stem cells can be difficult in these patients, as they usually have received multiple prior cytotoxic regimens. Studys have shown recombinant human thrombopoietin(rhTPO) improved mobilization of peripheral blood progenitor cells in patients with breast cancer.Data on mobilization efficacy in patients with lymphoma are, however, still limited.
Aims: To assess the efficacy and safety of rhTPO in combination with chemotherapy and granulocyte colony–stimulating factor(G–CSF) for peripheral blood progenitor cell mobilization in patients with relapsed Non–Hodgkin lymphoma.
Methods: A total of 79 patients were included in this prospective multi–center randomized controlled clinical study. After administration of mobilizing regimens (CTX $2.5g/m^2d_{1-2}$+VP16 $200mg/m^2d_1$), patients were randomized to TPO+G–CSF (TPO group,n =40) or G–CSF (control group, n=39). Seven point five $\mu g/kg/d$ of G–CSF and 15000U/d rhTPO were subcutaneously administered from day 2 after chemotherapy and until the stem cell collection was completed. PBPCs were collected by daily leukapheresis when the white blood cell count reached $\geq 10\times10^9/L$; leukapheresis was continued until acquisition of a target dose of $\geq 5\times10^6$ $CD34^+$ cells/kg. Mobilized PBPCs were transplanted into patients after additional high–dose chemotherapy with cyclophosphamide, carmustine, and etoposide (CBV). The primary endpoint was $CD34^+$ cell yield.

Secondary endpoints included proportion of target and minimum mobilization, time of neutrophil and platelet engraftment, number of platelet transfusions,and adverse events.
Results: Addition of rhTPO significantly improved total $CD34^+$ cell yield (8.56 *vs* 4.84×10^6/kg, P<0.01). In TPO group, higher percentages of patients achieved the minimum yield of $CD34^+>2\times10^6$/kg (100% *vs* 86%; P<0.05) as well as the target yield of $CD34^+>5\times10^6$/kg (72.9% *vs* 41.3%; P<0.05).No side effects associated with TPO were observed. The median time to neutrophil engraftment was the same in both groups (12 days). No differences in platelet engraftment(12.3 *vs* 14.1 days), number of platelet transfusions (3.2 *vs* 4.4), days of fever (4.6 *vs* 3.3) were seen between TPO and control groups, respectively.
Summary/Conclusions: These results indicate that rhTPO safely and effectively enhance mobilization of PBPCs with chemotherapy and G–CSF in patients with relapsed Non–Hodgkin lymphoma.

P713

A POLYCLONAL POPULATION OF PIGA MUTANT CD52 AND GPI-ANCHOR NEGATIVE T-CELLS CAN GIVE EARLY IMMUNE PROTECTION AFTER ALEMTUZUMAB-BASED T-CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Alemtuzumab, a monoclonal antibody targeting the glycophosphatidylinositol (GPI)-anchored CD52 protein, is used for *in vivo* and/or *in vitro* T-cell depletion before allogeneic stem cell transplantation (alloSCT) to reduce the risk of graft rejection and graft-*versus*-host disease. Following profound lymphodepletion, we observed a rapid recovery of T-cell numbers early after transplantation, despite presence of lytic circulating levels of residual alemtuzumab. In the majority of patients, a substantial portion of these T-cells completely lacked CD52 membrane expression, explaining why these cells escaped alemtuzumab-induced cytotoxicity.
Aims: To further characterize the CD52 negative T cells arising after alemtuzumab-based T cell depleted alloSCT and to unravel the mechanism underlying the loss of CD52 membrane expression.
Methods: To study the functionality of the CD52 negative T cells, these cells were isolated by flowcytometric cell sorting from peripheral blood (PB) samples taken with informed consent from patients within 6 months after alloSCT. Presence of the GPI anchor was determined using a fluorescently labeled GPI-specific aerolysing FLAER. CD52 positive T cells were similarly isolated as control cells. For specific experiments, CD52 negative virus-specific T cells were isolated based on staining with specific peptide/HLA tetramers. Functional activity was tested in cytokine release assay and cytotoxicity assays. To investigate whether absence of CD52 expression was the result of loss of CD52 gene expression, mRNA expression analysis was performed on the CD52 negative T cells.
Results: Cytokine production and cytotoxicity assays showed that the CD52-negative T-cells which were present early after alloSCT contain functional virus-specific T-cells with lytic capacity comparable to their CD52+ counterparts. mRNA expression analysis revealed similar levels of CD52 expression in CD52-negative and CD52+ cells. Since CD52 is tethered to the membrane via the GPI-anchor, we analyzed whether loss of CD52 membrane expression resulted from loss of GPI-anchor expression by flowcytometry using counterstaining with the GPI-specific aearolysin FLAER. This analysis revealed that loss of CD52 expression on post-transplant T-cells generally resulted from loss of GPI-anchor expression. Gene expression analysis of the 26 genes that comprise the GPI-anchor biosynthesis pathway revealed no overall loss of expression for any of these genes. Since loss of GPI-anchor expression in paroxysmal nocturnal hemoglobinuria (PNH) and aplastic anemia has been described to be often the result of mutations in PIGA, we performed mutation analysis on CD52/GPI-negative (CD4+ n=53, CD8+ n=12) and CD52/GPI-positive (CD4+ n=8, CD8+ n=7) T-cell clones isolated from 3 patients after alloSCT. mRNA was isolated from each clone followed by Sanger sequencing of the PIGA coding region. We were able to detect mutations in 42/53 CD4+ and in 10/12 CD8+ CD52/GPI-negative clones. None of the individual mutations was found more than twice within clones from the same recipient, demonstrating a highly polyclonal mutational landscape. In CD52/GPI-positive clones no mutations in the PIGA coding region were found. To investigate whether these mutations in PIGA were sufficient to induce loss of GPI-anchor expression, we retrovirally transduced 35 CD52/GPI-negative CD4+ T-cell clones with constructs encoding wtPIGA. Restored GPI-anchor expression and coinciding CD52 membrane expression was observed in all 35 clones upon transduction with PIGA, but not with empty vector.
Summary/Conclusions: We conclude that loss of CD52 membrane expression in T-cells isolated early after alemtuzumab-based T-cell depleted alloSCT is the result of various mutations in the PIGA gene and consequential loss of GPI-anchor expression. We showed that these CD52-negative populations contain functional virus-specific T-cells and may therefore be essential in immune protection early after transplantation.

P714

WHICH CONDITIONING REGIMEN IS MORE EFFECTIVE FOR HIGH-RISK PATIENTS WITH AML/MDS : COMPARING LOW-DOSE DECITABINE COMBINED WITH MODIFIED BUCY WITH MODIFIED BUCY FOLLOWED BY ALLO-HSCT

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only curative treatment options to hematologic malignancies. However, majority of patients with refractory or resistant hematologic malignancies can not achieve remission before transplantation. It is necessary to design a safe and affective conditioning regimen to reduce the tumor burden, improve the remission rate, decrease the transplantation related mortality and improve disease-free survival in patients with advanced acute myloid leukemia(AML) and myelodysplastic syndrom(MDS). One of the promising drugs of epigenetics is decitabine (DAC), which has a significant effect on a variety of hematologic malignancies including MDS and advanced AML. Furthermore, decitabine can not only up-modulate the tumor-associated antigen express on surface of leukemia cells to increase graft-*versus*-leukemia (GVL) effect but also can reduce the incidence of graft-*versus*-host disease (GVHD) by increase the number of regulatory T Cells (Tregs).
Aims: This clinical study will investigate the security and efficacy of conditioning regimen containing low-dose decitabine combined with modified BUCY regimen for advanced AML/MDS patients, explore the role of immunomodulatory activity post transplantation and compared this regimen with conventional modified BUCY regimen.
Methods: Between January 2012 and March 2015, a total of 156 patients were enrolled in this study. In which, there were 46 patients who received a conditioning regimen of low-dose DAC (DAC group) and a modified BUCY regimen(Con group) followed by allo-HSCT, and the second cohort consisted of 110 who only received a modified BUCY regimen.Comparing the baseline of two groups, there were no significant difference except there were more advanced stage patients in DAC group(63% *vs* 32.7%,p=0.007) A modified BUCY conditioning regimen include semustine (250 mg/m^2/d) for 1 d(-10d), cytarabine (2 g/m^2 q12 h) for 2 d (-9 d to -8 d), busulfan (0.8 mg/kg/6 h) for 3 d (-7 d to -5 d), and cyclophosphamide (1.8 g/m^2/d) for 2 d (-4 d to -3 d). Meanwhile, patients in the DAC group received the DAC treatment for 3 to 4 d with a total of 100 mg/m^2before modified BUCY regimen.
Results: In the DAC group, all patients engrafted successfully, including 29/46 (63%) non-remission (NR) patients. However, there were seven patients presented graft failure in Con group.The median time of neutrophil and platelet recovery in two groups were 12 (10-21) *vs* 12 d (range: 10-23 d)(p and 13 (10-35) days *vs* 14 d (range: 9-40 d) respectively.There were no significant differences between two groups. The transplantation-related mortality (TRM) rate was 0% *vs* 16.4%(p=0.004) for DAC group and Con group. The median follow-up were 200(20-985)d and 177.5(3-1093)d. The cumulative rate of aGVHD and cGVHD were 26.7% *vs* 46.8%(p=0.034) and 68.4% *vs* 70.7% (p=0.598) respectively. The cumulative relapse rate was 26.5% and 37.8%(p=0.706)respectively. The estimated 2-year overall survival (2yr-OS) and 2 year disease-free survival (2yr-DFS) rate were 76.6% *vs* 51.2%(p=0.01) and 63.3% *vs* 41.4%(p=0.046) respectively. Furtheremore, for the patients who were in advanced stage before transplant, the The estimated 2yr-OS and 2yr-DFS rate were 77.3% *vs* 44.8%(p=0.009) and 66.5% *vs* 46.7%(p=0.031) respectively.
Summary/Conclusions: 1. Low-dose decitabine combined with modified BUCY is a safe and effective conditioning regimen for high-risk patients with AML/MDS with low toxicity and well tolerance.All patients of DAC group engrafted and transplantation-related mortality (TRM) rate was 0.
2. 100% NR patients of DAC group achieved complete remission with full donor chimerism at d30. 3.Comparing with Con group, DAC group patients had ralative lower incidence of aGVHD and TRM but relative higher estimated 2-yr OS and DFS, especially for advanced stage patients.

Stem cell transplantation - Clinical 4

P715

ELEVATED EXPRESSION OF TIM-3 ON CD8 T CELLS CORRELATES WITH CYTOMEGALOVIRUS REACTIVATION AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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Background: T-cell immunoglobulin and mucin domain-containing molecule 3 (Tim-3) represents a novel mechanism of T-cell dysfunction and exhaustion in virus infections. However, the role of Tim-3 in the pathogenesis of early cytomegalovirus (CMV) reactivation after allogeneic stem cell transplantation (allo-HSCT) is not well understood.
Aims: In this study, we aimed to investigate the role of Tim-3 during CMV infection after allo-HSCT.
Methods: We detected 33 patients and 10 healthy controls in this study. Collected peripheral blood (PB) of patients 30 days, 60 days, 90 days after allo-HSCT who were neither aGVHD nor suffered from other virus infection, such as HBV, HIV, EBV, *et al.* Plasma samples were assessed for CMV-DNA by PCR, (Peripheral blood mononuclear cells) PBMCs were isolated for flow cytometry to detect Tim-3 expression on CD8+ T cells, apoptosis of Tim-3- and Tim-3+ T cells (Annexin V, AV) and IFN-γ secreted by Tim-3- and Tim-3+ T cells.
Results: The rate of CMV reactivation was 51.5% (17/33) of all the patients. Hence we divided them into three groups: CMV positive group, CMV negative group, healthy control group. An elevated frequency of Tim-3+CD8+ cells was observed in transplanted patients compared to healthy controls, and in transplanted patients, the Tim-3 expression on CD8+ T cells was significantly higher in CMV positive patients than CMV negative cases (7.048±3.060% for healthy controls, 15.55±6.314% for CMV negative patients, and 38.98±17.50% for CMV positive patients, P<0.05). The frequency of Tim-3-expression T cells correlates with viral load in CMV reactivation**.** After effective antivirus treatment and CMV became negative, the frequency of Tim-3+CD8+ T cells was decreased. IFN-γ secreted by Tim-3-CD8+ T cell was significantly higher than that produced by Tim-3+CD8+ T cells in each group (P<0.05). In healthy controls, the apoptosis of Tim-3+CD8+ T lymphocytes were significantly increased than that of Tim-3-CD8+ counterparts (2.926±0.654% *vs* 1.546±0.411%, P=0.001), while in allo-HSCT patients, there were no differences in apoptosis between Tim-3+CD8+ and Tim-3-CD8+ T lymphocyte (2.069±1.720% *vs* 1.632±0.789% in CMV negative group, 2.274±1.185% *vs* 1.871±0.265% in CMV positive group, P>0.05).
Summary/Conclusions: Tim-3 expression on T cells results in the changes of T cell function and level of apoptosis, and closely correlated with CMV infection after allo-HSCT.

P716

INTERFERON-GAMMA GENE POLYMORPHISM ASSOCIATE WITH MORTALITY AND GRAFT-*VERSUS*-HOST DISEASE SEVERITY IN HLA-MATCHED SIBLING BONE MARROW TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) with sibling donors is a life-saving intervention for patients with hematological malignancies. It is recognized that numerous genetic factors in patient and donor, including polymorphic variants of cytokine encoding genes, have been reported that contribute to the outcome of the procedure. Interferon gamma (IFNy) plays a central role in innate and adaptive immunity. *IFNG* gene has a regulatory element in its first intron that includes a microsatellite CA repeat which CA12 variant is associated with an increased production. Up to date there are few published data on the association between this polymorphism and allo-HSCT related complications performed in small series.
Aims: To evaluate the presence of the microsatellite polymorphism +875 (CA)n *IFNG* and its relationship with response and/or complications related to allo-HSCT.
Methods: Two hundred and twenty two (222) patient/donor pairs who underwent a sibling donor HSCT in our centers were genotyped for the presence of +875 (CA)n *IFNG* (rs3138557) variants. Transplants took place between 01/2000 and 03/2015 with a median follow up of 4.4 years.
Results: Median age of recipients was 33 years, being 90% ≥16 years old and 58% male. Prevalent diagnoses were acute myeloid leukemia 29%, acute lymphoid leukemia 23%, lymphoproliferative disorders 13%, and myelodysplastic syndrome 12%; 45% were in early stage of the disease. Myeloablative conditioning regimens were used in 59% and the prevalent source for stem cells was peripheral blood in 90% of patients. Genotypes distribution was 22% CA12/CA12, 41% CA12/CAno12 and 37% CAno12/CAno12 for recipients, and 17%, 54% and 29% for donors, respectively (Chi2 test *p=0.011*). When patients with genotype CAno12/CAno12, associated with low expression, were compared against the rest of genotypes, we observed that these patients developed less acute graft *versus* host disease (aGVHD) grades II-IV (21% *vs* 37%, Fischer exact test *p=0.053),* grades III-IV (6% *vs* 15%, *p=0.071*), chronic GVHD (26% *vs* 41%, *p=0.029*), and a later myeloid engraftment (≥15 days: 54% *vs* 26%, *p <0.001*). Moreover, this group had a significant reduction in overall survival (OS) (1-5 years, 69-40% *vs* 71-56%, Kaplan-Meier, log-rank, *p=0.03*), disease free survival (DFS 1-5 years, 54-40% *vs* 65-49%, log-rank, *p=0.07*) due to a higher relapse rate (1-5 years, 37-45% *vs* 22-32%, Gray's Test, cumulative incidence *p=0.02*), The predictive value was sustained (OS 59-31% *vs* 69-54%, *p=0.05*) and Relapse rate (38-45% *vs* 22-32%, *p=0.01*) when the cohort of patients that receive a non-myeloablative conditioning was analyzed separately.
Summary/Conclusions: The study of the polymorphism +875 (CA)n *IFNG* would be useful to identify patients with differential behavior towards allo-HSCT with sibling donors. While it is necessary to confirm our results in a larger series, genotype associated with a lower expression of IFNγ would be associated with less GVHD, later engraftment and shorter overall survival that could be related to a higher relapse rate, especially in those patients who received reduced-intensity conditioning regimens.

P717

CLINICAL OUTCOME AND IMMUNE RECONSTITUTION IN α/β T-CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION FROM MATCHED RELATED AND UNRELATED DONORS

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Background: The outcome of allo-SCT in patients with hematological malignancies is still hampered by GVHD and relapse. Specific depletion of αβ T-cells is proposed to result in a decreased incidence of aGVHD, whereas the remaining innate cells such as NK cells and γδT cells may provide control of infected and transformed cells the first months post SCT. This strategy has been pioneered in haploindentical transplantation with encouraging results. Within this study, we extend αβT- cell depleted allo-SCT to patients with a matched related and unrelated donor.
Aims: The primary aim is to develop an allogeneic SCT protocol with a low incidence of aGVHD without an increased incidence of infections or relapse to serve as a platform for post-allo interventions such as a pre-emptive DLI or transfer of genetically modified T cells.
Methods: 55 Patients with hematological malignancies (including AML, ALL, MM, NHL, MPN) who received an αβT-cell depleted allo-SCT of a HLA matched sibling (MRD) or HLA matched (9 or 10/10) unrelated donor (MUD) were analysed. αβT-cell reduction was performed by negative selection with anti-αβTCR antibodies in combination with magnetic microbeads, using the automated CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany). The maximal contamination with αβT-cells was $5x10^5$/kg. The conditioning regimen consisted of: ATG (Genzyme®) 6 mg/m^2+fludarabine 120 mg/m^2+busilvex AUC=90. Part of the patients received mycophenolic acid as GVHD prophylaxis for 28 days. Patients were retrospectively analyzed for clinical parameters including immune reconstitution, engraftment, infections, GVHD, relapse, NRM and OS and compared to an historical control cohort of recipients of T cell replete allo-SCT. A retrospective cohort of recipients of T cell replete allografts was used for comparison. In addition in a subset of patients NGS of the TCRβ chain was performed using the Illumina/MiSeq sequencing platform after isolation of diverse immune subsets within the αβT-cell repertoire.
Results: αβT-cell depletion with anti-αβTCR antibodies resulted in a 4.1 (1.7-5.2) log depletion of αβT cells and a recovery of 77% (43-98%) of the CD34$^+$ cells. The median contamination with αβT-cells was $16x10^3$/kg ($0.8x10^3$/kg - $200x10^3$/kg) and infused number of CD34$^+$ cells were $6.8x10^6$/kg ($1.2 x10^6$/kg – $10.4x10^6$/kg). The combination of ATG/fludarabine/busilvex was well tolerated with hematological recovery within 3 weeks. Primary engraftment (chimerism >95%) was observed in all patients (n=55). Immune reconstitution primarily consisted of NK cells. In addition, γδT cells were detectable at normal numbers the first half year post SCT, whereas the adaptive immune repertoire showed a delayed reconstitution. The incidence of CMV infections was 54% in patients after αβT cell depleted allo-SCT without MMF, 23% in patients after αβT cell depleted allo-SCT with MMF and 38% in T cell replete allo-SCT control cohort. The incidence of EBV infections was 30,8%; 9,5% and 8,7% respectively. The incidence of aGVHD >grade II within 100 days in patients of a αβT-cell depleted allo-SCT was 0%. During this short time of follow-up (1-20 months) we observed no significant differences in EFS, NRM and OS as compared to historical control cohorts. With NGS of the TCRβ repertoire, a surprising diversity was observed in defined immune subsets ranging from clonal expansion of regulatory T cells to broad repertoires in effector memory cells.

Summary/Conclusions: Here we present the clinical outcome of a large cohort (n=55) of patients having received an αβT-cell depleted allograft of MRD/MUD. We observe a swift reconstitution of innate cells (NK cells and γδT-cells) the first 6 months post transplantation, followed by a subsequent reconstitution of the adaptive immune repertoire. The incidence of severe aGVHD was 0%, without a significant increase in infections or relapse shortly post allo-SCT. These results will be confirmed during extended follow-up and in a planned prospective multicenter study.

P718

BASELINE LABORATORY VARIABLES SERVE AS POTENT PREDICTORS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION RELATED MORTALITY

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Background: Prognostic models for allogeneic hematopoietic stem cell transplantation (HSCT) outcomes have traditionally relied on physician-evaluated parameters, subject to interpretation. Furthermore, despite availability of comprehensive electronic medical data, their use has been limited by standard statistical techniques, requiring subjective pre-selection of variables prior to construction of prediction models.
Aims: Using Random Survival Forests (RSF), a machine learning (ML) algorithm, we sought to estimate predictive importance of a wide array of clinical and laboratory variables, including many not often assessed, in predicting HSCT related mortality (TRM). ML is a subfield of artificial intelligence, commonly applied in complex data scenarios.
Methods: We retrospectively analyzed a cohort of patients undergoing allogeneic HSCT in a single center. Patients' clinical data were crossed with laboratory data from institutional electronic medical records at baseline admission for transplantation. A total of 33 variables, mostly laboratory based (v=23), were included. An RSF model for TRM, with relapse as a competing event, was constructed and variable importance was extracted. Partial dependence plots were used to graphically explore the risk-adjusted relationship between predictors and TRM. Top RSF predictors were introduced into a Cox regression model, and cumulative incidence (CIn) was compared across individual variables.
Results: A cohort of 965 patients with a median age of 53 (16 -76) were analyzed. Indications for transplant were mostly hematologic malignancies mainly AML and MDS. The majority of patients received myeloablative conditioning (58%) and graft-*versus*-host-disease (GvHD) prophylaxis with cyclosporine and methotrexate (76%). Median follow-up time was 6.4 years. The top 15 predictors selected by RSF are presented in Figure 1a. TRM is strongly predicted by pre HSCT albumin, GvHD prophylaxis and estimated creatinine clearance, whereas these factors are less predictive of relapse. Partial dependence plotting uncovered non-linear relationships between TRM CIn and creatinine clearance (Figure 1b) and serum albumin. Seven of the top 10 RSF predictors were confirmed by a Cox regression model (Table 1) and TRM CIn plots (Figure 1c).

Table 1.

Variable		Hazard ratio	p-value
GvHD Prophylaxis	MTX *vs* MMF	1.32	0.0180
	- *vs* Other	0.734	
HLA mis-match	0 *vs* 1	2.356	0.0001
Diagnosis	AML *vs* ALL	0.934	0.0085
	- *vs* MDS/MPD	1.404	
	- *vs* Lymphoma	2.157	
	- *vs* Other	1.676	
Albumin (g/dl)	>3.7 *vs* <3.7	1.708	0.0002
Creatinine clearance (mL/min)	>60 *vs* <60	1.694	0.0007
Age	<20 *vs* 20-40	1.442	0.0085
	- *vs* >40	2.225	
Alk Phos (IU/l)	<120 *vs* >120	1.461	0.0073

Summary/Conclusions: Using a novel methodology across a comprehensive landscape of HSCT patient characteristics, we identified key predictors of TRM. Variables such as creatinine clearance and albumin display a strong non-linear relationship with the primary outcome-TRM, while TRM is linearly dependent on alkaline phosphatase. Some elements considered in existing prognostic scoring systems, such as hepatocellular transaminases, were not predictive. Taken together, these findings provide a new framework for evaluating transplantation risk in a data-rich environment, and reveal simple and objective determinants of transplant-related mortality.

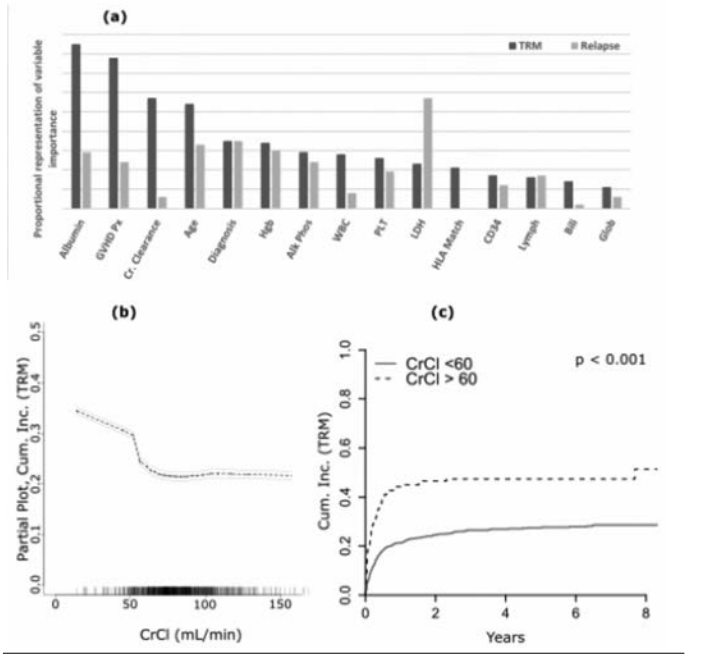

Figure 1.

P719

CYTOMEGALOVIRUS INDUCES STRONG ANTILEUKEMIC EFFECT IN ACUTE MYELOID LEUKEMIA PATIENTS FOLLOWING SIBLING HSCT WITHOUT ATG-CONTAINING REGIMEN

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Background: A considerable number of studies have demonstrated that cytomegalovirus (CMV) reactivation after allogeneic hematopoietic stem cell transplantation (Allo-HSCT) could enforce graft-*versus* leukemia (GVL) effect in acute myeloid leukemia (AML) patients. However, the use of antithymocyte globulin (ATG) as part of graft-*versus*-host disease (GVHD) prophylaxis may dampen this beneficial effect of CMV replication.
Aims: The purpose of this study is to investigate whether the ATG or non-ATG containing regimens can all benefit from this GVL effect.
Methods: In this context, we retrospectively analyzed the effect of CMV reactivation on relapse, survival and prognosis in a total of 227 AML patients who received a myeloablative (MA) conditioning regimen at a single research center between January 2010 and April 2013.
Results: Of these 227 patients, 110 cases received non-ATG-containing regimens and 117 cases received ATG-containing regimens. CMV reactivation occurred in 45 patients (41%) among non-ATG regimen -months, a lower risk of cumulative relapse incidence associated with CMV reactivation was observed in non-ATG group in multivariate analyses (OR 0.28, 95% CI 0.10-0.79; *P*=0.016). However, CMV reactivation after transplantation did not significantly decrease the cumulative incidence of relapse in our ATG group (OR 0.28, 95% CI 0.10-0.79; *P*=0.016).
Summary/Conclusions: Collectively, our results demonstrate that in AML patients following sibling HSCT, the CMV-induced beneficial effect on relapse occurs only in the MA regimens containing no ATG, although ATG promotes CMV reactivation. the cumulative incidence of relapse in our ATG group (OR 0.28, 95% CI 0.10-0.79; *P*=0.016).

P720

COMPARING TIME-DEPENDENT PREDICTIVE PERFORMANCE OF ALLOGENEIC HSCT RISK SCORING SYSTEMS

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Background: The European Bone Marrow Transplant (EBMT) score and Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) predominate in the assessment of risk of allogeneic hematopoietic stem cell transplantation (HSCT)-related mortality. Though validated, both scores have been shown to fluctuate in predictive capacity between different patient populations.

Aims: To evaluate and compare the long-term performance of the EBMT and HCT-CI scores across typical transplant indications.
Methods: This is a retrospective analysis of a cohort of patients from a single center. EBMT and HCT-CI scores were calculated for the entire cohort. Cox regression modeling, with scores as covariates, was used to assess the probability of overall survival (OS) as well as treatment-related mortality (TRM) with relapse as a competing risk. Score performance in predicting each outcome at multiple time-points was compared, using receiver operating characteristics to determine time-dependent areas under the curve (AUC). AUC measures the ability of a model to differentiate between those who do and do not experience the outcome event on a scale of 0.5 (no better than random chance) to 1 (perfect discrimination). Performance was further compared within specific subgroups.
Results: A cohort of 845 patients, with a median age of 52 (16 - 76), was assessed. Indications for transplant were primarily acute leukemia and myelodysplastic syndrome. EBMT scores reflected a normal distribution; 50%, 25%, 12% and 14% of patients had HCT-CI scores of 0, 1, 2 and 3 or greater, respectively. The majority of patients had advanced disease (45.0%) and received myeloablative conditioning (67.7%). Median follow-up was 6.2 years. Patients with early or intermediate disease had a median survival >4 years; those with advanced disease had only 0.5 year. Increasing scores corresponded to distinct and increasing risk of TRM (Figure 1a, b), Table 1). Neither score predicted relapse. Predictive performance, measured by AUC, was time-dependent. While in the first 2 years post-HSCT the EBMT score showed significantly greater discrimination, the scores subsequently converged. This was seen both in the entire population and within subgroups such as disease status and conditioning regimen.

Table 1.

	Risk score	% patients	TRM HR (95% CI)	p	OS HR (95% CI)	p
EBMT	0-2	19.4%	1			
	1					
	3	22.2%	1.59 (0.96-2.63)	0.071	1.29 (0.82-2.02)	0.270
	4	23.9%	1.86 (1.15-3.00)	0.010	1.76	0.011
	5	24.1%	2.55 (1.61-4.05)	<0.001	2.25 (1.46-3.47)	<0.001
	6-7	13.0%	3.87 (2.40-6.26)	<0.001	2.62 (1.66-4.14)	<0.001
HCT-CI	0	49.9%	1		1	
	1	24.7%	1.36 (0.97-1.89)	0.072	1.28 (1.03-1.59)	0.025
	2	12.4%	1.54 (1.05-2.27)	0.028	1.39 (1.07-1.80)	0.014
	≥3	14.4%	2.09 (1.48-2.96)	<0.001	1.72 (1.36-2.17)	<0.001

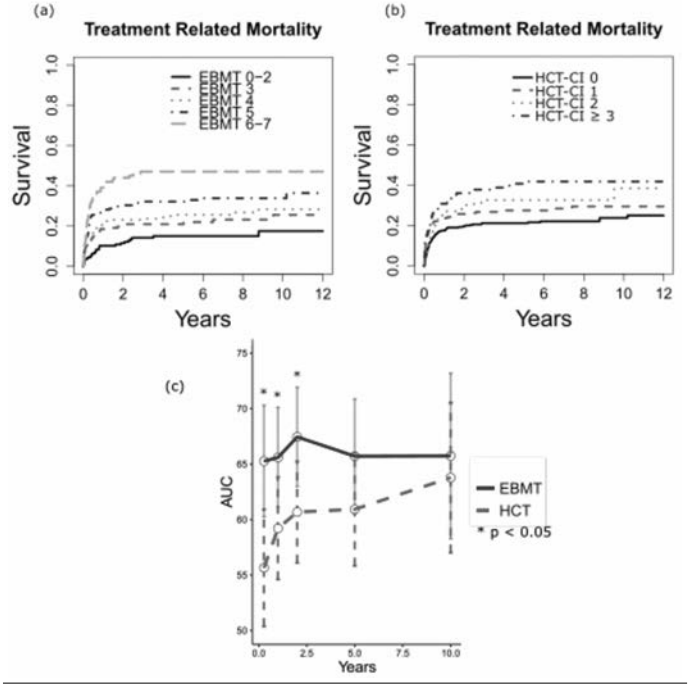

Figure 1.

Summary/Conclusions: In an allogeneic HSCT cohort with median follow-up time >6 years, predictive performance of the EBMT score is greater than that of HCT-CI in the first two years after transplantation. The HCT-CI score steadily improves until performances converge. The different components of the two prognostic models may account for the HCT-CI score's improving effectiveness over time: the EBMT score reflects primarily static features of the patient and procedure at time of transplantation; the HCT-CI score includes factors subject to subsequent improvement or deterioration. Altogether, both models provide stepwise risk estimation, however individual clinical prediction remains elusive due to limited discrimination (*i.e.* AUC).

P721

PROPHYLACTIC DLI *VS* NO DLI FOR REFRACTORY ACUTE LEUKEMIA UNDERGOING ALLO-HSCT WITH SEQUENTIAL INTENSIFIED CONDITIONING

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Background: The major obstacle is leukemia relapse for refractory leukemia undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). We previously introduced a strategy of sequential intensified conditioning and early rapid immunosupressant withdrawal for refractory leukemia undergoing allo-HSCT, with 5-year overall survival (OS) and 3-year relapse rate of 44.6% and 33.3%.
Aims: To reduce leukemia relapse, prophylactic donor lymphocyte infusion (DLI) was administered based on our historical strategy.
Methods: We conducted a prospective multi-center study to assess the efficacy of the new strategy for refractory advanced acute leukemia. Sequential intensified conditioning regimen was: fludarabine (30 mg/m^2/day, -10 to -6 days)+cytarabine (2.0 g/m^2/day, -10 to -6 days) plus total body irradiation (4.5 Gy/day, -5, -4 days)+cyclophosphamide (60 mg/kg/ day, -3, -2 days)+etoposide (15 mg/kg/day, -3, -2 days). Immunosupressant was withdrawn by 10%/week in patients without acute graft-*versus*-host disease (aGVHD) by day +30 post-transplantation. For patients without grade II/>II aGVHD by day +60 post-transplantation, DLI was administered at a median dose of 1.0×10^{8}mononuclear cells/kg if donor lymphocytes were available. DLI was given once to all patients regardless of minimal residual disease (MRD), and was then administered based on GVHD and MRD status. If patients were MRD negative, DLI was not given again; if patients were MRD positive and without GVHD, DLI was given monthly until GVHD occurred or MRD became negative or for a total of four times.
Results: A total of 153 patients with refractory advanced acute leukemia undergoing allo-HSCT from January 2009 to June 2014 were enrolled. All patients achieved hematopoietic reconstitution except for two who died of infection and two who died of RRT within 2 weeks post-transplantation. At the time of neutrophil reconstitution, all 149 evaluable patients achieved complete remission. The incidence and mortality of regimen-related toxicities were 100.0% and 1.3%. The 5-year overall and disease-free survival post-transplantation were 51.1%±5.7% and 49.2%±5.3%. The 5-year relapse rate and non-relapse mortality (NRM) were 27.3%±4.4% and 29.7%±5.3%. According to the availability of donor lymphocytes and the criteria for DLI, 144 patients surviving day +60 were divided into two groups (80 DLI *versus* 64 non-DLI). The relapse rate was less and OS was better in patients receiving DLI than in those not receiving DLI (22.7% *vs* 33.9%, P=0.048; 58.1% *vs* 54.9%, P=0.043). NRM was similar between DLI and non-DLI groups (P=0.104). Multivariate analysis revealed that lower bone marrow blasts on day 0, DLI and chronic GVHD were associated with less relapse and better OS.
Summary/Conclusions: The strategy of sequential intensified conditioning followed by early immunosupressant withdrawal and DLI could reduce relapse of refractory acute leukemia after allo-HSCT and improve survival.

P722

THE PRE-TRANSPLANT BIOMARKER RISK INDEX BASED ON SERUM FERRITIN, ALBUMIN AND CRP CAN PREDICT OUTCOMES INDEPENDENTLY OF THE HCT-CI AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Although the Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI) and Comorbidity-age index are useful tools for pre-transplant risk assessments in allogenic hematopoietic stem cell transplantation (allo HSCT), c-statistic estimates for non-relapse mortality (NRM) and overall survival (OS) of them are reported to be about 0.65. Therefore, a new index rather than the HCT-CI which can predict more reliably outcomes is required to aid bedside decision making.
Aims: The purpose of this study was to create a new risk index based on three biomarkers, which are pre-transplant serum ferritin (SF), albumin (Alb), and C-reactive protein (CRP), to predict NRM and OS after allo HSCT.
Methods: We analyzed 198 adult patients who underwent allo HSCT between

2005 and 2014 at our facilities in this study, including 96 with acute myelogenous leukemia, 47 with acute lymphoblastic leukemia, 26 with myelodysplastic syndrome, 4 with myeloproliferative neoplasms, 3 with chronic myeloid leukemia, 5 with adult T-cell leukemia/lymphoma, 1 with mixed lineage leukemia, 4 with aplastic anemia, 2 with chronic active Epstein-Barr virus infection and 10 with malignant lymphoma. Median age of patients was 52.5 years (range: 16-67). A total of 57 patients received related stem cell transplantations, 82 received unrelated stem cell transplantations, and 59 received cord blood transplantations. In addition, myeloablative and non-myeloablative conditioning regimens were administered to 114 and 84 patients, respectively. The 70 patients with non-remission acute leukemia/lymphoma or refractory anemia with excess blasts were categorized as having a high disease status, and the others were categorized as having a standard disease status. We scored the pre-transplant risk index using biomarkers (Biomarker index: Bio-I) which was calculated to be assigned a 1 point for >2000ng/ml of SF, <3.4mg/dl of Alb and >1.5mg/dl of CRP respectively. We divided patients into three risk groups using Bio-I, low (score 0, N=138), intermediate (score 1-2, N=49) and high (score 3, N=11). We evaluated the utility of the Bio-I as an index for predicting post-transplant outcomes.
Results: Median pre-transplant SF was 4894ng/ml (range: 8-35185 ng/ml), Alb was 3.65g/dl (range: 2.1-5.2g/dl) and CRP was 0.16mg/dl (range: 0.01-17.9mg/dl). The Bio-I exhibited sufficient c-statistic estimates for NRM (0.613) and OS (0.662) at 1 year. These values were similar to those reported for the HCT-CI. In univariate analysis, the Bio-I was successfully used to stratify the patients into three risk groups in NRM (p=0.001) and OS (p<0.001). In multivariate analysis adjusted for the HCT-CI and disease status, the high-risk Bio-I group was found to be an independent poor prognostic factor for NRM (hazard ratio (HR): 5.16, p<0.001). Furthermore, intermediate- and high-risk groups were shown to be independent poor prognostic factors for OS (intermediate: HR 1.67, p=0.034, high: HR 4.39, p<0.001). In addition, we created a comprehensive scoring system for OS which was calculated to be assigned each point (Bio-I low: 0, intermediate: 1, high: 2, HCT-CI 0-2: 0, ≥3: 1 and disease status standard: 0, high: 1). This comprehensive scoring system displayed a higher c-statistic estimate for OS than the HCT-CI at 1 year (0.754).
Summary/Conclusions: The Bio-I, which is based on SF, Alb, and CRP levels, is a useful tool for risk assessment before allo-HSCT because it is easy to calculate, exhibits sufficient sensitivity, can be used to stratify patients into three clear risk groups, and acts as an independent (including from the HCT-CI) risk factor for predicting post-transplant outcomes.

P723

HAPLOIDENTICAL TRANSPLANTATION FOR PEDIATRIC PATIENTS WITH ACQUIRED SEVERE APLASTIC ANEMIA

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Background: Matched sibling donor hematopoietic stem cell transplantation (MSD HSCT) is the upfront therapy for children with acquired severe aplastic anemia (SAA), but MSD is not available for most patients. The technique of haploidentical HSCT (haplo-HSCT) for SAA has been improved according to exciting results reported recently, although it is recommended to pediatric SAA patients after failure to at least 2 courses of IST based on ATG in current guidelines.
Aims: To study that who will benefit from haplo-HSCT most.
Methods: 52 SAA patients younger than 18 years received haplo-HSCT consecutively between February 2007 and November 2015 in our center. All the patients received haplo-HSCT with the protocol of using G-CSF primed bone marrow plus G-CSF-mobilized peripheral blood stem cells without *in vitro* T cell depletion. The conditioning regimen included BU(08.mg/kg q6hrsx2d), CY(50mg/kgx4d) and rATG(2.5mg/kg/dx4d). CsA, MMF and short-time MTX were used for GVHD prophylaxis.In 23 newly diagnosed patients, haplo-HSCT was administered as the upfront therapy and in the other 29 refractory patients, it was administered as non-upfront therapy.
Results: 51 patients achieved primary engraftment except one child died of regimen related toxicity on the day +1. Graft failure occurred on 3 patients (two was rejection and the other one was secondary poor graft function). The cumulative incidence (CI) of aGVHD grade II-IV and grade III-IV was 39.2±0.5% and 13.7±0.2%, respectively. The CI of cGVHD was 34.2±0.5%. The 3-year OS and FFS was 84.5±5.0% and 82.7±5.2% with a median follow-up time of 744.5d (100-3294) for surviving patients. ECOG was the only predictor of OS and failure free survival (FFS).The OS, FFS, aGVHD, cGVHD have no significant difference between the upfront therapy group and the non-upfront therapy group.
Summary/Conclusions: Both newly diagnosed and refractory pediatric SAA patients could benefit from haplo-HSCT, especially for the children with good general condition. Considering the excellent outcome, haplo-HSCT might be considered to be alternative therapy for pediatric SAA patients without MSD in selected centers. Further studies are needed to confirm this result in prospective trials.

P724

COMPARISON BETWEEN FLUDARABINE PLUS BUSULFAN AND FLUDARABINE PLUS MELPHALAN CONDITIONING REGIMENS BEFORE SINGLE-UNIT CORD BLOOD TRANSPLANTATION FOR ADULT ACUTE MYELOID LEUKEMIA PATIENTS

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Background: In recent year, reduced intensity conditioning (RIC) is developed and increasingly used in allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acute myeloid leukemia (AML) patients. Fludarabine plus busulfan (FB) and fludarabine plus melphalan (FM) are widely used RIC regimens. A previous study reported FB and FM provide comparable survival after allo-HSCT from HLA matched siblings for AML patients. However, outcomes of cord blood transplantation (CBT) following FB or FM are unknown in AML patients.
Aims: We conducted a nationwide retrospective study to compare FM and FB in AML patients who underwent CBT.
Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. The selection criteria included AML (excluding acute promyelocytic leukemia), aged 16 years or older, first transplantation, single-unit CBT (HLA 4-6/6 match) between January 2003 and December 2013, FB (fludarabine 120-180 mg/m^2, busulfan 8mg/kg (or intravenous injection in equivalent doses) and TBI 2-4Gy) or FM (fludarabine 120-180 mg/m^2, melphalan 80 mg/m^2 and TBI 2-4Gy). We compared outcomes (overall survival (OS), relapse, non-relapse mortality (NRM) and hematopoietic recovery) after CBT following FB or FM.
OS was estimated by the Kaplan-Meier method and was compared using a log-rank test. Relapse and NRM were considered competing risk events for each other and were compared using Gray's test. The cumulative neutrophil and platelet recoveries were also estimated and compared by Gray's test considering death without these events as a competing risk. In a multivariate analysis, the Cox proportional hazard model and Fine-Gray methods were used for OS and cumulative incidence of relapse, NRM and hematopoietic recovery respectively, using the following variables: age, gender, disease status, cytogenetic risk category, HLA disparity, nucleated cell dose, donor-recipient gender mismatch, ABO mismatch, and transplantation year.
Results: Of the 412 patients, 122 patients (29.6%) received FB and 290 patients (70.4%) received FM. Median age were 61 (20-78) and 60.5 (17-82) years old, respectively (P=0.66). Higher proportion of FM patients received HLA 6/6 matched CBT (P=0.03) and 4Gy TBI (P=0.002). There was no difference in other factors. Three-years OS (34.0% and 33.7%, P=0.81) relapse (34.9% and 36.3%, P=0.93), NRM (33.4% and 33.4%, P=0.89), 30-days neutrophil engraftment (63.1% and 67.2%, P=0.39) and 60-days platelet engraftment (45.1% and 50.0%, P=0.33) were not significantly different between FB and FM. In multivariate analysis, FB and FM showed similar OS (HR, 0.96; P=0.78), relapse (HR, 0.98; P=0.91), NRM (HR, 1.03; P=0.88), neutrophil engraftment (HR, 1.09; P=0.51) and platelet engraftment (HR, 1.18; P=0.31).
Summary/Conclusions: The current study showed FB and FM provide similar outcomes of CBT for AML patients.

P725

MINIMAL RESIDUAL DISEASE AND GRAFT-*VERSUS*-HOST DISEASE GUIDED SEQUENTIAL CHEMOTHERAPY PLUS DONOR LYMPHOCYTE INFUSION COULD IMPROVE SURVIVAL OF PATIENTS WITH ACUTE LEUKEMIA WHO RELAPSED AFTER ALLO-HSCT

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Background: Allogeneic hematopoietic cell transplantation (allo-HSCT) is an effective therapy for acute leukemia, but relapse remains a major problem. Our recently article confirmed that chemotherapy plus DLI was superior to chemotherapy alone in patients with acute leukemia who relapsed after allo-HSCT, but the disease-free-survival (DFS) was only 20% at 2 years post-DLI and the cumulative risk of re-relapse was nearly 60% at 2 years post-DLI. Some articles have confirmed that chronic graft-*versus*-host disease (GVHD) post-DLI was a favorable prognostic factor for DFS. Besides, minimal residual

disease (MRD) identified by multi-parameter flow cytometry (FCM) and real-time quantitative polymerase chain reaction (RQ-PCR) could represent leukemic load and predict relapse.
Aims: To perform a prospective study and investigate the impact of MRD and GVHD guided sequential chemotherapy plus DLI on re-relapse and DFS in patients with acute leukemia who relapsed after HSCT.
Methods: From January 1, 2013 to February 28, 2015, consecutive 70 patients with acute leukemia who relapsed after allo-HSCT and received chemotherapy plus DLI to treat relapse were enrolled in this study and were defined as study group. When patients relapsed after allo-HSCT, induction chemotherapy plus DLI would be given firstly, and then, if patients achieved complete remission (CR), sequential chemotherapy plus DLI would be given based on the state of MRD and GVHD post-DLI. Besides, from January 1, 2000 to December 31, 2008, 54 patients with acute leukemia who relapsed after allo-HSCT only received induction chemotherapy plus DLI to control relapse and were defined as historical group.
Results: In study group, cumulative risk of re-relapse, overall survival (OS) and disease-free survival (DFS) at 1-year post-DLI was 45.7%, 56.9% and 50.0%, respectively. As well, multivariate analysis suggested that no chronic GVHD post-DLI (P=0.019, HR=5.093) and persistent MRD-positive state post-DLI (P<0.0001, HR=14.178) were unfavorable factors for re-relapse. Furthermore, based on the risk factors, 70 patients were further stratified into low-risk group, intermediate-risk group and high-risk group. Cumulative risk of re-relapse was significantly lower, DFS and OS were significantly better in low-risk group than that in other two groups (re-relapse: 22.3% *vs* 80.0% *vs* 92.5%, P<0.0001; DFS: 75.1% *vs* 0.0% *vs* 0.0%, P<0.0001 and OS: 94.7% *vs* 60.0% *vs* 0.0%, P<0.0001). Besides, compared with historical group, cumulative risk of re-relapse was significantly lower, DFS and OS were significantly better in study group (re-relapse: 45.7% *vs* 70.4%, P<0.0001; DFS: 50.0% *vs* 24.1%, P<0.0001 and OS: 56.9% *vs* 29.6%, P<0.0001).
Summary/Conclusions: These data suggest that based on the state of MRD and GVHD post-DLI, sequential chemotherapy plus DLI could improve outcomes of patients with acute leukemia who relapsed after allo-HSCT.

Stem cells and the microenvironment 2

P726

TERMINAL NK CELL MATURATION IS CONTROLLED BY CONCERTED ACTIONS OF T-BET AND ZEB2

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Background: ZEB2 is a Zinc finger E-box binding transcription factor known to control epithelial-to-mesenchymal transition during development and disease. We recently have demonstrated that it is as well essential in multiple hematopoietic lineage differentiation decisions and for hematopoietic stem/progenitor migration.
Aims: We found that the Zeb2 is the most highly induced transcription factor during NK cell maturation. The aim of this project was to elucidate the role of Zeb2 in NK cells.
Methods: Using conditional loss and gain-of function mouse models we generated a cohort of mouse models with graded expression levels of Zeb2 specifically in the NK cell lineage.
Results: Targeted deletion of Zeb2 resulted in impaired NK cell maturation, survival and exit from the bone marrow. NK cell function was preserved but mice lacking Zeb2 in NK cells were more susceptible to B16 melanoma lung metastases. Reciprocally, ectopic expression of Zeb2 resulted in a higher frequency of mature NK cells in all organs. Moreover, the immature phenotype of Zeb2 null NK cells closely resembled that of Tbx2 null NK cells. This was due both to a dependence of Zeb2 expression on T-bet and to a probable cooperation of these factors in gene regulation. Transgenic expression of Zeb2 in Tbx21 null NK cells partially restored a normal maturation, establishing that timely induction of Zeb2 by T-bet is an essential event during NK cell differentiation. Finally, this novel transcriptional cascade could also operate in human as T-bet and Zeb2 are similarly regulated in mouse and human NK cells.
Summary/Conclusions: Here, we demonstrate that Zeb2 is essential to promote terminal NK cell maturation and that it functions downstream of T-bet.

P727

BIFURCATION INTO HEMATOPOIETIC STEM CELL TYPES IS DEPENDENT UPON THE DEVELOPMENTAL SIGNALING MOLECULES

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Background: All hematopoietic stem cells (HSC) are not alike. Clonal HSC transplantations and refined cell sorting based on HSC surface markers or reporters or responsiveness to signalling pathways have identified HSC subtypes with different functional properties. HSC subtype behaviours include preferred/biased lineage output following transplantation. The source of this HSC heterogeneity has yet to be identified. Since bone morphogenetic protein (BMP) affects the development of HSCs while they are generated, HSC subtypes may be differentially controlled by the BMP signaling pathway. However, the canonical BMP pathway is dispensable in the fetal liver and adult bone marrow, tissues where HSCs migrate during development.
Aims: Investigate whether HSC subtypes are programmed intrinsically during the developmental emergence of HSCs and/or whether their heterogeneity is determined by extrinsic factors encountered in different developmental hematopoietic tissues.
Methods: We examined whether HSCs in the embryo, fetus and adult are directly responsive to BMP signaling and the relationship of BMP signaling to HSC heterogeneity. To this objective, we used the *BRE GFP* transgenic reporter mouse model. GFP is expressed in *BRE–GFP* mice when BMP and the BMP receptor signal through Smad1/5 to activate transcription from the *BRE* sequence. GFP expression denotes BMP activation at the moment of cell observation/isolation and does not represent previous BMP activation history. Prospectively isolated cell types based on GFP expression and/or specific marker combinations were further transplanted into irradiated recipients or analyzed by RNA sequencing.
Results: We show that BMP signalling directly regulates HSCs as they are first generated in the aorta-gonad-mesonephros (AGM) region. However, in expansion conditions upon explant culture, we find that the AGM harbors two types of adult-repopulating HSCs: One type is BMP-activated and the other is a non-BMP-activated HSC type that is indirectly controlled by Hedgehog signalling through the VEGF pathway. Importantly, the two types of HSCs are

also found in the murine fetal liver and adult bone marrow *in vivo*. Clonal transplantation demonstrates that they have distinct haematopoietic lineage outputs. Transcriptomic analyses show that the two HSC types differ in intrinsic genetic programs, thus supporting a role for the developmental signalling pathways in the regulation of HSC heterogeneity and lineage output.
Summary/Conclusions: Our results reveal that the bifurcation in HSC types take place from early embryonic stages and suggest that their development is dependent upon the signalling molecules in the microenvironment they reside. Our findings provide insight into the molecular control mechanisms that define HSC types and have important implications for reprogramming cells to HSC fate and treatments targeting distinct HSC types.

P728

LACK OF MIR-127 DOWN-REGULATION IN THE TRANSITION FROM HEMATOPOIETIC STEM CELLS TO MULTI-POTENT PROGENITORS LEADS TO PANCYTOPENIA AND DEFECTIVE SELF-RENEWAL

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Background: The ability of balancing self-renewal and differentiation is a key hallmark of somatic stem cells; however, the molecular pathways underlying this regulation are not completely understood, including any role for microRNAs (miRNAs). To identify miRNAs involved in the maintenance of hematopoietic stem cells (HSCs) identity we employed Pbx1-conditional knockout (KO) mice. Pbx1 is a homeobox transcription factor that positively regulates HSC quiescence. Its absence in post-natal HSCs causes a premature myeloid differentiation and an excessive proliferation that ultimately leads to their exhaustion, indicating a profound self-renewal defect [1]. Moreover, Pbx1maintains proto-oncogenic transcriptional pathways involved in myeloproliferative disorders [2].
Aims: Our aim was to assess if miRNAs contribute to regulate the balance between self-renewal and differentiation in HSCs, which is malfunctioning in myeloproliferative disorders and/or leukemia.
Methods: To achieve our aim we employed Pbx1-conditional KO mice, whose stem cells display a profound self-renewal defect. We reasoned that miRNA profiling of Pbx1-null HSCs, which are defective in their main functions, would lead to uncover miRNAs playing a role in HSC biology. Therefore, TaqMan-based miRNA profiling of HSCs and of their immediate downstream progeny of multi-potent progenitors (MPPs) from wt and Pbx1-conditional KO mice was performed. The expression of the most differentially expressed (DE) miRNAs was validated through independent real time PCR analysis, and was assessed in additional BM cell populations. Finally, we performed *in vivo* functional studies through lentiviral-mediated over-expression in primary murine bone marrow (BM) cells followed by transplantation in irradiated recipient mice.
Results: Unsupervised hierarchical clustering indicates a clear distinction between HSCs and MPPs at the level of miRNA expression, in accordance with the hypothesis that miRNAs contribute to regulate the first transition step in the adult hematopoietic development. Within each group, Pbx1-null and wt cells cluster separately, suggesting that Pbx1 directly or indirectly might regulate the expression of miRNAs involved in HSC self-renewal. Importantly, we found a group of miRNAs that are concordantly DE both in the physiological HSC-to-MPP transition and in Pbx1-null HSCs compared to wt HSCs. Among this list, miR-127 is the most DE. Its level of expression is comparable to that of other miRNAs previously associated to HSCs. However, it is the only one whose down-regulation occurs at the first transition from HSCs to MPPs, and that is strongly HSC-specific, since it is not re-expressed further down in the hematopoietic hierarchy within the BM. Interestingly, it is up-regulated in $CD34^+$ cells from chronic myeloid leukemia patients. Lack of miR-127 down-regulation in $Lineage^-$ (Lin^-) cells, achieved through lentiviral-mediated constitutive expression, led to their *in vivo* competitive disadvantage over untransduced Lin^- cells in long-term transplantation assays, and to a self-renewal defect as assessed in secondary recipients. In addition, transduced Lin^- cells transplanted in the absence of competition gave rise to pancytopenia.
Summary/Conclusions: A tight regulation of miR-127 is crucial to maintain HSC functions.
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References
1. Ficara F *et al.*, Cell stem cell 2008, 2(5):484-496.
2. Ficara F, Crisafulli L, *et al.*, Journal of cell science 2013, 126(Pt 14):3181-3191.

P729

NAMPT-NAD+-SIRT2 PATHWAY-TRIGGERED DEACETYLATION OF LMO2 PROTEIN IS INDISPENSABLE IN THE EARLY HEMATOPOIESIS THROUGH COMPLETE TAL1 COMPLEX FORMATION

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Background: Nicotinamide phosphoribosyltransferase (NAMPT) is known as a rate-limiting enzyme in the biosynthesis of nicotinamide adenine dinucleotide (NAD^+) and supplies NAD^+ to sirtuins (SIRTs) that require NAD^+ to activate their protein deacetylase functions. We already reported that NAMPT-NAD^+-SIRT1 pathway is essential for granulopoiesis (Skokowa J, *et al.* Nat Med. 2009).
Aims: To analyze the roles of NAMPT-NAD^+-SIRT pathway in early hematopoiesis and elucidate new molecular mechanisms.
Methods: We used human induced pluripotent stem (iPS) cells model and a serum- and feeder-free monolayer hematopoietic differentiation system. We added NAMPT- or SIRT-inhibitors to the cells and analyzed hematopoietic differentiation.
Results: First of all, we cultured iPS cells in the presence or absence of the specific inhibitor of NAMPT, FK866. Interestingly, we found marked reduction of $VEGFR2^+CD34^+$ early hematopoietic progenitor cells at day 6 of differentiation by inhibition of NAMPT. As a results of qRT-PCR results which shows *SIRT2* mRNA expression is increasing in this differentiation stage, we tested SIRT2 specific inhibitor AC93253 and found that inhibition of SIRT2 also significantly suppressed differentiation into $VEGFR2^+CD34^+$ early hematopoietic progenitor fraction. Furthermore, the emergence of $CD43^+$ hematopoietic progenitor cells and colony forming ability at day 13 was completely suppressed by inhibition of SIRT2. These results indicate that NAMPT-NAD^+-SIRT2 signaling is needed for early hematopoiesis. To evaluate downstream targets of NAMPT-NAD^+-SIRT2, we analyzed mRNA and protein expressions of well-known early hematopoietic genes (*GATA2, RUNX1, LMO2, and TAL1*) in differentiated iPS cells on day 6 and found that both of them in these factors were not down-regulated by NAMPT or SIRT2 inhibition. With the idea of post-transcriptional modifications, we tested protein-protein interaction between SIRT2 and these candidate proteins in these cells using Duolink technique. We found that RUNX1, LMO2 and TAL1 proteins interact with SIRT2 protein. To identify the target of SIRT2-triggered deacetylation, we quantified and compared the Duolink signals from total- and acetylated- candidate proteins in the cells treated or not with SIRT2 inhibitor using flow cytometry. This analysis revealed that only LMO2 is deacetylated by SIRT2. LMO2 is known as a component of TAL1 complex, which consist of LMO2, TAL1, LDB1, E47 and GATA1/2 proteins and has transcriptional activity as a complex on hematopoiesis-specific target genes. Interestingly, mRNA expression levels of all these target genes were markedly downregulated by inhibition of NAMPT or SIRT2. Next, we predicted possible acetylated lysine residue in LMO2 protein and found K74 and K78 as strong candidates. We made LMO2 protein expression constructs containing acetylation- (KQ) and deacetylation- (KR) mimic mutants at these lysine residues and compared LMO2-LDB1 interaction between WT and mutant LMO2 constructs by co-immunoprecipitation. This experiment revealed that KQ-mutant had impaired interaction with LDB1 protein in comparison to WT and KR-mutant LMO2. These results indicate that deacetylation of LMO2 protein by SIRT2 is required for the interaction between LMO2 and LDB1 proteins.
Summary/Conclusions: NAMPT-NAD^+-SIRT2 pathway plays an indispensable role in the early hematopoiesis by deacetylation of LMO2 protein which enables the interaction between LMO2 and LDB1 proteins and complete TAL1 complex formation.

P730

B LYMPHOPOIESIS IS DEPENDENT ON TRANSCRIPTION FACTOR ERG

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Background: B lymphopoiesis is a process that has been extensively studied for decades, which has led to the discovery of several transcription factors that are indispensible for the formation of B-cells.
The transcription factor ERG has previously been shown to influence hematopoietic stem cells and various cancers. However, its role in B-cell development is, thus far, largely uncharacterized.
Aims: To characterize the role of the transcription factor ERG in the development of B lymphocytes with particular emphasis on elucidating underlying transcriptional mechanisms.
Methods: We have created an *Erg*$^{fl/fl}$*:CD2iCre* mouse model, in which the

DNA-binding domain of *Erg* is deleted exclusively in the lymphoid cells rendering the transcription factor non-functional in this cell compartment.
Phenotypic characterization of the knock-out mouse was done using a combination of flow cytometry and *in vitro* differentiation assays.
To elucidate the mechanisms behind the influence of ERG on B lymphopoiesis, we have examined gene expression changes in B cell progenitors following its deletion using a microarray-based approach. For examination of changes at the chromosomal level, we are using ATAC-sequencing, which assesses chromatin accessibility.
Results: The lymphoid-specific knock-out of *Erg* leads to a dramatic loss of B-cells, which can be detected as a decline in the number of mature B-cells in the peripheral lymphoid organs. In the bone marrow, a substantial reduction in B-cell progenitors is seen already at an early progenitor stage with the cell loss becoming gradually worse throughout differentiation. These findings are recapitulated in *ex vivo* experiments.
The loss of B-cells can partly be ascribed to changes in the propensity of B cell progenitors to undergo apoptosis and proliferation.
Accordingly, gene expression analyses show a reduced expression of cell cycle-related genes in the B-cell progenitors. Furthermore, we see a deregulation of genes associated with B-cell differentiation.
On the chromosomal level, the deficiency of ERG leads to marked changes in genome-wide DNA accessibility in the B-cell progenitors.
Summary/Conclusions: We have identified ERG to be of central importance for the development of B-cells. Loss of ERG in the lymphoid cell compartment leads to a severe reduction in B-cells, which can be detected already at an early progenitor stage. The deficiency of ERG leads to changes in key cell properties, gene expression and genome-wide chromosomal accessibility.

P731

EXPRESSION OF BCL2, BUT NOT STAT5 SIGNALING, RESCUES LYMPHOPOIESIS IN THE ABSENCE OF HHEX

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Background: The transcription factor Hhex (Hematopoietically-expressed homeobox gene) is important for the development of definitive hematopoietic progenitors and B-lymphocytes during embryogenesis. We have previously shown that Hhex is essential for adult lymphoid development, with its absence resulting in a severe defect stemming from a block in Common Lymphoid Progenitor (CLP) differentiation. This block was characterised by cell cycle arrest of B cell progenitors, as well as apoptosis and defective Stat5 phosphorylation in response to IL-7.
Aims: The aim of this study is to determine the roles of apoptosis and defective Stat5 signaling in the lymphoid developmental failure caused by loss of Hhex.
Methods: To investigate the pathways mediating this developmental block we have crossed Mx-Cre inducible conditional Hhex knock-out mice with transgenic mice expressing Bcl2 or constitutively active Stat5. We employed steady-state flow cytometric cell phenotyping, competitive bone marrow transplantation and *in vitro* differentiation experiments to assess lymphoid development. In addition, B-cell function was assessed by *in vitro* proliferation assays of splenic B-cells.
Results: We found that overexpression of Bcl2 rescued B-cell development in the absence of Hhex, as well as restoring the development of T, B and natural killer (NK) lineages during *in vitro* differentiation and *in vivo* transplantation experiments. In contrast, constitutively active Stat5 failed to rescue lymphoid development *in vitro* or *in vivo* in the absence of Hhex. Further analysis of Hhex-null, Bcl2-transgenic B-cells *in vitro* demonstrated that they were capable of proliferation, indicating that these rescued B-cells were functional.
Summary/Conclusions: These results indicate that Bcl2 expression can overcome the lymphoid deficiencies observed in the absence of Hhex, suggesting that the primary role of this transcription factor is to promote survival of lymphoid progenitors during early lymphoid development.

P732

ANAMORSIN IS ESSENTIAL FOR B-CELL TERMINAL DIFFERENTIATION

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Background: Anamorsin (AM, also called CIAPIN1) is a cell death-defying factor, which we have cloned as a molecule that conferred resistance to apoptosis induced by growth factor deprivation. AM-deficient mice die during late gestation, and AM KO embryos are anemic and very small compared to wild-type embryos (J Exp Med 2004; 199: 581–592), suggesting that AM is essential for embryo growth and hematopoiesis. The embryonic lethality of AM KO mice impedes detailed analysis of the roles of AM. AM is important in Fe-S cluster protein biogenesis (BBRC 2011; 408: 329–333), which is indispensable in many biological processes, suggesting that AM may be required for critical biological pathways.
Aims: To overcome the embryonic lethality of AM KO, here we generated AM conditional KO ($AM^{flox/flox}$) mice. The Cre-loxP system enabled cell type-specific genetic modification. Since we previously reported AM overexpression in ~30% of B-cell-type malignant lymphoma, here we focused on the role of AM in B-cell development.
Methods: We generated and analyzed CD19-Cre/$AM^{flox/flox}$ mice, with AM deleted specifically in $CD19^+$B cells (from Pro-B cells to mature peripheral B cells in B-cell development). B-cell development progresses through the pro-B-cell, pre-B-cell and immature-B-cell stages in BM and then exit from BM to spleen, where they pass through transitional stages; transitional type 1 (T1) and transitional type 2 (T2), and mature to either follicular B cells or marginal zone B cells.
Results: Compared with control CD19-Cre mice, CD19-Cre/$AM^{flox/flox}$ mice exhibited fewer $B220^+$B cells in the spleen (40.8±2.6% *vs* 56.8±3.8%, $P<0.001$), peripheral blood (23.2±2.5% *vs* 51.0±4.4%, $P<0.001$), and LN (8±2.3% *vs* 31.7±1.0%, $P<0.001$). To further examine how AM deficiency impacted B-cell differentiation, BM and spleen cells were categorized into B-cell subsets—from hematopoietic stem cells to mature follicular and marginal zone B cells—based on marker expression patterns using flow cytometry. Compared to control mice, CD19-Cre/$AM^{flox/flox}$ mice showed significantly fewer follicular type I (FO-I) cells ($B220^+$, $AA4.1^-$, $sIgM^{low}$, $CD23^+$, $sIgD^+$), which are the most matured follicular B cells in the spleen (7.9±0.1% *vs* 15.1±1.0%, $P<0.01$). Immunohistochemistry using monoclonal anti-B220 antibody in formalin-fixed paraffin-embedded spleen sections showed smaller B-cell follicles in CD19-Cre/$AM^{flox/flox}$ mice. Since AM was originally isolated as an anti-apoptotic molecule, spleen cells were treated with FITC-conjugated annexin V to determine the percentage of apoptotic cells, revealing greater apoptotic cell accumulation in CD19-Cre/$AM^{flox/flox}$ mice compared to controls (30.0±4.4% *vs* 19.8±1.3%, $P<0.05$). This suggested that follicular B cell development was blocked by increased apoptosis in the CD19-Cre/$AM^{flox/flox}$ mice. Furthermore, CD19-Cre/$AM^{flox/flox}$ mice lacked splenic $CD19^+B220^{-/low}$ cells, which display a plasmablast phenotype.
Summary/Conclusions: Although AM expression was consistent throughout the differentiation stages of $CD19^+$ B cells, AM deficiency predominantly affected terminal differentiation of B cells, especially from T2 to FO-I. Immature B cells mature through transient T2 B cells into follicular or marginal zone B cells, and their follicular *versus* marginal zone fate partly depends on the strength of BCR signaling. Reduced numbers of FO-I B cells and $CD19^+B220^{-/low}$ cells in the spleen are also reported in mice with defects in Bruton's tyrosine kinase (BTK), which is activated in downstream of BCR signaling. The phenotypic similarity between Btk-deficient mice and our CD19-Cre/$AM^{flox/flox}$ mice suggests that AM may affect B-cell development through the BCR-BTK pathway. Overall, our present results show that AM plays essential roles in B-cell terminal differentiation, suggesting the potential involvement of some Fe-S cluster proteins in B-cell terminal differentiation. Moreover, our novel $AM^{flox/flox}$ mice will enable further investigation of the functions of AM and the related Fe-S cluster proteins in various organs.

P733

DOWNREGULATION OF CD44 FUNCTIONALLY DEFINES HUMAN T-CELL COMMITMENT

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Background: T-cell development in the thymus is a complex process that depends on sequential transcriptional and epigenetic events that induce T-cell lineage commitment and simultaneously suppress alternative cell fates. Human T-cell development is less well studied than its murine counterpart due to the lack of genetic tools and the difficulty of obtaining cells and tissues. However, recent technological advances have enabled the identification of the transcriptional landscape of differentiating human thymocytes. Research has focused on mimicking the thymus environment in *in vitro* T-cell differentiation cultures starting from hematopoietic stem/progenitor cells (HSCs) derived from human cord blood or bone marrow.
Aims: We aimed to compare *in vitro* T-cell differentiation at the level of gene expression with gene expression of *in vivo* murine and human early T-cell development. Recapitulation of gene expression at the correct stage of differentiation would validate this model beyond the level of surface markers that are commonly used to identify sequential differentiation stages *in vitro* and *in vivo*, and helps identify novel markers that delineate specific stages and commitment points.
Methods: We have utilized the OP9-DL1 *in vitro* co-culture system to gradually differentiate $CD34^+$ HSCs from umbilical cord blood into the T-cell lineage. HSCs in this co-culture will recapitulate *in vivo* T-cell development as measured by incremental acquisition of surface markers CD7, CD5, CD1a, CD4, and CD8. We investigated and compared gene expression profiles of 11 human T-cell differentiation subsets collected by FACS sorting at 5 different time-points.

We determined the TCRβ rearrangement status and functionally defined T-cell commitment of several human early thymocyte subsets.
Results: The changes in gene expression of subsequent early T-cell developmental stages closely resemble those of human and murine *in vivo* thymocyte subsets, validating the OP9-DL1 co-culture as a true representative of *in vivo* human T-cell development. These analyses let us to define a gene signature that distinguishes pre- and post- αβ T-cell commitment. We searched for various candidate surface markers that could pinpoint the exact transition from pre- to post-T-cell committed human thymocytes; both in human thymocytes *in vivo* as well as in differentiating cells *in vitro*, loss of CD44 marks T-cell commitment in early CD7+CD5+ cells, before the acquisition of CD1a surface expression. The CD44-CD1a- post-committed thymocytes have initiated in frame TCR rearrangements and have completely lost the capacity to develop into myeloid, B- and NK-cells, unlike uncommitted CD44+CD1a- thymocytes.
Summary/Conclusions: The gene expression profiles of 11 human *in vitro* T-cell differentiation subsets closely resemble those of early T-cell development of murine and human thymocytes. Therefore, the OP9-DL1 co-culture closely mimics *in vivo* T-cell development. Moreover, this model has enabled us to pinpoint human αβ T-cell commitment using CD44. In conclusion, loss of CD44 represents a previously unrecognized stage that defines the earliest committed T-cell population in the human thymus.

P734

KNOCKDOWN OF THE ADHESION G-PROTEIN COUPLED RECEPTOR 56 IMPAIRS HUMAN CORD BLOOD STEM AND PROGENITOR CELL FUNCTION

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Background: Knowledge about the differences and similarities between normal hematopoietic stem and progenitor cells (HSPCs) and their malignant counterparts is an essential prerequisite for the development of novel therapeutic approaches for the treatment of hematologic diseases such as acute myeloid leukemia (AML). We recently identified the adhesion G-protein coupled receptor 56 (GPR56) as novel leukemia stem cell marker in AML. While GPR56 has been attributed a role in homing and endothelial to hematopoietic stem cell transition in mice and zebrafish its functional role in human primary HSPCs has not been fully characterized yet.
Aims: Here we set out to determine the functional role of GPR56 expression for normal HSPC function in the human hematopoietic system.
Methods: We knocked down GPR56 via lentiviral shRNA vectors in cord blood (CB) CD34+ cells. Gene knockdown was confirmed by FACS analysis upon lentiviral transduction prior to subjecting the cells to *in vitro* and *in vivo* assays. GFP+ cells were sorted into methylcellulose and colony formation potential was determined after 12 days. Furthermore, cells were injected in NSG mice and bone marrow was analyzed for total and lineage specific human engraftment at 4, 8, and 20 weeks post transplantation. RNA-Sequencing (RNA-Seq) was performed in CB CD34+ cells 3 days post infection to determine differences in gene expression.
Results: We observed a rapid decrease in the fraction of GFP+ cells infected with two different shRNA vectors against GPR56, but not when infected with shLuciferase control vector. Furthermore, colony formation capacity of CD34+ cord blood cells was severely impaired upon knockdown (KD) of GPR56 with greater reduction in the more immature GEMM and BFU-E colonies compared to G- and M-colonies. Moreover, we found that the repopulating potential of CB CD34+ cells was significantly impaired in cells with near complete KD of GPR56. Accordingly, we observed an inverse correlation between the level of GPR56 KD and contribution of GFP+ cells to engraftment. In addition, we observed significant differences in the lympho-myeloid-lineage-ratio to the disadvantage of the lymphoid lineage when harvesting human cells from mouse bone marrow 20 weeks post transplantation of GPR56 KD HSCs compared to controls. RNA-Seq of CB CD34+ cells upon GPR56 KD *vs* controls revealed enrichment for several GO terms connected to the regulation of hematopoiesis, apoptosis, and survival.
Summary/Conclusions: Together, our findings indicate that GPR56 is not only involved in survival and proliferation, but also in the maintenance of the multipotent undifferentiated state of human HSPCs.

P735

DISSECTING THE ROLE OF THE MICROENVIRONMENT IN APLASTIC ANEMIA USING AN *IN VIVO* HAEMATOPOIETIC STEM CELL NICHE MODEL

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Background: Aplastic anemia (AA) is a bone marrow failure disorder in which the microenvironment seems to play a role.
Mesenchymal stromal cells (MSC) have been investigated in AA for their essential function in bone marrow microenvironment. However, there are limited and controversial *in vitro* studies characterizing patient-derived MSC. Therefore, *in vivo* models would be tremendously useful to better understand the effective role of the stromal compartment in AA niche. Indeed, the establishment of simplified haematopoietic stem cell niche models potentially allows to recapitulate haematopoietic and stromal defects and to dissect the functional role of different cell populations involved in the development of specific diseases.
Aims: Using our recently described *in vivo* model which accurately mimics the miniature of a bone/bone marrow organ, we aim to investigate the role of MSC in AA reproducing pathological microenvironment and testing their ability to support haematopoiesis (Serafini *et al.*, 2014).
Methods: We isolated MSC from 7 newly diagnosed AA pediatric patients (AA-MSC) and 7 age-matched healthy donors (HD-MSC), after informed consent according to institutionally approved protocols. MSC cultures were characterized by evaluating clonogenicity, cell growth, immunophenotype, and differentiation potential *in vitro*. Additionally, we analyzed immunohistologically and functionally the haematopoietic and stromal compartment of the ossicles obtained after the *in vivo* implant of chondrogenic pellets derived from MSC of both groups.
Results: AA-MSC displayed morphology, phenotypic profile, proliferation and *in vitro* differentiation capacities similar to their normal counterparts, albeit exhibited a lower colony forming efficiency. Moreover, we showed that AA-MSC maintain the capacity to establish *in vivo* an ossicle constituted by cortical bone filled with normal host-derived haematopoiesis. Immunohistochemistry analysis of the haematopoietic tissue in the intertrabecular space within the ossicles revealed the presence of murine macrophages, myeloid cells, megakaryocytes, red blood cells and osteoclasts in similar proportion in normal and patient-derived sections. Moreover, within the AA ossicle-derived marrows, it was possible to determine the presence of haematopoietic clonogenic progenitors and quantify the same number of cells belonging to the erythroid, myeloid and megakaryocytic lineages as their normal counterparts. Also the human stromal compartment of the ossicles originated from AA-MSC and HD-MSC resulted similar. A comparable amount and disposition of adipose marrow was observed in AA ossicles and in controls. Ossicles derived from both sources of MSC preparations similarly presented osteoblasts lining the osseous trabeculae, bone marrow interstitial fibrosis, reticulin deposition and iron storages.
Summary/Conclusions: This is the first study which addresses the *in vivo* ability of AA-MSC to support haematopoiesis. Using our *in vivo* model, we have been able to demonstrate that AA-MSC generate normal ossicles characterised by cortical bone, marrow cavity, donor-derived marrow stroma, and host-derived haematopoietic tissue. Further studies are needed to expand this investigation to a larger cohort of patients, in order to understand if these observations could be confirmed in MSC preparations derived from AA patients of different age and/or disease classification. Moreover, this work demonstrates that our experimental system could be suitable to model *in vivo* haemopoietic diseases and paves the way for studies on AA and other disorders.

P736

A HIGH RESOLUTION GENETIC ATLAS OF BLOOD CELL VARIATION AND FUNCTION IN HUMANS

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Background: Human blood production releases around 10^{11} cells per day into circulation. These originate from a minute population of haematopoietic stem cells, which differentiate towards mature cells by the clonal expansion of progenitor cells, following lineages of increasingly restricted fate. The molecules and pathways involved in lineage choice have been studied in great detail, but our understanding of the networks of proteins controlling cell fating is partial.
Aims: We sought to identify genetic variants controlling iron metabolism, haemotopoeisis, blood cell differentiation and blood cell clearance by a hypothesis free genomewide study; the largest population association analysis of blood cell traits to date.
Methods: We studied 36 haematological traits (measuring the properties of reticulocytes, red cells, platelets and the white cell subtypes) derived from the full blood counts of 173,480 individuals of European ancestry, selected from the

UK Biobank and INTERVAL cohorts. We analysed 29.5 million genetic variants (many of which were rare, with minor allele frequencies as low as 0.01%), imputed from genotypes measured using the UK Biobank Affymetrix Axiom array.
Results: We identified 182,105 variants significantly associated with at least one trait and could explain these associations using 1,652 distinct sentinel variants, 148 of which corresponded to previously reported GWAS hits, validating almost all the blood trait associations known for European populations. The associations of the remaining 1,504 sentinel variants were novel, augmenting the number of known associations by over 10-fold. Remarkably, we identified 270 rare and 258 low-frequency variants (allele frequency <1% and 1-5% respectively), and 44 "high impact" variants with additive allelic effects of more than 0.5 phenotypic standard deviations.
Summary/Conclusions: Almost three quarters of the associated variants are in the non-coding part of the genome with a striking enrichment in DNAse-I hypersensitivity sites with an enhancer signature. Gene sets containing associations were enriched in haematological pathways, genes implicated in rare blood and immune disorders, and genes predisposing to human complex disease, confirming the importance of our results for the understanding of haematopoietic processes and pathogenesis.

P737

EFFICIENT GENOME EDITING OF MOUSE HEMATOPOIETIC CELLS USING CRISPR/CAS9 TECHNOLOGY
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Background: A number of techniques have been used to study hematopoietic malignancies including viral overexpression of human oncogenes, RNA interference (RNAi) and transgenic animals. Each of these have their caveats for cell specific targeting, particularly RNAi approaches that are characterised by incomplete genetic inactivation without changing the genetic code. Genome editing technologies, including clustered regularly-interspaced short palindromic repeats (CRISPR), have been used to stably modulate the genome of several model organisms. The genome engineering of hematopoietic cells is complicated mainly due to the low efficiency of the non-viral delivery methods.
Aims: To generate efficient and stable genome edited murine hematopoietic models using CRISPR/Cas9 technology.
Methods: Two different guideRNAs (gRNAs) targeting either Mcm6 (gRNA5 and 8) or Sirt1 (gRNA3 and 9) were designed and cloned into the pLCRISPR.EFS.GFP lentiviral vector. These genes were chosen as they are involved in hematopoietic progenitor and stem cell (HSPC) properties. GFP$^+$ 32D Cl3 mouse hematopoietic cells were FACS sorted at day 5 post-transduction. Knockout efficiency was confirmed by both western blotting and qRT-PCR. Functional analysis was performed by the SURVEYOR assay and Sanger sequencing. Murine HSPCs were isolated from 5FU treated C57BL/6 mice (CD45.2+), transduced with the most efficient gRNA for either Sirt1 or Mcm6 and injected into B6.SJL mice. Engraftment was measured by FACS analysis of blood sampling at 4 weeks post-transplant.
Results: CRISPR/Cas9 efficiency was first tested on the mouse 32D cell line *in vitro*. Decreased protein and mRNA levels of Sirt1 were detected in 32D GFP$^+$ bulk population transduced with gRNA9 (32D+gRNA9) at day 5 which was not evident with gRNA3. SURVEYOR assay revealed that Cas9-mediated cleavage efficiency was higher in 32D+gRNA9 cells (16.3% indels) compared to 32D+gRNA3 cells (12.5% indels). 32D+gRNA9 cells were further expanded and FACS sorted for single clones. 5/20 clones showed complete absence of Sirt1 at the protein level (25% of Cas9 efficiency). Efficient clones were further used for genotype analysis by Sanger sequencing to confirm the type of indels. On the other hand, we saw complete absence of Mcm6 protein expression in 32D GFP$^+$ bulk cells transduced with gRNA5 or gRNA8, and >2-4-fold mRNA reduction was observed when transduced with gRNA5 and 8, respectively. Our CRISPR/Cas9 system was further tested *in vivo* using primary cells. Murine CD45.2$^+$ HSPCs were isolated, transduced with equal viral titre of empty vector, Mcm6-gRNA8 or Sirt1-gRNA9 and transplanted into CD45.1$^+$ mice. Blood sampling revealed high levels of engraftment as determined by CD45.2% and CRISPR transduction efficiency determined by the GFP levels at 4 weeks post-transplant. Our results show that CD45.2$^+$GFP$^+$ cells engrafted at 4 weeks showing effective CRISPR/Cas9 mediated transduction and transplantation of Mcm6 and Sirt1 knockout primary HSPCs.
Summary/Conclusions: In this study we successfully generated *in vitro* and *in vivo* CRISPR/Cas9 models for efficient genome editing on hematopoietic cells. Our findings demonstrate a complete gene knockout *in vitro* but with variable Cas9-mediated cleavage which was further confirmed by our clonal analysis. This suggests that Cas9 function might depend on both the targeted gene and/or the gRNA. Also, transduction of HSPCs was sufficient to ensure engraftment of GFP$^+$ cells. Long-term serial analysis will be further conducted to ensure stable expression and test Cas9-mediated cleavage *in vivo*.

Red blood cells and iron - Biology

P738

COMPREHENSIVE PROTEOMIC ANALYSIS OF HUMAN ERYTHROPOIESIS
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Background: Erythropoiesis is a complex process starting from pluripotent medullary progenitors and leading to the production of enucleated erythrocytes. Several transcriptomic analyses of erythroid differentiation have been published to better understand the molecular mechanisms of erythropoiesis. However, it is now clearly established that the proteome cannot simply be deduced from the transcriptome and that post transcriptomic modifications are responsible for a large part of the proteome variations. Consequently, a direct proteomic analysis of the erythroid differentiation is required to accurately assess the modifications that occur during this process.
Aims: Our goal was to provide a deep analysis of the proteome evolution of human erythroid cells during their differentiation, from the BFU-E up to the orthochromatic erythroblast, and a quantitative analysis of the repartition of the proteins between the pyrenocyte and the reticulocyte after enucleation. The absolute quantification of proteins will be determined rather than their relative evolution all along the differentiation process.
Methods: We used CD34$^+$ cord blood progenitors and an optimized cell culture method allowing the production of highly synchronized cell populations of erythroid cells at various differentiation stages. Cellular populations from erythroid progenitors up to reticulocytes were submitted to mass spectrometry label-free analysis. To analyze the repartition of proteins after enucleation, pyrenocytes and reticulocytes were sorted by FACS. Equal numbers of reticulocytes and pyrenocytes were analyzed by mass spectrometry after iTRAQ labelling.
Results: Our analysis led to the absolute quantification, as protein copy number per cell, of more than 6100 proteins involved in many various biological process throughout the erythroid differentiation, with a false discovery rate of less than 1%. The range of quantified protein expression extended to around 7 logs. Absolute quantification was validated by several ways including the comparison of calculated values with those already published for several proteins and the classical differentiation markers expression pattern. All erythroid transcription factors were quantified. A comparison between mRNA and protein expression showed only a modest correlation as usual. Interestingly, this analysis revealed proteins with unexpected expression in erythroid differentiation such as optineurin, an autophagy inducer, or phospho1, a phosphatase involved in bone mineralization, which increased along differentiation, or leptin receptor, known to activate JAK2 and STAT5, which peaked at the proE/BasoI stage. Furthermore, the absolute quantification allows the calculation of protein ratio of different complexes. Regarding to segregation of proteins between reticulocytes and pyrenocytes, our study allowed the quantitative repartition of 1300 proteins. Flow cytometry imaging was used to calculate the cell surface area and the cytoplasmic volumes of pyrenocytes and reticulocytes allowing to determine whether a protein segregates passively or whether it is actively sorted. This method revealed proteins with unexpected localization and the active segregation of several proteins including most erythrocyte-specific membrane proteins which actively segregate with reticulocytes.
Summary/Conclusions: All these results significantly increase our knowledge of the protein expression pattern during erythropoiesis and should constitute a valuable data base for subsequent studies regarding both physiological and disordered erythropoiesis. A similar analysis is currently ongoing on murine erythroid differentiation to compare human and murine erythroid proteomes.

P739

AN IMBALANCE BETWEEN PLASMA ANNEXIN-A5 AND PHOSPHATIDYLSERINE EXPRESSION IN ERYTHROCYTES PROMOTES VASCULAR INJURY DURING SICKLE CELL DISEASE
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Background: Chronic hemolytic anemia, including Sickle Cell Disease (SCD) is characterized by painful vaso-occlusive crises (VOC), vascular injury, red blood

cell (RBC) aggregation and vesiculation, and intravascular hemolysis. At steady state, RBC release hemoglobin, heme and microparticles (MP) in plasma. This increases again during VOC. Stressed RBC and the MP they release express phosphatidylserine (PS) at their surface and contain cytotoxic heme, with deleterious impact on endothelial cells and vascular function. On the other hand, annexin-A5 is an intracellular protein that associates with membranes during cell stress and can be found in plasma. Annexin-A5 acts as a PS inhibitor, neutralizing PS-mediated effects of stressed cells and MP, and is generally thought to be an anti-inflammatory and anti-thrombotic protective agent.

Aims: We aimed to determine whether PS expression in red blood cells and MP in SCD is counterbalanced by annexin binding, and whether annexin-A5 in particular is modulated during vaso-occlusions. More specifically, we wanted to assess whether the plasma levels of annexin-A5 were all consumed during vaso-occlusions, or whether some annexin-A5 remained bioavailable for new interactions.

Methods: 1) We collected plasma and erythrocytes from a cohort of SCD patients during steady state or VOC, and matched controls. We studied plasma MP, plasma annexin-A5 levels and erythrocyte and MP expression of PS. 2) We designed a novel ELISA-based assay to capture PS+ MP with an anti-annexin-A5 antibody. It quantified annexin-A5-covered MP in plasma, but also estimated ligand-free functional annexin-A5. We characterized plasma MP by FACS with labeling of ligand-free PS with annexin-A5 and anti-CD235a, and size by NTA (50-1500 nm). 3) We tested recombinant annexin-A5 to block ROS production induced by SCD MP in cultured endothelial cells. 4) We used a model of hypoxia-induced vaso-occlusion in transgenic mice with SCD and tested PS neutralization *in vivo* with recombinant annexin-A5.

Results: In controls, RBC displayed little labeling with exogenous annexin-A5 by FACS. ELISA revealed that MP were few and covered with endogenous annexin-A5. Moreover, some annexin-A5 remained bioavailable in plasma. In SCD, plasma levels of annexin-A5 were increased, and MP levels were increased significantly, materializing hemolysis as expected. Surprisingly, SCD RBC and plasma MP all showed increased PS expression, as mean fluorescence intensities (MFI) by FACS. Hence, SCD RBC and MP bore more 'free' PS at their surface, available to exogenous annexin-A5 binding. In acute stage VOC, MP levels were increased even further and bore cytotoxic heme. RBC PS externalization increased even further in intensity, and we still found virtually no ligand-free annexin-A5 in plasma. *In vitro*, recombinant annexin-A5 inhibited the pro-oxidant effects of SCD MP on cultured endothelial cells. *In vivo*, recombinant annexin-A5 released vaso-occlusions triggered by hypoxia in transgenic mice with SCD, characterized by echo-Doppler measurements of renal perfusion.

Summary/Conclusions: We concluded that the equilibrium between PS expression and annexin-A5 is compromised in SCD. Endogenous plasma anenxin-A5 appears to be entirely consumed by excess PS externalization, unable to quench new bouts of PS expression during hemolysis, and insufficient to neutralize the high levels of PS+ MP produced by stressed RBC. In SCD, the therapeutic use of recombinant annexin-A5 may thus help compensate the imbalance between PS+ MP and annexin-A5.

P740

STAPHYLOCOCCUS AUREUS PROTEIN ISDH INHIBITS THE CD163 PATHWAY FOR HAPTOGLOBIN-FACILITATED HEMOGLOBIN SCAVENGING

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Background: Hemolysis induced by bacterial α-toxin is a common complication in septic infections with *Staphylococcus aureus* and other bacteria, which utilize heme as a source for exogenous iron. The *S. aureus* uptake of heme occurs by a sophisticated heme-extraction mechanism that involves proteins from the iron-regulated surface determinant (Isd) system. Sequestration of heme from the hemoglobin-haptoglobin (Hb-Hp) complex is initiated by binding of Hb-Hp to the Isd protein H (IsdH). IsdH contains three the near-iron transporter (NEAT) domains; N1 and N2 anchors to hemoglobin, while the third NEAT domain N3 extracts heme. Normally, Hb-Hp is rapidly scavenged by the macrophage receptor CD163. However, since IsdH bind Hb-Hp close to the binding site of CD163, IsdH can potentially interfere with the uptake.

Aims: The objective of this study was to test the effects of recombinant IsdH on Hb-Hp binding to CD163 and cellular uptake.

Methods: To study effects of IsdH, recombinantly expressed full-length $IsdH^{N1N2N3}$ and two truncated variants of $IsdH^{N1}$ and $IsdH^{N2N3}$ were used. Endocytotic uptake of fluorescently labelled Hb-Hp by CD163-expressing Chinese hamster ovary (CHO) cells in presence of IsdH was evaluated by confocal microscopy and image-cytometry. Binding kinetics of IsdH was further characterized by SPR analysis.

Results: Our present data show that the high-affinity binding of IsdH to Hb-Hp strongly inhibits concomitant binding to and uptake by the human macrophage receptor CD163. Full-length $IsdH^{N1N2N3}$ showed the strongest inhibition of Hb-Hp uptake in CD163 expressing CHO cells and displayed the highest overall affinity for binding to Hb-Hp compared to the truncated versions. The single domain, $IsdH^{N1}$, which is not directly involved in the heme extraction, was also a potent inhibitor of both CD163 binding and endocytosis of Hb-Hp (Figure 1).

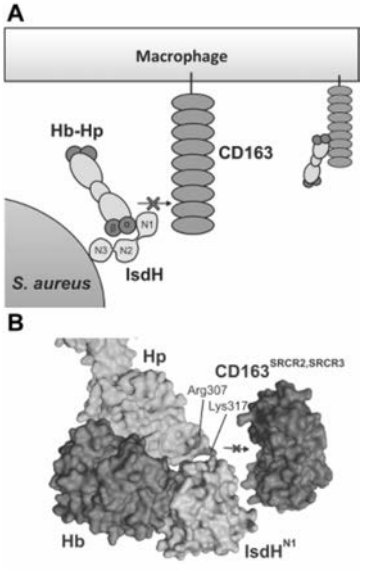

Figure 1.

Summary/Conclusions: Inhibition of the CD163 uptake of Hb-Hp could benefit the bacterium by keeping hemoglobin accessible a source of iron. Furthermore, dampening of the CD163-mediated hemoglobin degradation pathway, which converts the pro-inflammatory hemoglobin to anti-inflammatory metabolites, may further spark a septic inflammation supporting bacterial growth. Finally, it may skew the diagnostic negative correlation between hemolytic activity and the plasma concentration of haptoglobin. We suggest that inhibition of CD163-mediated Hb-Hp uptake is an additional function of IsdH.

P741

BODY IRON STATUS HAS A LARGER IMPACT ON UTILIZATION EFFICIENCY OF DIETARY IRON FOR ERYTHROPOIESIS THAN ERYTHROPOIETIC STIMULATION

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Background: In a previous study, we demonstrated that dietary iron uptake and mobilization of stored iron are both up-regulated through suppression of serum hepcidin levels during erythropoietic stimulation by administration of Epoetin beta pegol (C.E.R.A.), a long-acting erythropoiesis-stimulating agent. It was also demonstrated that up-regulation of ferroportin (FPN) in reticuloendothelial macrophages and up-regulation of divalent metal transporter 1 (DMT1) and FPN in enterocytes are followed by hepcidin suppression. To determine the contribution of dietary iron for erythropoiesis under iron intervening conditions and erythropoietic stimulation, we labeled dietary iron with stable iron isotope ^{57}Fe and assessed it by inductively coupled plasma mass spectrometry (ICP-MS). By using them, we demonstrated that the utilization of dietary iron for erythropoiesis was increased under erythropoietic stimulation and decreased under iron loading. However, it is not elucidated which intervention has stronger impact on the utilization efficiency of dietary iron for erythropoiesis.

Aims: In this study, we compared the impact of body iron status and erythropoietic stimulation on the utilization efficiency of dietary iron for erythropoiesis.

Methods: To assess dietary iron-derived hemoglobin synthesis, a diet containing 200 ppm of ^{57}Fe instead of natural iron (^{57}Fe-diet) was used. A diet containing 200 ppm of natural iron (native Fe diet) was used as a control. C57BL/6NCrl mice were fed the native Fe diet and were intravenously administered 0.2 or 0.4 mg/mouse of iron-dextran (iron-loading condition) or dextran (control). Five days after iron loading, the diet was switched to the ^{57}Fe-diet immediately after intravenous injection of 2 or 10 μg/kg of C.E.R.A. or vehicle. On 10 days after C.E.R.A. treatment, mice were euthanized by exsanguination under anesthesia with isoflurane, and hemoglobin levels were measured. To quantify dietary iron-derived hemoglobin synthesis, the content of hemoglobin containing ^{57}Fe (^{57}Fe-hemoglobin) was measured by ICP-MS. The utilization efficiency of dietary iron for erythropoiesis was assessed as a ratio of ^{57}Fe-hemoglobin to hemoglobin levels.

Results: On 10 days after C.E.R.A. administration, hemoglobin levels were increased in C.E.R.A. 10 μg/kg-treated groups compared with vehicle-treated groups, as well as ^{57}Fe-hemoglobin levels. On the other hand, iron-loading did not affect hemoglobin levels. However, ^{57}Fe-hemoglobin levels were decreased in the iron-dextran 0.4 mg/mouse-treated groups as well as in the

iron-dextran 0.2 mg/mouse-treated groups under steady-state erythropoiesis. The utilization efficiency of dietary iron for erythropoiesis was significantly lowered in iron-dextran 0.4 mg/mouse-treated groups compared with dextran-treated group, whereas the utilization efficiency of dietary iron for erythropoiesis was not significantly different between vehicle- and C.E.R.A.-treated groups.
Summary/Conclusions: In the previous study, we demonstrated that both erythropoietic stimulation and iron intervention influenced the dietary iron utilization for erythropoiesis. Furthermore, in the present study, we demonstrated that iron intervention, in spite of mild iron loading conditions which did not affect the hemoglobin levels, reduced the utilization efficiency of dietary iron for erythropoiesis and it had larger effect than erythropoietic stimulation. It suggested that iron loading could potentially drop the utilization efficiency of dietary iron for erythropoiesis and therefore it is important to optimize the iron replacement therapy for renal anemia patients.

P742

AG-519 IS A POTENT ACTIVATOR OF MUTANT PYRUVATE KINASE ASSOCIATED WITH HEMOLYTIC ANEMIA

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Background: Pyruvate kinase (PK) deficiency is an autosomal recessive enzymopathy that is the most common cause of hereditary nonspherocytic hemolytic anemia. PK deficiency is a rare disease characterized by a life-long chronic hemolysis with severe co-morbidities. It is hypothesized that mutations in PK result in insufficient energy production, in the form of adenosine triphosphate (ATP), to maintain red cell membrane homeostasis, leading to chronic hemolysis. Treatment is generally palliative, focusing on the resultant anemia, and there are no approved drugs that directly target mutated PK. AG-519 is an orally available, allosteric activator of the red cell isoform of PK (PK-R). AG-519 is currently in a randomized, double-blind, phase 1 study in healthy volunteers (NCT02630927), with the objective of identifying a safe and pharmacodynamically active dose and schedule to be used in subsequent clinical studies in subjects with PK deficiency.
Aims: We describe the mechanism of action and cellular effects of AG-519 in *in vitro* and *ex vivo* settings on mutant PK-R proteins associated with PK deficiency.
Methods: Mutant PK-R enzymes were expressed in E. coli and the kinetic parameters of the purified enzymes were evaluated in the presence or absence of AG-519. For thermostability studies, mutant enzymes were pre-incubated with control or AG-519 and then subjected to elevated temperature (53°C) followed by assessment of residual activity over time. Peripheral blood was obtained from patients with PK deficiency and the red cells were incubated with AG-519 for up to 24 h, followed by assessment of PK-R activity and ATP levels.
Results: We demonstrate that AG-519 can potently activate a spectrum of recombinantly-expressed PK-R mutant proteins associated with PK deficiency. All tested mutant enzymes exhibited at least 2-fold activation compared to baseline. The binding of AG-519 attenuated the thermostability defect of several mutant alleles of PK-R, including the commonly observed R510Q mutant, which had a half-life of ~2% of that of wild-type PK-R when incubated at 53°C. Pre-incubation of the R510Q protein with AG-519 restored the half-life to that of the wild-type enzyme. PK-deficient red cells are characterized by changes in metabolism associated with defective glycolysis, including a deficiency in ATP. PK-deficient red cells from several patients with distinct compound heterozygous PK-R mutations exposed to AG-519 *ex vivo* had increased PK-R enzyme activity (up to 4-fold over control) and increased ATP levels (up to 100% over control). These experiments demonstrate that AG-519 can effectively activate mutant PK-R within patient red cells. In these *ex vivo* settings, ATP levels in AG-519-treated cells can reach levels that are typical of normal, non-PK-deficient red cells (Figure 1).

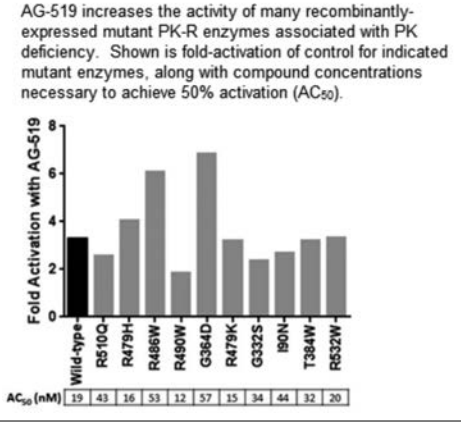

Figure 1.

Summary/Conclusions: These data support the hypothesis that drug intervention with AG-519 may restore glycolytic pathway activity and normalize red cell metabolism *in vivo*. This therapeutic approach may be an effective way to correct the underlying pathology of PK deficiency and, importantly, provide clinical benefit to patients.

P743

REDPLEX: A TARGETED NEXT GENERATION SEQUENCING-BASED DIAGNOSIS FOR PATIENTS WITH HEREDITARY HEMOLYTIC ANEMIAS

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Background: Mutations in more than 30 genes cause hereditary hemolytic anemias (HHA), a highly heterogeneous group of rare anemias characterized by complex and often unexplained genotype-phenotype correlations. In the group of HHA are included: (1) hyporegenerative anemias (HA), as congenital dyserythropoietic anemias (CDA); (2) hemolytic anemias due to red cell membrane defects (HAMD), as hereditary spherocytosis (HS) and hereditary stomatocytosis (HST). Although the workflow to diagnose these conditions is a normal clinical practice, differential diagnosis, classification, and patient stratification among HHA are often very difficult. Indeed, the variety of unspecific and overlapping phenotypes often hampers a correct clinical management of the patients. Beyond achieving a definitive diagnosis, knowing the genetic basis of these patients can be valuable also for guiding treatment.
Next generation sequencing (NGS) refers to non-Sanger-based high-throughput DNA sequencing technologies. This technology plays a major role either in disease gene discovery or in clinical use for establishing a genetic diagnosis. Particularly, the major current application of NGS in diagnostics is through targeted (t)-NGS, in which a selected fraction of genes is sequenced.
Aims: The main aim of our study is the development of a fast, accurate, reliable and cost effective diagnostic tool for HHA based on t-NGS.
Methods: In order to assess the reliability of this diagnostic method we created a t-NGS gene panel, named RedPlex, composed by 34 *loci* causative or candidates of HHA. *In silico* design of RedPlex was performed by Agilent Sure Design web tool. For each *locus*, all predicted exons and 100 flanking nucleotides were always included in the electronic design. Sequence length was set at 150×2 nucleotides, and the predicted target size amounted to 538 regions (239.764 kb). Targeted enrichment was performed on 44 patients by HaloPlex Target Enrichment System (Agilent Technologies). High-throughput sequencing was performed by Illumina NextSeq 500. SureCall software (Agilent Technologies) was used for bioinformatic and computational analyses. All focused variants were confirmed by Sanger sequencing and by the analysis of inheritance pattern.
Results: RedPlex panel showed high sensitivity and specificity. It was able to capture at least 99.4% of 538 exons with high and uniform coverage. Indeed, approximately 97.0% and 90.0% of regions were covered by at least 100 and 200 reads, respectively.
Several non-synonymous variants of unknown significance were identified in the largest genes. However, we were able to obtain a conclusive diagnosis in 32 out 44 patients with ambiguous phenotypes; instead, 12 patients remained undiagnosed. We also identified some patients showing multiple disease-associated variants suggesting complex inheritance.
Summary/Conclusions: NGS studies have identified a greater-than-expected number of genetic variations in the human genome. This suggested that existing clinical monogenic testing systematically can miss very relevant information. Our analyses demonstrated that RedPlex represents a reliable diagnostic tool for HHA patients. Indeed, it resulted to be a robust platform that overcomes for power, costs, speed, sensitivity and specificity the gene-by-gene strategy. Moreover, this approach also allowed the identification of "polygenic" conditions, *i.e.* patients in which the phenotypic variability could be explained by the presence of modifier variants associated to causative mutations.

P744

SOLUBLE FAS AND FAS LEGEND LEVELS IN YOUNG PATIENTS WITH SICKLE CELL DISEASE: ROLE IN NEPHROPATHY AND CARDIOPULMONARY COMPLICATIONS

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Background: Sickle cell disease (SCD) is characterized by chronic inflammation due to ischemic tissue damage, accentuated during acute complications. Fas and its ligand (FasL) are members of tumor necrosis factor receptor superfamily and a major pathway for induction of apoptosis. Fas/FasL interactions may be related to augmentation of inflammatory response.
Aims: We assessed the levels of sFas and sFasL in 35 children and adolescents with SCD compared with 35 healthy controls in relation to hemolysis, iron overload, sickle vasculopathy including kidney disease.
Methods: SCD patients, in steady state, asymptomatic for heart disease and pulmonary hypertension were studied. All patients were subjected to detailed

medical history and thorough clinical examination with special emphasis on disease duration, history of sickling crisis, acute chest syndrome, stroke, evidence of pulmonary or cardiac disease, spleen status, transfusion hsitory and hydroxyurea/chelation therapy. Serum ferritin, urinary albumin creatinine ratio (UACR), high-sensitivity C-reactive protein (hs-CRP) and soluble Fas/FasL (sFas/FasL) levels were assessed. Screening for pulmonary hypertension and cardiovascular abnormalities was performed by the noninvasive echocardiograpghy to evaluate left ventricular function, pulmonary artery pressure, tricuspid regurgitant jet velocity (TRV). A TRV ≥2.5 m/s was used as a proxy for patients at risk for pulmonary hypertension.
Results: A total of 20% of patients were splenectomized, 31.4% had pulmonary hypertension and 14.3% had heart disease. Nephropathy was found in 54.3% of patients sFas and sFas/sFasL ratio were significantly higher in SCD patients compared with the control group (p<0.001) while sFasL was significantly lower in patients (p=0.022). sFas/sFasL ratio was significantly higher in patients compared with controls and in patients with pulmonary hypertension, nephropathy and those who had history of frequent sickling crisis or serum ferritin ≥2500 (p<0.001). SCD patients treated with hydroxyurea had lower sFas/sFasL ratio than untreated patients. sFas/sFasL ratio was positively correlated to transfusion index, white blood cells, hs-CRP, serum ferritin and UACR. Multiple linear regression analysis showed that WBCs and serum ferritin (p<0.001) were independently related to sFas/sFasL ratio in patients with SCD. The cutoff value of sFas/sFasL at 8.75pg/mL could differentiate SCD patients with and without nephropathy while the cutoff value at 22pg/mL could differentiate SCD patients with and without pulmonary hypertension risk with high sensitivity and specificity.
Summary/Conclusions: sFas/sFasL ratio may be considered as a marker for vascular dysfunction in SCD patients and is related to inflammation, iron overload and albuminuria level. Thus, it may be a reliable method to assess renal impairment in SCD. Our findings could be of pathophysiological importance, because they provide evidence that diminishing inflammation in general, and perhaps the levels of sFas in particular, may have a role to play in altering SCD related-vasculopathy.

P745

ELEVATED PLASMA ASYMMETRIC DIMETHYLARGININE LEVELS IN CHILDREN WITH BETA-THALASSEMIA MAJOR MAY BE AN EARLY MARKER FOR ENDOTHELIAL DYSFUNCTION

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Background: Thalassemia, resulting from defects in the production of alpha- and beta-globin chains of hemoglobin, is the most common form of inherited anemia all over the world, *as well as in Turkey*. Beta-thalassemia major requires regular blood transfusions to maintain an adequate supply of hemoglobin and sustain life, and patients suffer from the long-term consequences of iron overload which may lead to various complications, including damage of parenchymal organs and cardiovascular system. *In vitro* studies have revealed that iron-loading has been linked with endothelial dysfunction due to peroxidative tissue injury resulting from blood transfusions, circulating cholesterol oxidation products, and possibly coronary artery disease. Asymmetric dimethylarginine (ADMA) is an early marker for endothelial dysfunction and also regarded as an independent predictor of future cardiovascular events. In addition, endothelial adhesion molecules [soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and P-selectin] are also considered as early endothelial dysfunction markers, and there is scarce data that highlights the significance and role of these molecules in endothelial dysfunction development in pediatric beta-thalassemia major patients.
Aims: Based on all these informations, in the present study we aimed to assess circulating levels of ADMA between patients with beta-thalassemia major and control group and determine its correlation with markers of endothelial adhesion molecules (sICAM-1, sVCAM-1 and P-selectin), and Pentraxin-3 as prognostic factors for vascular risk stratification and subclinical atherosclerosis.
Methods: A total of 31 children with beta-thalassemia major aged between 4-16 year old (study group) and 36 age and gender matched healthy controls were enrolled in the study. Fasting blood samples were obtained from the antecubital vein. The samples for the measurement of sICAM-1, s-VCAM-1, P-selectin, Pentraxin-3 and ADMA were centrifuged for 15 min at 2000 g, aliquoted, and immediately frozen at -80 ºC until analysis. Plasma ADMA was measured along with soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), P-selectin, and Pentraxin-3. Circulating levels of sICAM-1, s-VCAM-1, P-selectin, Pentraxin-3 and ADMA were determined with commercially available ELISA kits (ADMA were by Immunodiagnostic Systems, Boldon, UK; Pentraxin-3 was by R&D Systems, USA; sICAM-1, s-VCAM-1 and P-selectin were by eBioscience, San Diego, CA, USA).
Results: Age, gender and body mass index were similar in two groups. The beta-thalassemia major patients had significantly higher serum ferritin, sICAM-1, s-VCAM-1 and ADMA levels than controls (p <0.001 for ferritin, s-VCAM-1, ADMA; and p =0.003 for sICAM-1, respectively). There was a significant positive correlation between ADMA and ferritin, VCAM-1 and ICAM-1 levels while a negative correlation was noted between ADMA and BMI, and Hb concentrations (p <0.001 for Hb, ferritin, VCAM-1; p =0.031 for BMI; and p =0.001 for ICAM-1). On the other hand, there was no significant correlation between ADMA and age, WBC count, platelet count, serum P-selectin and Pentraxin-3 measurements (p >0.05).
Summary/Conclusions: In conclusion, as far as we know, this is the first study evaluating ADMA and endothelial adhesion molecules together in children with beta-thalassemia major and this makes the *results of our study more meaningful compared to previous studies.* These findings support the hypothesis that a serious degree of endothelial activation and damage underlie the pathophysiology of beta-thalassemia major and endothelial dysfunction may participate in the progression of common complications encountered in these subjects.

Red blood cells and iron - Clinical 2

P746

A NATION-WIDE POPULATION STUDY OF REFERENCE INTERVAL FOR HEMOGLOBIN LEVEL

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Background: Attempts to define the 'norm' for hemoglobin level have proposed different reference intervals depending on influential factors such as age, sex, pregnancy, altitude and ethnicity. Changes in life style and public health affect those factors and reference intervals require an update accordingly.
Aims: Reference interval for hemoglobin can be variable and an obstacle for this problem is the absence of large database that enables exclusion of individuals that are not 'normal'. Here we attempt to provide an update of reference interval regarding age and sex groups based on large national database, which so far is the largest used for establishing a reference interval of hemoglobin level.
Methods: The National Health Insurance (NHI) program is a government-operated mandatory health insurance program of Republic of Korea, providing general health screening once every two years. Participation rate of general health screening of NHI is steadily increasing, reaching 74.8% of registered individuals in 2014. Between the period of January 2009 and December 2013, total of 6,759,566 individuals and their hemoglobin value were analyzed after applying the exclusion criteria. The exclusion criteria were in brief, a) diagnosed diseases; respiratory diseases including tuberculosis, hypertension, obesity, anemia, diabetes mellitus, dyslipidemia, hepatitis, nephrotic diseases, b) life style; smoking, alcohol consumption and c) physiologic conditions; pregnancy. Questionnaire used for general health screening is available online (http://minwon.nhis.or.kr/exam/i/file/english_n.pdf). Reference intervals were based on median value and 2.5~97.5 percentile. Data were categorized by sex and age group by 10 years. All statistical analyses in this study were done using SAS v9.4 (SAS Institute Inc., Cary, NC, USA).
Results: Reference intervals for each respective groups are shown in Figure 1. The data included threefold more women than men, as men were much more excluded by smoking and alcohol consumption criteria. The reference interval for whole population using median (2.5~97.5 percentile) was 14.8(12.5~16.8) g/dL in men and 12.8(10.6~14.7) g/dL in women. Men had higher median hemoglobin level of 2g/dL than women at the age group of 40s, which the difference has decreased thereafter. Hemoglobin level decreased along with increasing age, as expected.

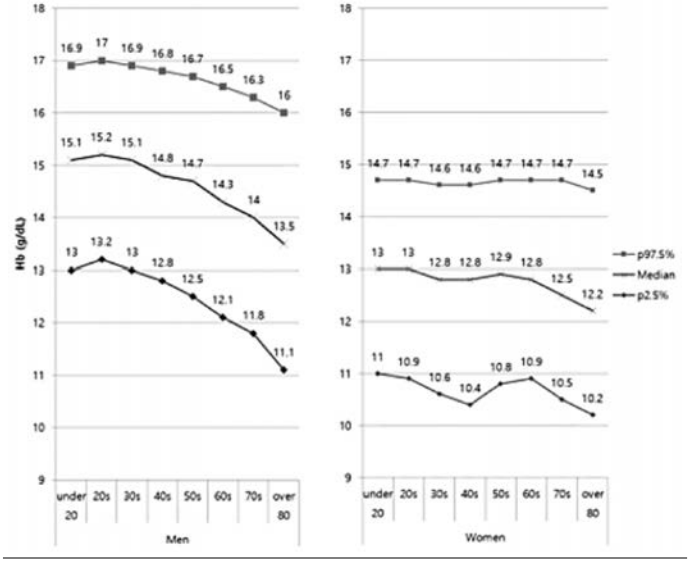

Figure 1.

Summary/Conclusions: Establishing a reference interval requires consideration of many variables other than age, sex, geographic location and ethnicity such as standardization of methods. Laboratory standardization is well established in Republic of Korea and this reference interval of hemoglobin level using large national database is capable of accurately representing this specific population. An updated reference interval will improve diagnostic orientation and decisions for therapy.

P747

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA SCREENING PRACTICE FROM UK CENTRES: A REPORT FROM THE UK PNH NETWORK

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is an acquired clonal stem cell disorder with an associated complement mediated morbidity and mortality. International flow cytometry guidelines recommend PNH screening in 'at risk' patients. These include patients with bone marrow failure syndromes; Intravascular hemolysis; unexplained hemolysis with iron deficiency, oesophageal spasm, thrombocytopenia or granulocytopenia; unusual thrombosis with unexplained cytopenia or hemolysis; or other acquired direct coomb's test negative hemolytic anaemias.
Aims: The aim of this retrospective audit is to determine application of the International flow cytometry guidelines in UK practice.
Methods: The UK PNH network members (hematologists with an interest in PNH) provided anonymised data for analysis. PNH screens analysed from January 2014 to December 2014 were included.
Results: 1579 PNH screens were assessed (53% male; mean age 53 yrs), of which 9.4% were positive. Screening indications included aplastic anaemia (AA) (5.3%) of which 40% were positive, cytopenias (36.7%) of which 11% were positive, thrombosis (28%) of which 2% were positive, haemolysis (5.7%) of which 12% were positive, MDS (0.4%) of which 6% were positive, and other reasons (6.9%) with 6% positive. 257 (16%) screens had no clinical details provided. PNH clone size varied with the majority less than 1% (53%). 22% had a clone size of 1-10%, and 12% had a clone of 10-50%. 18 (12%) had a clone of more than 50%, of these 5 were screened for haemolysis, 7 cytopenias, 4 no clinical details, 1 thrombosis and 1 AA. 377 patients with repeat testing were assessed, 91% of whom had a known PNH clone. The majority had clinical PNH (218), of whom 25% had evolved from preceding AA and 3% from MDS.
Summary/Conclusions: This is the largest audit of PNH screening requests from UK centres. It's reassuring that recommendations are adhered to with very few patients screened inappropriately. 40% of AA patients had a PNH clone in keeping with current evidence. The subgroup analysis highlights the importance of PNH clone monitoring in patients with underlying bone marrow disorders, as they may subsequently develop clinical PNH and require treatment. Ongoing education is essential for the screening and monitoring for this rare but potentially fatal disease.
Disclosure: Alexion UK provided funding to support the UK PNH network meetings.

P748

DIAGNOSTIC VALUES FOR IRON DEFICIENCY ANEMIA IN CHILDREN AND WOMEN USING SERUM HEPCIDIN AND ZINC PROTOPORPHYRIN (ZPP)

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Background: Iron deficiency with or without anemia is a major health problem, affecting more than 2 billion people worldwide. Widely used tests *e.g.* serum ferritin and sTfR are confounded by inflammation and erythropoietic activity respectively. Serum hepcidin and ZPP are potentially useful biomarkers in the diagnosis of iron deficiency (ID) and iron deficiency anemia (IDA). However, diagnostic values for iron deficiency anemia from participants in resource limited settings are not available.
Aims: We determined the optimal cutoff values, sensitivity and specificity of these two biomarkers in children and their mothers in a low middle income setting where both conditions are highly prevalent.
Methods: Healthy rural community dwelling children aged 12-59 months and their mothers from randomly selected villages in a province of South India were prospectively enrolled after obtaining informed consent. Participants were categorized biochemically into healthy, ID and IDA groups based on previously published criteria. Serum hepcidin concentration was quantified by enzyme immunoassay kit (PenninsulaLabs) according to the manufacturer's protocol. Washed red cells were used to measure ZPP in triplicate using a hematofluorometer (AvivBiomedical). Receiver operator characteristics were performed using the sTfR/log Ferritin index as a gold standard and optimized using the Youden index.
Results: The demographic characteristics, hematological and biochemical parameters of the different study groups are shown in Table 1. Mean hemoglo-

bin was significantly lower in IDA compared to healthy or ID subjects (p<0.001). In children, a serum hepcidin cut off value ≤13ng/mL diagnosed IDA with 92% sensitivity and 87% specificity (area under ROC curve (AUC^{ROC}): 0.972 (95%CI,0.939-0.990)) and a cutoff value ≤23ng/mL detected ID with 71% sensitivity and 80% specificity (AUC^{ROC}:0.768 (95% CI,0.703-0.824)). In women, serum hepcidin cutoff value ≤4.5ng/mL diagnosed IDA with 97% sensitivity and 92% specificity (AUC^{ROC}:0.975 (95% CI, 0.956-0.994)) and cutoff value of ≤7.4ng/mL had 90% sensitivity and 69% specificity (AUC^{ROC}: 0.792(95% CI,0.694-0.890)) to diagnose ID. Similarly, ZPP in children at a cutoff value of ≥83 µmole/mole heme diagnosed IDA with 94% sensitivity and 84% specificity (AUC^{ROC}: 0.941 (95%CI,0.90-0.97)). In women, ZPP at a cutoff value ≥123 µmole/mole heme diagnosed IDA with 98% sensitivity and 69% specificity (AUC^{ROC}: 0.894 (95% CI,0.848-0.939)). In both women and children, the AUC^{ROC} for ZPP to diagnose ID was less than 0.5.

Table1. Hematological and biochemical parameters.*Median (Interquartile range); [a]p<0.05 compared with respective healthy subjects, [b]p<0.05 compared with respective ID subjects.

	Healthy		Iron deficiency (ID)		Iron deficiency anemia (IDA)	
	Women(n=90)	Children(n=101)	Women(n=100)	Children(n=109)	Women(n=100)	Children(n=104)
Age±SD	26±3.7	3.7±0.9	26±3.9	3.4±0.9	25±3.8	2.4±0.8[a,b]
Gender M:F	na	49:52	na	40:69	na	56:48
Hb±SD(g/dl)	13.2±0.7	11.9±0.6	12.8±0.6[b]	12.1±0.7	10±1.2[a,b]	9.3±1.2[a,b]
Ferritin*(ng/mL)	39(33,48)	35(32,45)	13*(7,18)	13*(10,18)	3.7*[a] (2.9,5.3)	3.7*[a](2.6,5.1)
Biomarkers						
Hepcidin*(ng/mL)	17.7(9,38)	42.6(25, 62)	4.9*(1.5,11)	13.4*[a](6.2,28)	0.6*[a,b] (0.2,1.3)	1.2*[a,b](0.5,3.6)
ZPP*(µmol/molheme)	66(53,83)	49(39,60)	65(50,85)	48*[a](40,59)	155*[a,b](101,243)	151*[a,b](104,263)

Summary/Conclusions: Cutoff values for diagnostic markers hepcdin and ZPP to diagnose IDA in two high risk groups provide guidance for practitioners in limited resource settings. Serum hepcidin has higher sensitivity and specificity to diagnose IDA than ID. ZPP may diagnose IDA with higher sensitivity and specificity, it is a technically simple, rapid, and cheap point of care test to detect IDA in both practice and community settings.

P749

UTILITY OF RED CELL INDICES TO DIAGNOSE IRON DEFICIENCY IN RURAL INDIAN CHILDREN

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Background: The prevalence of anemia in Indian children aged 6-59 months is high (56%) with 62% cases attributable to iron deficiency. However, this condition is underdiagnosed in India, in part due to unavailability of diagnostic tests for iron deficiency anemia (IDA) in rural settings. Red cell distribution width (RDW) appears to be sensitive in the diagnosis of iron deficiency and values >14.3% was found to predict iron deficiency in anemic toddlers. RDW measurements are provided routinely while measuring complete blood counts and are operator independent. Thus, we investigated the potential of RDW as a point of care test to diagnose iron deficiency anemia.
Aims: To determine utility and establish cutoff values for RDW to diagnose IDA in rural Indian children
Methods: Healthy community dwelling children aged 1-5 years living in randomly selected villages in a South Indian province provided a venous blood sample. A complete blood count inclusive of RDW was performed using a field based automated cell counter. Serum ferritin (SF) and Ferritin Index (FI) were measured at the central laboratory. Subjects were classified into healthy (HL); iron deficient (ID) and iron deficiency anemia (IDA) as shown in Table 1.The coefficient of variation in red cell size (RDW-CV) was expressed as a percentage. Receiver's operator characteristics (ROC) were performed to examine the sensitivity and specificity of RDW CV when compared with a gold standard SF to diagnose ID or IDA and optimized using Youden index.
Results: Majority of the children in this study had ID (66%) or IDA (27%) with only a small portion of the population that demonstrating normal ferritin values. Baseline demographic, hematological and biochemical parameters of children enrolled in the study are tabulated (Table 1).

Table 1: Demographic, hematological and biochemical characteristics in HL,ID and IDA

Baseline characteristicsMarker	Age*(y)	Hb* (g/dl)	MCV*(fl)	SF ** (ng/ml)	RDW*
HL (n=44) (Hb >11g/dl andSF >30ng/ml)	3.4±0.85	11.8±0.58	76.9±3.2	35.2 (32-41)	13.4%±1.1
ID (n=466) (Hb >11g/dl and SF <30ng/ml)	3.1±0.90	11.9±0.69	75.3±4.3	11.8 (7.8-16.6)	13.9%±1.4
IDA (n=192) (Hb <11g/dl and SF <12ng/ml or FI >1.03)	2.4±0.90	9.8±0.82	66.6±6.0	4.3 (3.1-6.9)	16.7%±2.1

**Mean±SD; **Median+IQR.*

Mean RDW CV was found to be significantly higher in IDA (16.7%) compared to HL (13.4%) and ID (13.9%) (p <0.001). IDA was diagnosed at cutoff of RDW >14.6% with 90% sensitivity and 84.9% specificity (AUC^{ROC}: 0.94, 95%CI 0.89-0.97) (Figure 1). However, RDW >13.5% detected iron deficiency with only low sensitivity (65.9%) and specificity (55.9%) (AUC^{ROC} :0.63, 95%CI 0.52-0.71). Those subjects with an RDW >14.6% were more likely to have iron deficiency (OR 6.9, 95%CI 2.4-19.6, p value 0.003).

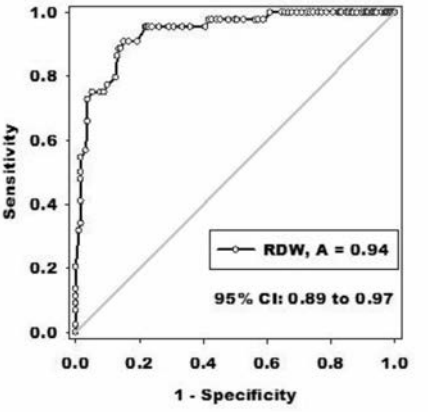

Figure 1. Receiver operator characteristic curve to predict ID anaemic children.

Summary/Conclusions: In a rural population of children, with a very high prevalence of ID and IDA, we found that an RDW CV value >14.6% diagnosed IDA with 90% sensitivity and 85% specificity. Use of this RDW cut off value could guide primary health care decisions regarding iron supplementation in rural children with ID/IDA.

P750

GROWTH AND DEVELOPMENT DURING A 5-YEAR OBSERVATIONAL STUDY OF IRON CHELATION THERAPY WITH DEFERASIROX IN PEDIATRIC PATIENTS WITH TRANSFUSIONAL HEMOSIDEROSIS (ENTRUST)

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Background: Regular transfusion and iron chelation therapy (ICT) are often indicated for pediatric patients (pts) with chronic anemias such as β thalassemia major (βTM), sickle cell disease (SCD) and Diamond–Blackfan anemia (DBA). Delayed growth and development can occur as a result of iron overload, indicating optimal management of iron loading is required. Growth retardation has also been reported in young patients (<3 yrs) receiving intensive ICT with DFO (de Virgillis S *et al. J Pediatr* 1998) and thus regular monitoring of growth is essential. In a 5 yr clinical trial of deferasirox in βTM pediatric patients (2–<16 yrs), growth and development progressed normally (Cappellini MD *et al. Blood* 2011). Here we report growth and development during deferasirox treatment in real-life clinical practice in young pts with transfusional hemosiderosis from the 5-yr multinational, observational ENTRUST study.
Aims: To assess growth and development during long-term deferasirox treatment in an unselected population of transfusion-dependent children with chronic iron overload.
Methods: Over 5 yrs, pts with transfusional iron overload aged 2-<6 yrs at enrollment received deferasirox in accordance with local (country-specific) prescribing information. An initial deferasirox dose of 10-30 mg/kg body weight was recommended and adjusted based on serum ferritin (SF), therapeutic goals, tolerability and changes in pt weight. Parents/guardians provided written, informed consent. Pt height and weight were recorded at baseline and end of each year and mean annual growth velocity was calculated for pts on treatment; data were analyzed by geographical region (Europe, Americas, Middle East/Africa, Asia).
Results: In total, 267 pts (mean age at study entry 3.2 yrs; 52.1% male) with βTM (n=176, 65.9%), SCD (n=52, 19.5%), DBA (n=12, 4.5%) and other anemias (n=27, 10.1%) were enrolled and received ≥1 deferasirox dose. Most pts (n=172; 64.4%) received an average blood intake >7 mL/kg/month. Median SF level decreased from 1702 ng/mL at baseline to 1127 ng/mL after 5 yrs. Overall, mean deferasirox dose increased from a planned starting dose of 26.2±5.9 mg/kg/day to a final actual dose of 28.6±7.7 mg/kg/day. Actual average dose was generally aligned with weight; however, initially deferasirox doses received were suboptimal to manage iron intake and dose adjustments based on weight gain were delayed. Mean growth velocity was 4.86–5.95 cm/yr in male and 5.44–6.08 cm/yr in female pts (Table 1), which is in line with public growth curves for children aged 2-≤11 yrs; as observed across all geographical

regions. Mean weight increased steadily over time (Table 1) in line with mean growth velocity. Median serum creatinine increased steadily over time (28.0 µmol/L at baseline to 39.4 µmol/L) in line with median height, and by association with increasing muscle mass. One patient with βTM experienced an adverse event of below normal height that was not suspected to be drug-related.

Table 1. Growth velocity, height, and weight during treatment with deferasirox in male and female patients aged 2-<6 years at baseline.

	Mean (SD) Weight (kg)	Mean (SD) Height (cm)*	Mean (SD) growth velocity (cm/year)
Male patients (n=137)			
Baseline	15.82 (2.94)	98.76 (9.53)	n/a
Year 1	17.39 (3.07)	104.35 (9.23)	5.95 (3.70)
Year 2	19.20 (3.36)	109.44 (8.83)	5.37 (2.36)
Year 3	20.93 (3.72)	114.58 (8.50)	5.27 (2.28)
Year 4	23.02 (4.47)	119.37 (8.29)	4.88 (3.95)
Year 5	25.22 (4.71)	124.43 (7.76)	4.86 (2.47)
Female patients (n=124)			
Baseline	14.87 (2.98)	97.38 (9.04)	n/a
Year 1	16.53 (3.16)	101.85 (8.85)	6.08 (3.74)
Year 2	17.90 (3.25)	107.22 (8.20)	5.91 (6.77)
Year 3	20.10 (3.91)	112.62 (8.37)	5.67 (3.19)
Year 4	21.92 (4.93)	117.74 (9.20)	5.57 (3.16)
Year 5	24.07 (5.32)	122.63 (9.57)	5.44 (4.80)

*Negative height increments were considered 'no growth' by assigning a nominal value of 0.01. Negative height increments were attributed to different measurement methods as standard guidance was not given. n/a, not available; SD, standard deviation

Summary/Conclusions: In accordance with previous deferasirox trials, this long-term, 'real-life' safety study in young pediatric pts with transfusional iron overload showed expected growth velocities and weight gain for a prepubertal population across all geographical regions. Careful monitoring of pts and appropriate weight-based adjustment of deferasirox dose ensures effective management of long-term ICT and control of transfusional iron loading.

P751

SEARCH AND YOU SHALL FIND – VARIANT HEMOCHROMATOSIS GENE MUTATIONS AS A CAUSE OF HYPERFERRITINEMIA IN BLOOD DONORS

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Background: Blood donors in the capital region of Denmark undergo frequent ferritin measurements to prevent blood donation-related depletion of iron storages. Somewhat surprisingly, some patients actually demonstrate hyperferritinemia, despite regular blood donations. Hyperferritinemia is often the result of inflammatory conditions or liver diseases, but hereditary hemochromatosis (HH) is also a frequent cause of elevated serum ferritin concentrations. HH is in most cases an autosomal recessive genetic disorder characterized by mutations in different genes related to iron metabolism. HH is associated with an increase in intestinal iron absorption and excessive iron deposition in variant tissues (*e.g.* liver, heart and endocrine glands) with risk of concomitant organ dysfunction. The primary HH gene defect is a missense mutation (C282Y) in the HFE gene and C282Y homozygosity is by far the most frequent genotype in HH. However, several different HFE genotypes can also lead to HH. Furthermore, non-HFE phenotypic HH with iron overload can result from other genetic defects in proteins related to iron metabolism, including mutations affecting hepcidin (HAMP), ferroportin 1 (SLC40A1), hemojuvelin (HFE2) and transferrin receptor 2 (TFR2) gene expression.
Aims: The purpose of our study was to explore the relevance of extensive HH mutational analyses in a selected population of healthy Danish blood donors with hyperferritinemia.
Methods: 49 consecutive donors (f=6, m=43) were included prospectively from the Capital Regional Blood Center. Inclusion criteria were either a single ferritin value above 1000µg/L or repeated hyperferritinemia with at least one value above 500µg/L. Included donors were evaluated with a full physical examination, a medical history and extensive biochemical investigations, including a complete blood count, parameters of iron metabolism and relevant organ function tests. Genetic testing was carried out by next generation sequencing using a hemochromatosis gene panel including mutational status of the HFE gene, the HFE2 gene, the HAMP gene, the SLC40A1 gene and the TFR2 gene.
Results: 40 of 49 donors were mutation positive, combining for a total of 67 mutations. 18 patients had 1 mutation, 18 patients had 2 mutations, 3 patients had 3 mutations and 1 patient had 4 mutations. Interestingly, there were only 11 patients with C282Y homozygosity. Thus, of the 40 mutation positive donors, 29 had phenotypic evidence of HH, albeit with a more atypical HH genotype. An overview of the mutational frequencies is shown in Figure 1.
Summary/Conclusions: Our view on the genetic background of HH is constantly expanding due to the documentation of several different genotypes resulting in phenotypic HH. Screening of blood donors with hyperferritinemia could be a straightforward way of identifying individuals with a high probability of HH since iron overload in healthy blood donors adherent to the blood donor program could be suggestive of significant malfunction of iron metabolism. Although standard genetic testing for C282Y mutational status is available at a relatively low-cost, it obvioulsy does not account for the genotypic variations in HH pathogenesis. Thus, a more complete genetic workup should be considered in order to improve HH diagnostic testing in individuals with unexplained hyperferritinemia.

P752

A PHASE 1, SINGLE AND MULTIPLE ASCENDING DOSE STUDY OF SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF AG-519, AN ALLOSTERIC ACTIVATOR OF PYRUVATE KINASE-R IN HEALTHY SUBJECTS

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Background: Pyruvate kinase (PK) deficiency is an inborn error of metabolism affecting children and adults that results in lifelong hemolytic anemia, and is associated with serious long-term comorbidities such as poor growth and development in children and chronic iron overload in adults. PK deficiency is caused by a functional deficiency of the red blood cell isoform of PK (PK-R). As a result of this defect in glycolysis, levels of 2,3-diphosphoglycerate (2,3-DPG) in the blood are elevated and adenosine triphosphate (ATP) levels are low. AG-348, an orally available allosteric activator of PK-R currently being tested in a phase 2 clinical trial in adult patients with PK deficiency (NCT02476916), has been shown to increase ATP and decrease the levels of 2,3-DPG in healthy volunteers. A drug discovery effort aimed at eliminating the aromatase inhibitory activity observed with AG-348 resulted in the identification of AG-519.
Aims: AG-519 is currently in a randomized, double-blind, phase 1 study in healthy volunteers (NCT02630927), with the objective of identifying a safe and pharmacodynamically active dose and schedule to be used in subsequent clinical studies in subjects with PK deficiency. Here we report the single ascending dose phase of this study.
Methods: Healthy men and women (non-childbearing potential) aged 18–60 years and providing informed consent are eligible. Four dose levels (from 50 mg to up to 1250 mg) will be explored sequentially, starting with the lowest dose. At each dose level, 8 subjects will be enrolled and randomized to receive a single oral dose of AG-519 (n=6) or placebo (n=2), with an option to enroll 2 additional dose cohorts (n=8 each) to assess alternative single dose levels. Safety assessments include adverse events (AEs), vital signs, electrocardiogram and clinical laboratory parameters. Serial blood samples will be drawn to measure plasma AG-519 concentrations and whole blood 2,3-DPG and ATP concentrations for pharmacokinetic and pharmacodynamic assessments.
Results: Eight subjects have been enrolled at 50 mg and a further 8 subjects enrolled at 250 mg to date. Blinded safety reviews indicated no safety concerns in healthy volunteers receiving a single dose of AG-519 given as 50 mg or 250 mg, or placebo. Plasma AG-519 exposure was dose-dependent, with low to moderate variability in the pharmacokinetic parameters of AG-519. Investigation of the pharmacodynamic effects of AG-519 after a single dose indicated that there was a substantial dose-dependent decrease in blood 2,3-DPG concentration (Figure 1). There was no effect on blood ATP concentration, indicating that ATP change requires more than one dose, as observed in the single ascending dose study for AG-348. Based on these encouraging results, single dose escalation is ongoing, and the multiple ascending dose component has been initiated, with dose escalation proceeding in order to determine maximal tolerated dose and/or optimally effective dose and effects on sex hormones. Further data from the single ascending dose and multiple ascending dose cohorts will be presented.

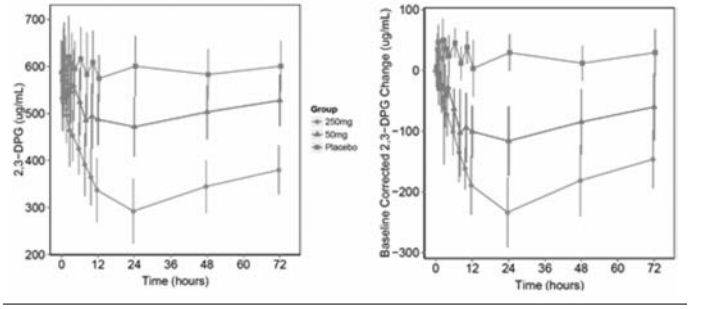

Figure 1. Actual and change from baseline concentration-time profiles of 2,3-DPG in blood following a single dose of AG-519 (n=6 for 50 mg and 250 mg, n=4 for placebo.

Summary/Conclusions: AG-519 is well tolerated, with exposure-dependent decreases in 2,3-DPG concentrations when given as a single daily dose in healthy subjects. Fourteen day dosing results from this trial will further inform the potential for AG-519 as a second potential molecule for the treatment of PK deficiency.

Non-malignant hematopoietic disorders

P753

ANALYSIS OF MOLECULAR MECHANISMS OF DOMINANT-INTERFERING FAS MUTATIONS

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Background: The autoimmune lymphoproliferative syndrome (ALPS) is clinically characterized by lymphadenopathy, hepatosplenomegaly, autoimmunity, hypergammaglobulinemia, and an increased number of $CD3^+TCR\alpha\beta^+CD4^-CD8^-$ double-negative T (DNT) cells. Defects in the FAS signaling pathway lead to impairment of lymphocyte apoptosis and hence to accumulation of activated and autoreactive lymphocytes. In approximately 70% of ALPS cases, mutations in the trimeric FAS receptor have been identified (classified as ALPS-FAS). Most ALPS patients harbor heterozygous dominant interfering mutations with in part unclear mechanisms.
Aims: To better understand the pathogenesis of ALPS-FAS and the molecular mechanisms of dominant-interfering FAS mutations we tried to identify and to functionally analyze novel FAS mutations in a cohort of 35 patients with an ALPS-like clinical phenotype.
Methods: DNA was isolated from blood of 35 patients with an ALPS-like clinical phenotype using standard methods and *FAS* was subjected to Sanger sequencing and compared to the annotated wildtype sequence. Activated and expanded T cells were stimulated with recombinant FAS ligand, staurosporine or were left untreated and apoptosis was measured by Annexin V-FITC/PI staining via flow cytometry. Immunophenotyping was carried out by flow cytometry. Immortalized T cell lines were generated using *Herpes virus saimirii*.
Results: We identified six patients with heterozygous *FAS* mutations. Five mutations were novel (p.R102C, p.R250G, p.NVQ266-268KQT, p.W281L, p.Q283K), one was a known splice site mutation frequently found in ALPS patients (p.E218MfsX*4). One mutation affected the extracellular domain of the receptor (p.R102C), the others occurred in the death domain. All patients with *FAS* mutations fulfilled the two required ALPS diagnostic criteria: 1) chronic (>6 months), nonmalignant, noninfectious lymphadenopathy or splenomegaly or both and 2) elevated DNT cells (≥1.5% of total lymphocytes). Secondary diagnostic criteria were also fulfilled: all patients had a typical ALPS immunophenotype and impaired FAS signaling led to resistance of primary patient T cells to FAS ligand-mediated apoptosis. All six patients can be classified as ALPS-FAS cases.
Summary/Conclusions: With heterozygous *FAS* mutations, statistically one in eight FAS trimers is made of wildtype proteins only. This is a mechanism by which the FAS signaling in ALPS patients is impaired. Among the identified *FAS* mutations was a known splice site mutation leading to skipping of exon 8, frameshift and premature termination in exon 9. This mutation abrogates recruitment of the adapter molecule FADD to the receptor. The R250G mutation is novel, but other amino acid exchanges (Q,P,L or termination) have been described at this site. By analogy, p.R250G should reduce binding of FADD. Reported structural analyses indicate that p.Q283K also dominantly interferes with FADD recruitment. Two further mutations affected the death domain and may interfere with binding of proteins via death domain interaction (p.W281L and p.NVQ266-268KQT). The latter mutation resulted from six consecutive missense mutations leading to the exchange of three amino acids. To our knowledge, six consecutive missense mutations have not been described for ALPS patients or in general. One patient harbored a mutation in the extracellular domain of the receptor (p.R102C). This may lead to a wrong formation of a cystin-bridge. Further analysis of *FAS* mutations regarding their structure and functional impact are performed and will be reported.

P754

UNSMAKING PRIMARY IMMUNE DEFICIENCIES IN EARLY-ONSET EVANS SYNDROME USING IMMUNOPHENOTYPING AND NGS: TOWARDS A CLINICAL AND GENETIC CLASSIFICATION

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Background: Evans syndrome (ES) is a rare autoimmune disorder in children with generally unknown aetiology at onset. In France, a collaborative immunopediatric network recently presented comprehensive data on a large series of 156 non selected childhood ES (OBS'CEREVANCE cohort): 70% of the cases were suspected to be secondary to potential immune defects.
Aims: This prospective study reports the genetically identified primitive immune deficiencies (PID) in those 156 non selected children with ES.
Methods: Complete data from birth to last follow-upare registered in real-time. Relevant immune events are defined, leading to the subgroup of suspected secondary ES: lymphoproliferation, systemic or organ-specific auto-immunity, hypogammaglobulinemia, non microbial pulmonary, digestive or neurological diseases. Etiological investigations at the initial diagnosis were consistent with national guidelines and repeated during the follow-up. Complete immunophenotyping was performed in laboratories of the national CEREDIH network. All the children underwent screening for autoimmune lymphoproliferative syndrome (ALPS). Functional and genetic studies (Sanger sequencing or NGS) were mainly addressed to the French CEDI reference laboratory, Necker, Paris and to the INSERM unit 1163, Institut IMAGINE, Paris.
Results: In January 2016, 156 children from 26 centres diagnosed between 1981 and 2014 with ES were analysed. The median age at initial cytopenia was 5.4 (0.2-17.2) years old. The median follow-up from ES diagnosis was 6.5 years (0.1-28.8). 14 children had aetiologies that might explain the autoimmune cytopenia (13 pediatric systemic lupus erythematosus (pSLE), and one 22q11.4 deletion). Lymphoproliferation at presentation led to the exploration of apoptosis pathways in 55 patients and to ALPS biomarkers in most patients. Six patients (11% of the tested patients) had an apoptosis defect, related to a germ line FAS mutation (ALPS-FAS) in 5 cases (9%) and to a somatic KRAS mutation in 1 case. A genetic search (Sanger sequencing or NGS) has been performed on 43 patients with clinical relevant criteria. A monogenic defect was found after the ES diagnosis in 8 patients: 2 STAT3 gain of function mutations, 4 CTLA4 deficiencies, 2 LRBA deficiencies. In the 35 other patients, genetics studies are ongoing. ES was thus secondary to pSLE in 13 patients (8.3%) or to an identified PID in 15 patients (9.6%). In 52% of the patients in the present cohort, and even in apparently primary cases (chronic isolated bi-cytopenia), ES could be related to an as yet unidentified genetic defect.
Summary/Conclusions: At the present time, in this cohort, a PID could be identified in 10% of ES patients. This proportion will certainly grow up in a near future following this genetic strategy based on well characterized clinical and immunological features. More specific therapies (mTOR inhibitors in ALPS patients, or CTLA4-targeted therapies in CTLA4 and LRBA deficient patients) can already be proposed. The current challenges are therefore to rapidly complete this genetic approach and to further define new candidate genes, to identify additional targeted therapies.

P755

MRI SKELETAL MANIFESTATIONS IN GAUCHER DISEASE: UTILITY OF SPANISH-MRI (S-MRI) SCORE AT DIAGNOSIS AND FOLLOW-UP

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Background: Gaucher disease (GD), the most prevalent lysosomal storage disorder is characterized by multisystem involvement, secondary to the accumulation of glucosyil-ceramide in the reticule-endothelial system. Bone disease, is on of the most frequent manifestations, it's caused by several factors: derived cell accumulation, vascular events and cytokine release – chronic inflammatory state. MRI has demonstrated to be the gold standard for assesses bone involvement, including bone infiltration, bone crisis, infarcts and avascular necrosis; others like vertebral collapses secondary to infiltration-osteopenia and osteoporosis are also common. The Spanish-MRI score (S-MRI), described by Roca *et al.* in 2006 is a semi quantitative tool combining the assessment of the bone marrow burden (infiltration), (BMB) in vertebra, pelvis and femurs (0-12 points), with the presence of bone vascular events (4 points for every lesion). Despite the value of S-MRI to assess the initial involvement of GD patients,

their value during follow up is also remarkable. The MRI was incorporated in our unit since 1993 for routinely evaluation.
Aims: To analyses our experience with MRI bone-assessment in our unit, especially focus on the role of S-MRI at diagnosis, follow-up and identification of bone lesions.
Methods: All patients included in the Spanish Registry of Gaucher disease had signed an inform consent for the management of the information relative to the disease. A total of 131 patients have been evaluated by MRI in the radiodiagnostic center of the unit, following the same protocol and by the same radiologist specialized in muscle-skeletal MRI, summarizing ~340 MRI studies.
Results: From 131 patients, in 64 (49.6%) was part of the initial assessment previous to start any therapy, 63(48.1%) were under enzymatic replacement therapy (ERT) and 4 under substrate reduction therapy (SRT). Mean age: 37.5 y (13-74), M/F: 70/61, type 1 GD: 124, type 3 GD: 7. The genotype N370S/L444P was the most frequent (37.1%). At first MRI 104 (79.4%) present at least one lesion related with GD, mean S-MRI: 11.6 (2-28) points, mean BMB 7.1 (0-12) points, vascular complications was presents in 67 (64.4%) patients and 44 (33.8%) presents lesions not related with GD. During follow-up 62 (47.3%) patients were evaluated sequentially. A reduction in the BMB scored by S-MRI was observed after 5 years of therapy (Figure 1). 14 patients on therapy (12 ERT and 2 SRT) and one without therapy present a vascular event, mean time on therapy (8.4 y; 1.5-16). Of them, three cases registered a bone crisis in small feet bones (two in calcaneus, scaphoids); two cases developed multiple foci lesions in lower limbs, mainly in tibia after severe infections. Into the not previously GD-related lesions we want to highlight the presence of hemangiomas in vertebra in 11 (8.4%), discopathies in 27 (20.6%) patients, two cases with neuronopathic-like arthropathy without manifestations of peripheral neuropathy but with severe bone involvement and 3 patients with bone alterations that lead a neoplasm diagnosis.

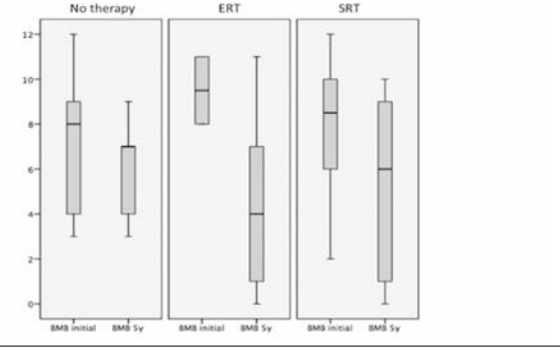

Figure 1. Evolution of BMB after 5 years on MRI follow-up (62 patients).

Summary/Conclusions: The bone disease is the most important issue in type 1 Gaucher disease patients, the most debilitating alterations have been identified since the description of the disease, despite the bone related MRI lesions, there were many findings registered. It's difficult to say if GD predispose to others manifestations like here described. In our experience, MRI assessment permits to optimize the follow-up of the patients on therapy and identified early complications.

P756

SEVUPARIN DEMONSTRATES BINDING TO KEY ADHESION RECEPTORS INVOLVED IN PATHOGENESIS OF SICKLE CELL DISEASE

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Background: Sevuparin is a novel drug candidate with potential as therapeutic agent for patients with sickle cell disease (SCD). Sevuparin has an anti-adhesive mechanism of action and is currently studied in a clinical phase II study in SCD patients hospitalized for acute painful vaso-occlusive crisis (VOC). Sevuparin's anti-adhesive effects have previously been demonstrated in both *in vitro* and *in vivo* models. SCD is the most common haemoglobinopathy, affecting millions of people worldwide. A single point mutation in the β-globin gene results in synthesis of abnormal sickle haemoglobin (HbS), which polymerizes and causes sickling of red blood cell (RBC) under hypoxic conditions. RBCs containing predominantly HbS (SS-RBCs) also demonstrate an adhesive phenotype, where degree of adhesiveness correlates with frequency of vaso-occlusive events in SCD patients. The pathogenesis of VOC involves decreased blood flow caused by microvascular obstructions from the adhesion between the different blood cells and the vessel endothelium as well as endothelial cell activation. The abnormal adhesion has been studied and several key adhesion proteins identified; Selectins are involved in the initial attachment of cells to the vessel wall. More firm binding is exerted via the integrins, such as thrombospondin (TSP), von Willebrand Factor (vWf) and fibronectin (FN). Among the selectins, P-selectin is the most extensively studied adhesion receptor in SCD, contributing to SS-RBC adhesion and vaso-occlusion both *in vitro* and *in vivo*. Blocking P-selectin has demonstrated improvement in blood flow. However, both E-selectin and L-selectin have been indicated to be involved as well. E-selectin is upregulated during inflammatory processes and participates in adhesion between white blood cells. L-selectin contributes to lymphocyte and neutrophil trafficking and is cleaved from leukocytes during cell activation. Several other, non-membrane bound proteins, such as FN, vWF and TSP, have been shown in pre-clinical model systems to contribute by serving as anchoring or bridging proteins between the blood cells and the activated endothelium in SCD.
Aims: To determine the binding characteristics of sevuparin with key adhesion proteins known to be involved in SCD vaso-occlusion and thereby provide evidence of the multiple anti-adhesive properties at a molecular level for this new investigational treatment.
Methods: The binding characteristics were determined by Fluorescence Correlation Spectroscopy (FCS). Pharmacokinetic studies were performed in mice.
Results: Dissociation constants were determined by FCS, using a competitive titration approach. Kd for the key adhesion proteins was determined to 0.49±0.38μM for P-selectin, 4.48 μM for E-selectin, 0.45±0.43μM for L-selectin, 2.25±0.34μM for Fibronectin, 0.48±0.34μM for von Willebrand Factor and 0.42±0.18μM for Thrombospondin. To understand which of the interactions would be relevant in the clinical setting, a pharmacokinetic study was performed in mice. From this, the target plasma concentration of 2.5 μM was calculated. Applying the target plasma concentration to the Kd values reported above, sevuparin would bind to the adhesion proteins P-selectin, L-selectin, vWF and TSP while the interaction with FN and E-selectin would be limited. Figure 1. Illustration of possible multimodal mechanism of sevuparin action. The Figure 1 demonstrates the potential interactions between sickle blood cells and the blood vessel wall endothelium.

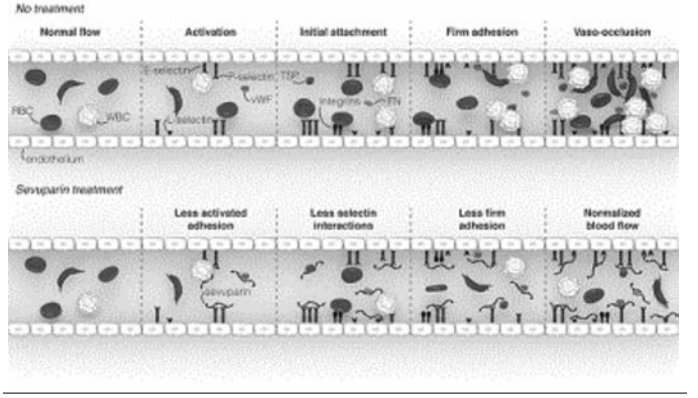

Figure 1.

Summary/Conclusions: Binding experiments with isolated adhesion receptors suggest that multiple interactions contribute to the anti-adhesive effect demonstrated *in vitro* and *in vivo* with sevuparin. Sevuparin shows significant binding to P-selectin, L-selectin, vWF and TSP, but a weaker binding to FN and E-selectin. An illustration of the potential multimodal mechanism of sevuparin action can be found in Figure 1.

P757

GLUTATHIONE S TRANSFERASE GENE POLYMORPHISMS AND THE RISK OF CNS COMPLICATIONS OF SICKLE CELL DISEASE IN EGYPTIAN PATIENTS

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Background: Sickle cell disease (SCD) is characterized by multisystem complications with marked variability in disease severity between individuals. Clinical manifestations of SCD are mainly resulted from sickling of Hb S, which is augmented by oxidative stress. The glutathione system plays an important role in scavenging the products of intracellular oxidation, so protects Hb S from oxidation, minimizing manifestations of SCD. SCD patients with genetically defective glutathione system due to glutathione S transferases (GSTs) gene polymorphisms, are expected to develop sever forms of SCD.
Aims: Exploring the possible association of the GST gene polymorphisms (*GSTM1*, *GSTT1* and *GSTP1)* and the severity of manifestations of SCD in Egyptian patients, as the GST gene polymorphisms may be considered as a critical genetic modifier in SCD patients.
Methods: We studied the frequency of the GSTs (GSTM1, GSTT1 and GSTP1) genes polymorphisms in 100 Egyptian adult SCD patients and 80 age-and sex-matched controls, GSTM1 and GSTT1 gene polymorphisms were examined by multiplex polymerase chain reaction (PCR) with using a housekeeping B globin gene as internal control. A polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP] was used to detect GSTP1 polymorphism. We studied the association between the GSTs gene polymorphisms in

the SCD patients, and the risk of developing severe clinical manifestations, including CNS complications in the form of transient ischemic attacks (TIAs) and strokes, avascular necrosis (AVN) of the head of femur (diagnosed by MRI of the hip), and renal injury by estimation of Albumin /creatinine ratio (A/C ratio).
Results: Among our study population, GSTM1, GSTT1 and GSTP1 genotypes distribution was similar between SCD patients and controls. CNS complications were observed in 66.7% of the SCD patients, 33.3% of the patients had no history of CNS complication. AVN of the head of femur was observed in 61.5% of the patients, while 38.5% of the patients had no previous AVN of the head of femur. The A/C ratio was ranging from 0.01 to 4, mean=0.5±0.6 and median =0.3. The *GSTM1 null genotype was significantly associated with CNS complications* (P value=0.03), *it was also associated with AVN of the head of the femur (Odd ratio=7) and raised A/C ratio* (mean=0.6±0.82 *vs* 0.49±0.42 for null genotype and non-null genotype respectively), however it was not of statistical significance (P-Value=0.2 and 0.8 respectively). *GSTT1 null genotype was also associated with CNS complication* (Odd ratio=5.1)*, AVN of the head of femur* (Odd ratio=4.2), *and raised A/C ratio* (mean=0.55±0.65 *vs* 0.49±0.44 for null genotype and non-null genotype respectively), however it was not statistically significant (P-Value=0.4, 0.5 and 0.9 respectively). Non wild GSTP1 polymorphisms (Homozygous and heterozygous) were not associated with sever clinical manifestations of SCD in the form of CNS complications, AVN of the head of the femur and A/C ratio (P value=0.5, 0.6 and 0.8 respectively).
Summary/Conclusions: As GSTM1 and GSTP1 null genotypes were associated with unfavorable clinical outcomes, GST gene polymorphisms may be used as a genetic marker, of a predictive value for defining patients at risk of developing sever SCD complications, due to impaired anti-oxidative defense mechanism. Patients with GSTM1 and GSTP1 null genotypes may get benefits of using prophylactic treatments aimed at reduction of oxidative stress to protect them against severe complications. However, further studies on a large scale including different ethnicities are required to prove our observation.

P758

LUSPATERCEPT INCREASES HEMOGLOBIN, REDUCES LIVER IRON CONCENTRATION, AND IMPROVES QUALITY OF LIFE IN NON-TRANSFUSION DEPENDENT ADULTS WITH BETA-THALASSEMIA

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Background: Luspatercept (ACE-536), a fusion protein containing a modified activin receptor type IIB, is being developed for the treatment of β-thalassemia. Luspatercept binds to GDF11 and other ligands in the TGF-β superfamily to promote late-stage erythroid differentiation. Luspatercept corrected the effects of ineffective erythropoiesis in a thalassemia mouse model (Suragani R, Blood 2014) and was well tolerated and increased hemoglobin (Hgb) in a phase 1 clinical study (Attie K, Am J Hematol 2014).
Aims: This is an ongoing, phase 2, multicenter, open-label, dose-finding study followed by a long-term extension study to evaluate the effects of luspatercept in adults with β-thalassemia, including a subgroup that was non-transfusion dependent (NTD, <4 Units RBC/8 wk). Endpoints included Hgb, liver iron concentration (LIC) by MRI, safety, and patient-reported quality of life questionnaires (FACT-An, SF-36).
Methods: Inclusion criteria included age ≥18 yr and Hgb <10 g/dL (NTD pts). Luspatercept was administered SC every 3 wks for up to 5 doses in the dose-ranging study (completed) with a 2-month follow-up unless enrolled directly into a 2-year extension study (ongoing). Six sequential cohorts (n=35 total) were treated at escalating doses from 0.2 to 1.25 mg/kg. An additional expansion cohort (n=29) was treated with a starting dose of 0.8 mg/kg with escalation up to 1.25 mg/kg. Of the 64 patients treated in the dose-ranging study (including 34 NTD), 51 were enrolled in the extension study.
Results: Data (as of 25 Sept 2015) were evaluable for 31 of 34 patients who were NTD. Median age was 37 yr, ranging from 20 to 61 yr, and 68% had prior splenectomy. Median Hgb was 8.4 g/dL (range 6.5-9.6 g/dL) and mean (±SD) LIC was 5.6±3.8 mg/g dw. 12 patients were receiving iron chelation therapy (ICT) at baseline. Mean increase in Hgb was greater in patients treated with 0.8-1.25 mg/kg than in patients treated with 0.2-0.6 mg/kg. Of the patients treated in the longer-term extension study, 11/17 (65%) achieved a ≥1.0 g/dL increase in mean Hgb over any 12-week period, and 8/17 (47%) increased by ≥1.5 g/dL. 5/14 (36%) patients with baseline LIC ≥5 mg/g dw had a mean decrease in LIC ≥2 mg/g dw (mean decrease 3.2 mg/g dw, 32.1%) over the 16-week base study period. 14/14 (100%) patients with baseline LIC <5 mg/g dw maintained LIC <5 mg/g dw. Four NTD patients with long-term, persistent leg ulcers experienced complete or partial healing with luspatercept treatment. Increases in the FACT-An anemia subscale and the SF-36 physical component summary (PCS) at Week 24 (n=15, last observation carried forward) correlated with Hgb increases ($p<0.05$). Luspatercept was generally well tolerated, with no related serious adverse events reported to date. Adverse events in the NTD subgroup were mostly mild-moderate and the most frequent related adverse events (≥3 patients) were bone pain, headache, musculoskeletal pain, myalgia, and arthralgia.
Summary/Conclusions: Luspatercept treatment was well-tolerated and led to increased hemoglobin levels, decreased LIC, and improved quality of life in NTD adults with β-thalassemia. The increase in hemoglobin correlated with improvement in QOL anemia symptom score. These changes represent a significant reduction in disease burden reflected in improved patient quality of life for patients with NTD β-thalassemia; further studies in this population are planned.

P759

AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS) AND ALPS-RELATED SYNDROME (ARS). DIFFERENCES ON CLINICAL AND BIOLOGICAL FEATURES AND TREATMENT RESPONSE: A SINGLE CENTER EXPERIENCE.

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Background: ALPS is a rare, inherited disorder of the immune system due to defective Fas mediated-apoptosis, characterized by lymphoproliferation, autoimmunity and frequently cytopenias. Immunological/autoimmune disorders with an ALPS phenotype but not completely fitting ALPS full diagnostic criteria, are frequently seen by heamatologists and often represent a therapeutic challenge.
Aims: is to describe clinical and biological features and treatment response of a cohort of ALPS and ARS patients seen at our Centre.
Methods: patients with ALPS and ARS treated at the our Centre from January 2001 to December 2015, were considered eligible to the study. ALPS was defined according to the revised diagnostic criteria established by a work shop in 2009. ARS has been arbitrarily defined by the presence of i) cytopenia+at least one "Required Criteria" or one "Primary Additional Criteria" or b) two "Required criteria"+one Secondary Additional Criteria. Anagraphical, clinical, biochemical data were collected for each patient. Response to immunosuppression was defined partial or complete according to a melioration/resolution of cytopenia or other symptoms.
Results: 90patients (49 males, 41 females) whose median age at diagnosis was 10 yrs (0-39.8) with ALPS (37) or ARS (53) were evaluated. Lymphoproliferation was present in 71% (64/90)patients and consisted of isolated lymphoadenopathy (31%>28/90-), isolated splenomegaly (54%>49/90), or both (19%>17/90). Lymphoadenopathy was more frequent (49%)in ALPS than in ARS (19%; $p<0.01$) and so was splenomegaly (84% in ALPS *vs* 34% in ARS patients, ($p<0.01$). The median percentage of Double Negative T-cells/total lymphocytes was and again was higher in ALPS 2.6% (0.6-14.8) over ARS (2.2% 0.6-19.5) ($p<0.01$). Cytopenia was present in 80% subjects; of these 32% (26) were ALPS and 64% (46) ARS ($p=0.05$) and involved one (62%) or more cell lineages (38%). Isolated thrombocytopenia was significantly more frequent in ARS (79%) *vs* ALPS (21%)($p=0.01$), while neutropenia (52%) and hemolytic anemia (44%) were more represented in ALPS *vs* ARS ($p=0.03$ and $=0.01$ respectively). Trilinear cytopenia was present almost exclusively in ALPS (86%) *vs* ARS (14%) ($p<0.01$). Vitamin B12 values above1500 ng/l and autoimmune symptoms were more frequent in ALPS than in ARS ($p=0.02$ and $p<0.01$). Fourteen out of 89 patients (16%)did not need any treatment. Of the remaining 75, 14 (19%) responded to steroids and/or immunoglobulin, while 61(81%)required a second/third line therapy. Of these 61, 41 (67%) received MycophenolateMofetil (MMF) which resulted in a partial/complete response in 70%. Response rate was 78% in ALPS and 61% in ARS (p=ns). Twenty eight of 61 (46%) received Sirolimus, including 15 patients who had previously failed MMF treatment. Partial/complete response was seen 92% of ALPS and in 77% of ARS patients (p=ns).
Summary/Conclusions: Lymphoprolipheration and autoimmune symptoms characterizes mainly ALPS, while cytopenia is a peculiarity of ARS. Isolated thrombocytopenia is more frequent in ARS patients, while neutropenia/haemolytic anemia or trilinear cytopenia is more typical of ALPS. MMF and Sirolimus represent a valid option for the treatment of ALPS and ARS being effective in more than ¾ of the cohort thus sparing steroids. The definition of the genetic profiles of these patients might enable treatments tailored on the different pathogenic mechanisms.

P760

INCREASED LEVELS OF CIRCULATING IMMATURE GRANULOCYTES IN SEPSIS HAVE A SIGNIFICANT IMPACT ON SURVIVAL: RESULTS OF THE FRENCH MULTICENTRIC SEPTIFLUX 2 STUDY

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Background: In industrialized countries, overall survival of sepsis is 27%, and each year, as many patients die of sepsis as of heart attack. In the first hours of sepsis, increased percentage of band cells and late immature granulocytes (Imm Gran), the cytologic equivalent of CD10dim and CD16dim immature granulocytes, has long been described. Recently, we suggested in a proof-of-concept monocentric study that Imm Gran CD10dim and CD16dim quantification by flow cytometry may have a prognostic interest in septic patients. We also showed that this cell compartment contains myeloid derived suppressor cells (MDSCs).
Aims: Here we demonstrate prospectively that the prognostic value of this the CD10dim and/or CD16dim granulocyte counts in a multicentric study including 12 centers and 781 patients.
Methods: Prospectively, between November 2013 and June 2015, all patients admitted for less than 24 hours in 12 french hospitals for Sepsis, severe Sepsis or septic shock were eligible. A blood count and a eight colors cytometry staining (CD64/CD10/CD14/CD16/CD24/CD11b/CD3/CD45) were performed to quantify CD3 cells, CD64+ granulocytes, CD10dim and CD16dim immature granulocytes, monocytes and although the inflammatory monocytes (CD16+). The intensity of CD10 and CD16 was also compared (mean, mode, CV).
Results: 1062 patients were screened and 281 excluded mainly due to unconfirmed infection. 781 were analyzed. Patients were segregated in 3 groups regarding the severity of sepsis: 343 Sepsis (67+/-75 years), 192 severe sepsis (67+/-67 years) and 246 with septic shock (65+/-15 years). In each group we have followed the clinical deterioration (8%, 12% and 9%) at 48 hours and the mortality rate (0%, 1% and 9%) respectively. Corresponding to the emergence of CD16dim Imm Gran, an increased CV of CD16 expression (CV=52, p<0.01) was found in deteriorating patients compared to patients with a stable or improved condition at 48 hours (CV=44.2, p<0.01). At day 0, the absolute count of Imm Gran was 2 G/L *vs* 4.7 G/L (p<0.01) in patients who survived or not at day 7, and 1.9 G/L *vs* 4.6 G/L at day 30 (p<0.01). The proportion of Imm Gran was 19.8% *vs* 66.4% (p<0.01) in patients who survived or not at day 7, and 19.1% *vs* 55.2% at day 30 (p<0.01). Thus, both absolute count and proportion of Imm Gran had a negative prognostic value on mortality at day 7 and 30. A CD3 lymphopenia had also a significant impact on survival, 0.5 G/L *vs* 0.3 G/L (p<0.01) at day 7 and 0.6 G/L *vs* 0.4 G/L (p<0.01) at day 30.
Summary/Conclusions: These results demonstrate in a multicentric manner on a very large prospective cohort of patients that increased levels of CD10dim and CD16dim Imm Gran, as quantified by daily routine flow cytometry in hospital laboratories, have a strong pejorative prognosis value on survival at day 7 and 30 in Sepsis. Early Sepsis management being known to prevent deterioration of patients, we strongly suggest that setting up the precise quantification of immature granulocytes by flow cytometry could benefit to septic patients with a tailored specific management.

Bleeding disorders

P761

USEFULNESS OF A THIRTY ONE-GENE PANEL BY NEXT-GENERATION SEQUENCING FOR THE MOLECULAR DIAGNOSTIC OF INHERITED BLEEDING COAGULATION DISORDERS

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Background: Molecular testing of Inherited bleeding coagulation disorders (IBCDs) not only offers confirmation of diagnosis, but also aids in genetic counseling, prenatal diagnosis and in certain cases genotype–phenotype correlations are important for predicting the clinical course of the disease and to allow tailor-made follow up of individuals. Until recently, genotyping has been mainly performed by Sanger sequencing, a technique known to be time consuming and expensive. Currently, Next-Generation Sequencing (NGS) offers a new potential approach that enables the simultaneous investigation of multiple genes at manageable cost.
Aims: To analyze the applicability of a 31-gene NGS panel in the molecular diagnosis of patients with IBCDs.
Methods: We enrolled a total of 30 patients (19 males and 11 females; median age, 20 years, ranging from 1 to 61 years old), with previously confirmed coagulation factor deficiencies. IBCDs genotype was studied using NGS technology. A custom target enrichment library was designed to capture thirty-one genes known to be associated with IBCDs. Probes were generated for 343 targets to cover 103.3 kb regions (all exons and flanking regions) of these genes. Sanger sequencing was performed to validate all causative variants identified by NGS.
Results: The use of this 31-gene panel approach allowed us to identify the causative variants of the IBCDs in all patients. Overall, thirty pathogenic variants were found (five hypodysfibrinogenemias, five von Willebrand disease, three FVII deficiencies, one FV, FX, FXII, FXIII, Prekallykrein deificiency, each other, two FXI deficiencies, eight Hemophliia A and 2 Hemophilia B), including eight novel mutations affecting F8, FGA, FGG, F11, F10, F5 and VWF genes and twenty-two previously reported variants were detected. Most of them, 27 were missense/nonsense and 3 were frameshift changes due to microdeletions. NGS and Sanger sequencing were 100% concordant (Table 1).

Table 1.

Hemostasis function	Genes
Intrinsic pathway	*KLKB1, HRG, KNG1, F12, F13A1, F13B, F11, F8, F9, VWF*
Extrinsic pathway	*F2, F3, F5, F7*
Vitamin-K dependent-carboxylation	*GGCX, VKORC1, F10*
Combined factor synthesis	*MCFD2, LMAN1*
Fibrinolysis	*PLAT, PLAUR, PLG, PLGRKT, TFPI*
Fibrinogen synthesis	*FGA, FGB, FGG, FIBCD1*
Complement system	*C1QBP, CLEC3B, CPB2*

Summary/Conclusions: Inherited coagulation disorders could be successfully molecularly characterized by using our 31-gene Next Generation DNA Sequencing panel. This approach allowed the diagnosis of the disease at a molecular level in all patients, and the identification of novel genetic variants in 26% of the cases included. Our results demonstrate that this approach could be an accurate, reproducible, and reliable tool in the rapid genetic diagnosis of IBCDs.

P762

APPLICATION OF A MOLECULAR DIAGNOSTIC ALGORITHM FOR HEMOPHILIA A AND B USING NEXT-GENERATION SEQUENCING OF THE WHOLE F8, F9 AND VWF GENES

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Background: Molecular diagnosis should be performed for all patients with hemophilia A (HA) and/or B (HB), highlighting any excess risk of inhibitor development associated with specific mutations, and enabling carrier testing for female relatives and prenatal or possible pre-implantation genetic diagnosis or to distinguish von Willebrand disease (VWD). Currently, Next-Generation Sequencing (NGS) offers a powerful approach that enables the simultaneous exploration of exons, introns and regulatory regions of various genes.
Aims: To evaluate the usefulness of a molecular diagnostic algorithm for hemophilia A and B based on NGS to analyze full-length *F8*, *F9* and *VWF* genes.
Methods: Overall of 100 DNA samples were analyzed. Ninety-five unrelated hemophilic patients (HA=78 and HB=17) and 5 females for her hemophilia carriers' status in whom the index case were unknown. Our molecular algorithm first step catalogues severe HA patients carrying intron 22 or intron 1 inversions detected by long range PCR. In a second step, severe HA patients without previous alterations as well as rest of different types of FVIII or FIX deficiency and female carriers were considered for NGS studies. (Figure 1). Whole regions (exonic, flanking, regulatory and intronic) were sequenced by the NGS enrichment target. In those patients without pathogenic variants, multiplex ligation-dependent probe amplification (MLPA) was performed.
Results: In the global analyses, genotyped were identified in 94 out of 100 samples (94%). Fourteen novel variants were detected. Only in 6 mild HA patients, pathogenic variants were not found. All severe HA patients (n=20) were genotyped. In 11 of them, IVS22 were confirmed (55%). None IVS1 was found. In the remaining 7 out of 8 patients (IVS22 and IVS1 negative), genetic variants were found by NGS analyses and it allowed us to detect 3 nonsense, 2 microdeletion, 1 duplication and 1 missense pathogenic variant. MLPA approach reached a complex mutation (gross deletion and duplication) in the last severe HA patient. Most of HA patients included were mild/moderate phenotype (n=54 and n=4; 75%). In 52 out of 58 patients (90%), missense pathogenic variants were found. In 5 of them, a new accurate diagnose was established due to detection of pathogenic *VWF* variants: one case with a VWD type 2N, one carrier of VWD type 2N and 3 cases of VWD type 1. In the remaining 6 cases, intronic variants are being analyzed. About HB patients, genotype was confirmed in all patients (n=17). All variants were missense except one nonsense variant. Most of them (n=10; 63%) affected to the catalytic domain. About hemophilia carrier status, 5 females were included because their index cases were not known. Three pathogenic variants were confirmed in *F8* gene (IVS-22, p.Arg427* and c.5374-19delT; IVS-16), in three of them. In the remaining two symptomatic females (mild FVIII deficiency), a pathogenic variant (c.2561G>A; p.Arg854Gln) in exon 20 of *VWF* gene was found. Accordingly, a new type 2N-VWD diagnose was established.

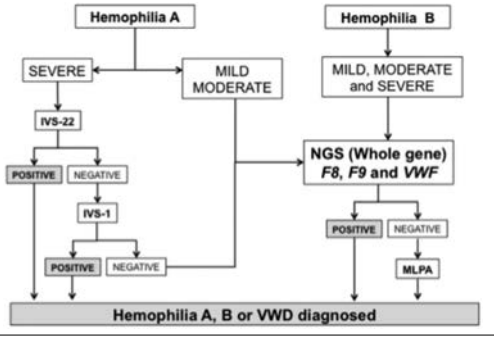

Figure 1.

Summary/Conclusions: The use of this molecular-algorithm approach allowed us to identify the causative variants of the HA and HB patients, correctly redefine VWD and to establish the status of female carriers.
Pfizer supported this study.

P763

ASSAY DISCREPANCY IN MILD HAEMOPHILIA A PATIENTS WITH MUTATIONS IN FVIII A DOMAIN

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Background: Significant discrepancy between one-stage clotting assay (FVIII:C1) and chromogenic method (FVIII:CR) for FVIII activity measurement is common in mild haemophilia A (HA) patients. Different missense mutations are identified in families with discrepant HA. Such mutations are located mainly within exons encoding C2 and A2 domains of FVIII. Missense mutations in A2 domain can cause instability of molecule leading to reduced FVIII:CR. The differences in FVIII levels may lead to an incorrect biochemical phenotype and diagnosis of haemophilia. The global haemostasis tests may represent an option for better prediction of the mild HA clinical phenotype.
Aims: Genetic background was determined in patients with discrepant FVIII activity in Slovenian patients with mild haemophilia A. The structural elements in FVIII molecule were characterised. Further specific and global coagulant assays were investigated to determine their usefulness.
Methods: *F8* gene mutations were determined with Sanger sequencing and analyzed with *in silico* methods. FVIII activity tests were performed with one stage clotting assay (APTT-SP reagent and FVIII deficient plasma, both Instrumentation laboratory-IL, USA) on ACL TOP 500 instrument (IL, USA) and chromogenic assay (Electachrome Factor VIII, IL, USA) using spectrofotometric manual method (Multiskan EX, Thermolabsystem, Finland). FVIII antigen (FVIII:Ag) was determined with Asseracgrom FVIII:Ag kit (Stago, France). APTT wave clot analysis was performed on ACL TOP 500 instrument and thromboelastometry on ROTEM®delta instrument (Pentapharm GmbH, Germany).
Results: Twelve patients from 7 families presented with FVIII:C1 measurement significantly higher than those obtained with FVIII:CR method. FVIII activity ratio range was from 2.6-4.2. The p.Ser308Leu variant in A1 domain (previously described) was identified in seven patients. All of them had reduced FVIII:C1 ranged from 21 to 48% and normal FVIII:Ag ranged from 52 to 112%. Three novel missense variants clustered in A2 domain (p.Val670Phe, p.Tyr683His, p.Asp685Gly) were associated with fourfold discrepancy. Four patients with those variants had FVIII:Ag from 26 to 62% however, they would be classified as having moderate form of HA using FVIII:CR method. All three genetic variants are located at the interface between A2 and A3 domain (Figure 1). APTT was normal in 5 patients carrying the p.Ser308Leu variant. The wave clot analysis shows that 92% of our patients demonstrated normal acceleration of APTT reaction (max2 parameter) in the contrast with nondiscrepant HA, where max2 is normal in only 17% (data not shown). For INTEM test of ROTEM analysis, clotting time has sensitivity of 42%. Clinical data classified all our patients as mild haemophiliacs with bleeding episodes provoked only by surgical interventions or trauma.

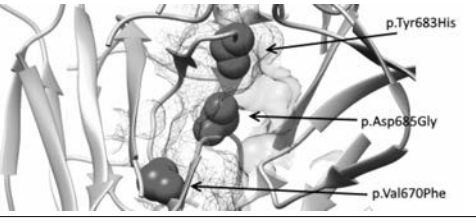

Figure 1. Close-up view of the FVIII A2 (orange) – A3 (magenta) domain interface. Amino acids at position 670, 683 and 685 contribute to FVIII interdomain.

Summary/Conclusions: Three novel genetic variants were described causing a discrepant biochemical phenotype of HA. The nature and position of variants are strongly associated with phenotype and contribute to the FVIII molecule instability. High results of FVIII:Ag showed that the endogenous synthesis and secretion of FVIII is less affected. The APTT test could be in normal range in discrepant patients, however, wave clot analysis shows good differentiation between discrepant and nondiscrepant patients and could provide additional information in managing HA. ROTEM poorly discriminates between normal individuals and mild HA patients and the method is unlikely to be helpful in discrepant HA identification. One-stage and chromogenic method should be used in diagnostics of non-severe HA to avoid the misdiagnosis and to complement the genetic findings.

P764

ARTHROPATHY IN PATIENTS WITH MODERATE AND SEVERE VON WILLEBRAND DISEASE

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Background: The 'Willebrand in the Netherlands' study group has previously

published that almost a quarter of patients with moderate and severe Von Willebrand disease (VWD) reported joint bleeds. Those bleeds had a negative impact on health related quality of life and joint integrity, according to patient-reported and retrospective medical file data. However, besides that, little information is available for VWD patients on the prevalence and severity of arthropathy and its influence on joint function and daily life activities.
Aims: To assess the prevalence and severity of arthropathy and its impact on joint function and daily life in moderate and severe VWD patients (trial number NTR 4548).
Methods: Dutch patients with moderate and severe VWD (VWF activity<30 IU/dL and <10 IU/dL, respectively) and documented treatment for at least one joint bleed were invited to participate. The same number of controls (moderate and severe VWD patients without joint bleed treatment) were selected and matched for age (±2 years), gender and FVIII level (±10%). A single experienced physiotherapist conducted the Haemophilia Joint Health Score (HJHS, 0-124). X-rays were made from all joints with prior bleeds, contralateral joints and one control joint. One radiologist scored the X-rays according to Pettersson (PS, 0-13 per joint). Arthropathy was defined as a clinical HJHS score ≥3 or PS>0. All participants completed the Haemophilia Activity List (HAL, 0-100) questionnaire. The Visual Analogue Score (VAS, 0-10 cm) was used to assess joint pain.
Results: 48 patients and 48 controls were included, 60% males, mean age 46 years (range 18-80). Mean FVIII levels were 26 IU/dL in the patients and 31 IU/dL in the controls (p=0.19). More patients had type 3 VWD (19/48 *vs* 3/48 controls). In the control group of patients without documentation on joint bleed treatment, 14/48 patients did report one or more joint bleeds but none of them more than five. In contrast, 56% of the 48 patients had more than five joint bleeds. Arthropathy occurred in 37/48 (77%) patients and 35/48 (73%) controls (p=0.51). Overall, arthropathy occurred in both severe (47/64) and moderate VWD (25/32) and in all three VWD types (22/28 type 1; 30/46 type 2; 20/22 type 3). The median HJHS was significantly higher in the patients compared to the controls (5 *vs* 1.5, p<0.01, maximum score 47 *vs* 29). PS>3 occurred in 2 controls compared to 12 patients (p<0.01) and overall most in type 3 VWD patients (9/22 type 3 *vs* 3/46 type 2 *vs* 2/28 type 1). The total HAL score as well as the scores on the three separate HAL components were significantly lower for the 48 patients compared to the controls (median scores HAL Sum: 88 *vs* 100, p<0.01; Upper Extremity Activities: 93 *vs* 100, p=0.01, Basic Lower Extremity Activities: 87 *vs* 100, p<0.01; Complex Lower Extremity Activities: 80 *vs* 100, p<0.01). Clinically relevant joint pain (mean VAS score >3) was reported by 17 patients and 9 controls (p=0.07).
Summary/Conclusions: Arthropathy, according to our stringent definition, was seen in 77% of patients with moderate and severe VWD treated for joint bleeds. Notably, arthropathy was also found in 73% of matched control VWD patients without joint bleed treatment. Joint function and –integrity in the VWD patients treated for joint bleeds was affected, consistent with higher HJHS and joint X-rays scores. These patients experienced a significant impact on daily life activities (HAL, both upper and lower extremities) and 35% also reported clinically relevant joint pain (VAS).

P765

A COMPREHENSIVE NEXT GENERATION SEQUENCING TEST FOR THE DIAGNOSIS OF INHERITED BLEEDING, THROMBOTIC AND PLATELET DISORDERS

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Background: Inherited bleeding, thrombotic and platelet disorders (BPDs) are rare diseases affecting approximately 300 individuals per million births. With the exception of haemophilia and von Willebrand disease patients, a molecular analysis for BPD patients is often unavailable. Many specialised tests are usually required to reach a putative diagnosis and they are typically performed in a slow, step-wise manner to control costs. The results of these tests may be used to prioritise genes for Sanger sequencing if a genetic diagnosis is required. This approach causes significant delays and a conclusive molecular diagnosis is often never reached, which can compromise treatment and impede rapid identification of affected relatives.
Aims: Our aim was to design a platform through which accurate and rapid testing of inherited BPDs would be possible.
Methods: We designed a high-throughput sequencing (HTS) platform targeting all 87 known BPD disease genes. The platform can call single nucleotide variants, short insertions/deletions and large copy number variants (though not inversions), which are subjected to automated filtering for diagnostic prioritisation.
Results: We sequenced 159 and 141 samples respectively from individuals with and without previously known causal variants. Among the latter group, 61 cases had phenotypes strongly indicative of a particular molecular aetiology while the remainder had an aetiology that was *a priori* highly uncertain. All the previously detected variants were recapitulated and, when the aetiology was suspected but unknown, a molecular diagnosis was reached in 56 of 61 cases.
Summary/Conclusions: The ThromboGenomics platform provides a comprehensive and affordable DNA-based test to diagnose patients suspected of having a known inherited BPD thereby significantly reducing the current diagnostic delay.

P766

THE ROLE OF CD72 IN THE REGULATION OF B CELL ACTIVATION THROUGH CD40 IN PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: CD72 is considered to be an important B cell co-receptor for its prominent role in immune regulation. Previous studies show that CD40 signals play a more important role in mechanism of primary immune thrombocytopenia (ITP) by activating B cell and inducing B-cell growth with Ig secretion. NF-κB activation is a pivotal pathway in CD40 signaling. However, the effect of CD72 on B cells activated by CD40 in ITP remains unknown.
Aims: This study aimed to explore the effect of CD72 on B cell activation through CD40 in ITP.
Methods: Activation-associated surface markers (CD80, CD86 and CD40), the proliferation, apoptosis, plasmablasts and platelet-associated IgG were analyzed by flow cytometry in patients with active ITP and health controls. In cultures vitro, peripheral blood mononuclear cells (PBMCs) were stimulated by anti-CD40 in the presence or absence of CD40L, IL-4 and IL-21, autologous platelets. Production of IgG and IgM in the culture supernatants was determined by enzyme-linked immunosorbent assay. The levels of NF-κB P65 and IκBα mRNA were assessed by real-time quantitative polymerase chain reaction.
Results: Our data showed that CD40 expression was significantly decreased on CD19+ B cells after CD72 ligation in ITP patients compared to controls. The higher levels of activation markers CD80, CD86 on CD19+ B cell stimulated by anti-CD40 was reduced by anti-CD72 in ITP patients and controls. CD40 stimulation significantly promoted the survival of CD19+ B cells, and reduced the apoptosis of CD19+ B cells in ITP patients compared to that observed in controls. CD72 ligation corrected the effect of CD40 stimulation on apoptosis and proliferation of B cells. The CD40-mediated the differentiation of B cells into plasmablasts was blocked by CD72 ligation in ITP patients and controls. However, the role of CD72 on B cell differentiation was stronger in ITP patients than in controls. CD72 ligation had only a minor effect on reducing platelet-associated IgG in ITP patients. Following stimulation with anti-CD40 and IL-21, synthesis of IgM by B cell was diminished in the presence of anti-CD72, whereas the IgG levels were barely reduced. The reduced ratio had no difference in ITP patients and controls.CD72 signaling significantly reduces NF-κB P65 and IκBα expression at the mRNA levels in PBMCs activated by anti-CD40 in ITP patients compared to controls.
Summary/Conclusions: These findings indicate that CD72 is a key molecule in regulating B cell activation, proliferation, apoptosis and antibody secretion mediated by CD40 signaling in ITP patients.Thus, CD72 may be involved in the pathogenesis of ITP and antagonizing CD72 could be a novel strategy for the therapy of ITP.

P767

IMPACT OF BRUTON'S TYROSINE KINASE INHIBITORS ON COLLAGEN-INDUCED PLATELET AGGREGATION: A PHARMACOKINETIC/PHARMACODYNAMIC PERSPECTIVE

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Background: Bruton's tyrosine kinase (BTK) is expressed in platelets (PLTs) and mediates collagen-induced aggregation of PLTs. TEC, a member of the TEC family kinases, is also expressed in human and mouse PLTs. Studies in PLTs from BTK-deficient X-linked agammaglobulinemia patients (Quek 1998; Lipsky 2015), and BTK and/or TEC KO mice suggest that the 2 kinases likely possess redundant functions in collagen-induced aggregation; however, BTK is thought to play a more dominant role than TEC, as BTK deficiency alone reduces robust aggregation at lower collagen concentrations (Atkinson 2003). Ibrutinib (ibr) is a once-daily, first-in-class, covalent inhibitor of BTK approved for various B-cell malignancies. Additional BTK inhibitors (BTKis) have been evaluated in clinical trials, with bleeding as a common adverse event (AE) observed not only for ibr but also for ONO-4059 (Walter 2016), ACP-196 (Byrd 2016), and BGB-3111 (Tam, ASH 2015). Early-phase clinical data reported to date for these follow-on molecules have been limited due to small patient numbers and minimal follow-up, where ACP-196 and ONO-4059 had bleeding events of petechiae and contusion among the most common AEs (hematoma was also frequently reported with ONO), and BGB similarly had a 33% bleeding rate. These compounds are potent BTKis with additional activity against TEC;

therefore, inhibition of BTK or both BTK and TEC may contribute to bleeding.
Aims: This study investigated how ibr and other clinical-stage BTKis, with a focus on selectivity over TEC, impact PLT function and their potential implication in bleeding events in patients on BTKi therapy.
Methods: On-target potency and selectivity of BTKis over TEC were investigated with recombinant enzymes using 3 different assays: LabChip® MSA, TR-FRET, and ^{33}P HotSpot activity. Cell activity was tested in human whole blood for BCR activation and in PLTs by light transmission aggregometry (LTA). The activity of BTKis was also evaluated by measuring cellular TEC phosphorylation levels in the presence of BTKis.
Results: Ibr had potency of approximately 1 nM or below with 0.7- to 11-fold selectivity for BTK over TEC in all 3 biochemical assays. ACP-196 had variable potency (3-135 nM) towards BTK; however, the selectivity profiles against TEC were similar to ibr, with <3-fold differences in the ratios (Table 1). Ibr, ACP-196, and ONO-4059 had IC_{50}s 20-40 nM in BCR assays, but in LTA with PLTs from healthy human donors, a 3-fold difference was observed compared with ibr (Figure 1). However, this difference in patients with CLL, in whom ACP-196 is administered twice daily, plasma C_{max} levels were approximately 1-2 µM, 3- to 5-fold higher than those after ibr (0.2-0.4 µM) in CLL patients (Table 2). Moreover, inhibition of TEC phosphorylation in PLTs was similar between ibr and ACP-196.

Table 1. Recombinant kinase assays.

	Ibrutinib (nM)			ACP-196 (nM)		
	BTK	TEC	*Ratio*	BTK	TEC	*Ratio*
Kinase Activity IC_{50} (LabChip MSA/TR-FRET/ ^{33}P Hotspot)	0.3/1.1/0.1	0.5/0.8/1.1	*1.7/0.7/11*	3.0/135/33	8.0/10/130	*2.7/<0.1/3.9*
Binding affinity	0.8	0.9	*1.1*	10	8	*0.8*

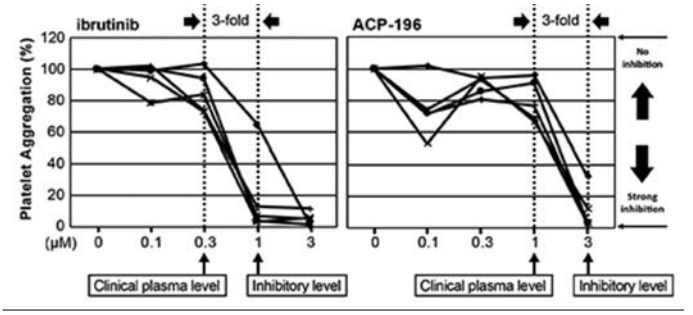

Figure 1. Platelet aggregation after ibrutinib or ACP-196 treatment.

Table 2. Pharmacokinetic parameters.

	Dose	$C_{max,ss}$ (µM)	AUC_{ss} (µM•hr)	LTA-induced inhibition (µM)
Ibrutinib	420 mg QD: CLL	0.3	1.7	0.3-1
ACP-196*	100 mg BID: CLL	1.8	2.0	1-3

*Byrd et al. *N Engl J Med.* 2016.

Summary/Conclusions: BTKis tested did not show a definitive selectivity advantage against TEC in enzymatic or cellular phosphorylation assays. When compared at the clinically and pharmacologically relevant plasma concentrations, both ACP-196 and ibr had similar effects on PLT aggregation. These results appear consistent with clinical data on bleeding events for both ACP-196 (Byrd 2016) and ibr (Byrd 2014). Only double-blinded placebo-controlled studies can address the potential risks and benefits of these subtle differences.

P768

QUALITATIVE AND QUANTITATIVE ANALYSIS OF INTRAHEPATIC BLOOD FLOW CHANGES IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA (HHT)

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Background: Hereditary hemorrhagic telangiectasia (HHT) is a rare inherited abnormality characterized by systemic vascular dilatation resulting in arteriovenous fusion including hepatic vascular malformations (HVaMs) with the risk of bleeding events. Due to current efforts to sub-classify a high risk group of asymptomatic patients with HVaMs on the one side and the experienced benefits of bevacizumab on the other side, cost-effective screening of HHT patients for hepatic involvement become more and more attractive.
Aims: To analyze HVaMs in a group of patients (n=18) applying CEUS and adding quantitative time intensity curve analysis (TIC analysis).
Methods: US/CEUS imaging data of 18 patients (2 men/16 women; mean age 59.4±12.1y; range, 21-83y) diagnosed with HHT in the Ear-Nose-Throat (ENT) department of the University Hospital of Regensburg between Jan 2015 and Jan 2016 were retrospectively analyzed with a high-end US scanner. SonoVue 1.0-2.4 ml was applied i.v. as contrast agent. Findings on B-Mode, color coded Doppler sonography (CCDS) and Power Doppler (PD) were analyzed in respect of suggested sonographic grading and staging criteria and by considering the "color spots". In order to perform TIC analysis eight regions of interest (ROIs) with a diameter of 5 mm were placed in the perfused hepatic artery, portal vein, shunt region and hepatic parenchyma, two ROIs per region. For quantitative investigation, the area under the curve (AUC) and time to peak (TTP) was considered.
Results: HVaMs could be identified in all patients, being localized particularly in the central area, segment 8 and the left lobe of the liver. All patients showed dilated hepatic artery (mean diameter 11,5 mm). Another major Caselitz criteria "intrahepatic arterial hypervascularization" was found in only 61,1% of the cases, while minor criteria "tortuous course of the extrahepatic artery" was described in 83,3%. The other 3 minor criteria (Vmax and RI hepatic artery; Vmax portal vein) were on average somewhat above the norm. Altogether, in only 55,6% of our patients the two major criteria were positive, while 16,7% did not reach diagnostic criteria for HVaMs. Buonamico reported higher sensitivity for "color spots" compared to Caselitz criteria, but 27,8% of our patients showed no spots at all. With regard to sonographic staging by Buscarini L. and grading criteria by Buscarini E., stage III and grade 3 could be demonstrated most frequent (~ 40%). We found arterioportal HVaMs in more than half of the patients (61,1%), however always in combination with arteriovenous malformations. In 16,7% even portovenous malformations were associated. HVaMs with early hyperenhancement during the arterial phase could be demonstrated by CEUS in all patients. Significant lowest TTP and highest AUC were detected in the hepatic artery and highest TTP and lowest AUC in the hepatic parenchyma and the portal vein. TTP and AUC values of the shunt region were between those of hepatic artery and portal vein. US/CEUS covered all subtle patterns and proved more accurate in diagnosis of challenging lesions like high-flow angiomas when compared to MRI, CT or angiography (Figure 1).

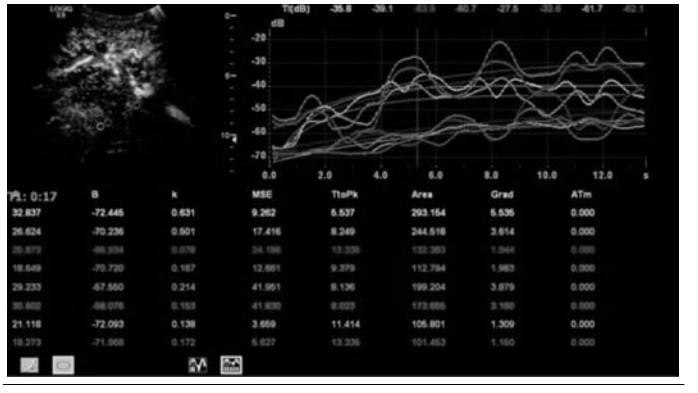

Figure 1.

Summary/Conclusions: For the first time we analyzed CEUS findings of a group of 18 HHT patients regarding macro- and microcirculation. Our data demonstrate significant differences in TTP and AUC values in the four selected regions: hepatic artery, shunt region, portal vein and hepatic parenchyma.

P769

C-EDGE IBLD: EFFICACY AND SAFETY OF ELBASVIR/GRAZOPREVIR (EBR/GZR) IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS (HCV) INFECTION AND INHERITED BLOOD DISORDERS (IBLD)

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Background: Complications from chronic HCV infection remain a major cause of morbidity and mortality among individuals with IBLD, including those with hemophilia (HEM), beta thalassemia (BTHAL), von Willebrand disease (VWD), and sickle cell anemia (SCA). Inability to tolerate ribavirin and frequent comorbidities have limited HCV treatment options in these patients. The efficacy and safety of a once-daily, fixed-dose combination of EBR 50 mg (NS5A inhibitor) and GZR 100 mg (NS3/4A protease inhibitor) has been demonstrated in a broad population of HCV-infected patients and supported evaluation in the IBLD population.

Aims: To assess the safety and efficacy of EBR/GZR in patients with HCV infection and SCA, BTHAL, or HEM/VWD.
Methods: C-EDGE-IBLD is a double-blind, placebo-controlled study that randomized treatment-naïve and peg-interferon/ribavirin treatment-experienced (TE) HCV genotype (GT)1, 4, or 6-infected patients in a 2:1 ratio to either an immediate-treatment group (ITG; 12 weeks of EBR/GZR) or deferred-treatment group (DTG; 12 weeks of placebo followed by EBR/GZR). Randomization was stratified according to cirrhosis status and IBLD group, defined as 1) HEM (A or B) or VWD, 2) BTHAL, or 3) SCA. The primary endpoints for this study were proportion of patients in the ITG who achieved an SVR12 (HCV RNA <15 IU/mL 12 weeks after study treatment completion) and a comparison of the safety and tolerability of EBR/GZR in the ITG relative to placebo treatment in the DTG. All patients provided written informed consent.
Results: Mean age was 44 years; 75% male; 18% black; 40% GT1a; 44% GT1b; 11% GT4; 26% cirrhotic; 50% TE; 6% HIV/HCV co-infected; 43% HEM/VWD; 38% BTHAL; 18% SCA. SVR12 was achieved by 93.5% (100/107) patients receiving EBR/GZR in the ITG. Of the 7 patients who failed to attain SVR12, 6 patients relapsed and 1 patient discontinued treatment early with noncompliance and did not participate further in the study. SVR12 was high in all blood disorder groups (Table 1). There were 3 (2.9%) patients with serious adverse events (SAEs) (1 drug-related, 2 related to IBLD) in the ITG with no discontinuations due to safety events. In the DTG, 1 patient discontinued due to AEs and 1 withdrew consent; 6/52 (11.5%) had SAEs (1 drug-related, 3 related to IBLD). No patient in either arm prematurely discontinued from the trial due to worsening of underlying IBLD. There was 1 hepatic event of clinical interest (alanine aminotransferase >3× baseline and >100 U/L) in each arm.

Table 1. SVR12 in the ITG (full analysis set population).

Description	SVR12† [n/N (%)]
Overall ITG	100/107 (93.5)
HEM/VWD	42/47 (89.4)
BTHAL	40/41 (97.6)
SCA	18/19 (94.7)
Cirrhosis	26/26 (100)

†Hepatitis C virus RNA IU/mL 12 weeks after completion of treatment.

Summary/Conclusions: EBR/GZR is well tolerated and effective in patients with HCV GT1, 4, or 6 with or without cirrhosis with IBLD.

P770

DOES HIGH HISTONE LEVEL LEAD US TO DETERMINE COAGULOPATHY EARLY AFTER TRAUMA IN PEDIATRIC PATIENTS?
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Background: Acute traumatic coagulopathy occurs after trauma with impairment of hemostasis and activation of fibrinolysis. Some endogenous materials may take role on this failure of coagulation system. Extracellular histone is one of the molecules taking attention recently.
Aims: This study investigated the association between plasma histone complexed DNA fragments (hcDNA) and coagulation abnormalities in pediatric trauma patients.
Methods: This case control study conducted in pediatric patients with multipl trauma or isolated brain injury. Fifty trauma patients and 30 healthy controls were enrolled. Demographic data, anatomic injury characteristics, coagulation parameters, computerized tomography findings, trauma and thrombosis scores were recorded. Venous blood samples for hcDNA were collected into two hours after injury and assessed by enzyme linked immunosorbent assay.
Results: Eighteen patients had isolated brain injury, 32 patients had multiple trauma. Twenty seven patients had mild, 11 patients had moderate and 12 patients had severe trauma according to Glasgow Coma Scale. Although no patient had overt disseminated intravascular coagulation, 13 patients had acute coagulopathy of trauma shock (ACoTS). Plasma hcDNA levels were significantly higher in trauma patients than healthy controls (0.474 AU and 0.145 AU, respectively). There was an association between plasma hcDNA levels and trauma severity according to Glasgow Coma Scale, Pediatric Trauma Score, Injury Severity Score. AcoTS patients had higher plasma histone levels than without AcoTS (0.703 AU and 0.398 AU; respectively). Plasma hcDNA levels were significantly correlated with the International Society of Thrombosis and Hemostasis score, length of intensive care unit stay, prothrombin time, D- dimer levels, and also there were negative correlations with fibrinogene levels and pediatric trauma scores.
Summary/Conclusions: This study indicated for the first time that hcDNA levels increase in pediatric trauma patients associated with coagulopathy. Further studies are needed to clarify the role of high hcDNA levels in determining the functional significance of these changes in disseminated intravascular coagulation and predicting mortality.

LB771

AAV-MEDIATED GENE THERAPY FOR HEMOPHILIA B-EXPRESSION AT THERAPEUTIC LEVELS WITH LOW VECTOR DOSES
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Background: Published data from Nathwani *et al.* (NEJM 2014) demonstrated long-term expression of Factor IX in men with hemophilia B infused with an AAV8 vector expressing wild-type Factor IX. However, levels of expression ranged from 1.4%>2.2% normal at the lowest dose (2 x 10^{11} vector genomes [vg]/kg body weight) to 2.9-7.2% normal at the highest dose (2 x 10^{12}vg/kg). Moreover, 4/6 subjects infused at the highest dose required a course of prednisolone to reduce rising transaminases associated with the highest dose (but not observed at the lower doses of 2 x 10^{11} or 6 x 10^{11} vg/kg). Data from a natural history study of patients with hemophilia by den Uijl *et al.* suggest that circulating levels of ~12% are required to eliminate spontaneous joint bleeds (Haemophilia 2011).
Aims: We sought to develop a high specific activity vector that would drive therapeutic levels of FIX activity at doses low enough to avoid the need for immunomodulation with steroids.
Methods: We developed a vector containing: 1) a novel bioengineered AAV capsid with tropism for liver; and 2) a Factor IX expression cassette that carries a strong liver-specific promoter driving the expression of FIX Padua (R338L). The Padua variant shows an ~8-fold increase in specific activity compared to wild-type FIX (Simioni *et al.*, NEJM 2009). Based on studies in non-human primates, we predicted that therapeutic FIX activity levels, >10%, would be attained at doses as low as 5 x 10^{11} vg/kg. We further predicted that the ability to observe therapeutic levels of FIX activity at lower doses of vector would limit potential need for a course of immunomodulation with steroids.
Results: Here we report data on the first three subjects with severe hemophilia B infused with this novel vector. The first two subjects, ages 23 and 18 respectively, had no prior history of liver disease, while the third, age 47, had a history of HCV infection but had cleared spontaneously. These subjects had been screened for neutralizing antibodies to the novel AAV capsid and found to be negative. Subjects were infused intravenously with *SPK-FIX* at a dose of 5 x 10^{11} vg/kg over a period of ~1 hour. FIX activity levels (shown in Figure 1) in the first two subjects have plateaued at 28% and 30% respectively at 18 and 7 weeks post infusion, while subject #3 is currently at 16% 3 weeks after infusion. Subject #3 treated himself with an extended half-life product for a suspected ankle bleed 2 days after vector infusion; other than this there have been no other factor infusions and no bleeds in the 28 cumulative weeks of observation. There have been no transaminase elevations higher than 1.5 times upper limit of normal, and steroids have not been administered to any subjects thus far. ELISPOTs were used to monitor T cell responses to AAV and to FIX in all subjects and have shown no or very low responses. Of note, the time course of rise in Factor IX levels to a plateau level has been remarkably consistent among the three subjects to date.

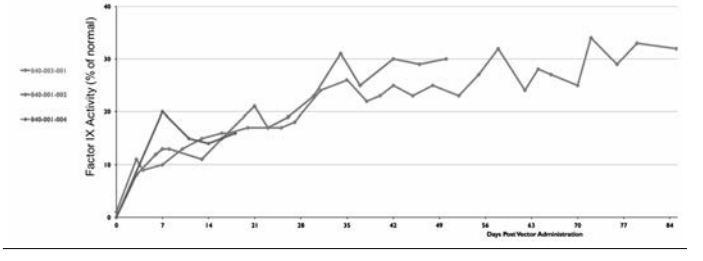

Figure 1.

Summary/Conclusions: Conclusion/Summary: We report the first clinical results using a novel bioengineered AAV capsid expressing a high specific activity FIX transgene. This has led to plateau FIX activity levels of 28% and 30% in the first two subjects infused, with no factor use since vector infusion. The development of a vector that can direct high level clotting factor expression at low doses, so that immunosuppression is not required, represents an important goal for liver-directed gene therapy.

Coagulation - Basic Research

P771

RECOMBINANT THROMBOMODULIN AMELIORATES HEMATOLOGICAL MALIGNANCY-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION MORE PROMPTLY THAN CONVENTIONAL THERAPY WITHOUT CAUSING SEVERE HEMORRHAGIC EVENTS

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Background: Disseminated intravascular coagulation (DIC) is a lethal complication in patients with hematological malignancies. Although standard therapy against DIC remains to be established, soluble recombinant thrombomodulin (rTM), which serves as a receptor for thrombin, has been developed as a promising anti-DIC agent. However, the position of rTM needs to be determined in the treatment of DIC associated with hematological malignancy.
Aims: We retrospectively compared outcome of hematological malignancy-related DIC treated with rTM or other conventional anticoagulant therapies in order to clarify the effectiveness of rTM.
Methods: Consecutive DIC episodes of 217 in 195 patients with hematological malignancies (AML except for APL, 69; APL, 26; ALL, 27; NHL, 44; myeloma, 12; CML, 7; other; 10) hospitalized between January 2004 and Dec 2015 in University of Tsukuba Hospital were analyzed using medical records. Diagnosis of DIC was based on the DIC scoring system proposed in the Japanese Ministry of Health and Labor Welfare criteria (Kobayashi *et al.,* Bibl Haematol 1983). DIC was induced by hematological malignancy itself (h-DIC) or severe infection secondary to hematological malignancy (i-DIC) in 140 and 77 episodes, respectively. In 125 episodes, 380 units/kg/day of rTM was administered intravenously from the onset of DIC for median of 6 (range, 1-41) days. Other DIC episodes were treated with conventional anticoagulant therapies (low molecular weight heparin, 65; gabexate mesilate, 16; other anticoagulants, 11) for median of 11 (range, 1-30) days. Every anticoagulant therapy was accompanied by treatment for DIC-causing disease. We compared recovery time from DIC (the day when the DIC score was decreased to 5 or less), overall survival, and severe hemorrhagic events related to the treatment, between rTM- and conventional anticoagulant-treated groups.
Results: Bleeding tendency was documented in 63 DIC episodes at the onset. In h-DIC, recovery from DIC was significantly more prompt in rTM-treated group than conventional therapy group with the recovery rates of 55% (95% CI: 43-65) and 32% (95% CI: 21-44), respectively at day 7 after the therapy initiation (P=0.03, Figure 1). By contrast, recovery from i-DIC was significantly worse than that from h-DIC, and was not influenced by anticoagulant therapies; recovery rates from i-DIC at day 7 were 23% (95% CI: 13-36) in the rTM-treated group and 33% (95% CI: 17-50) in the conventional therapy group (P=0.6). Day 60 overall survival rates in h-DIC were 81% (95% CI: 69- 88) and 79% (95% CI: 65 - 88) in the rTM-treated and conventional therapy groups. In i-DIC, on the other hand, 35% (95% CI: 21-49) and 38% (95% CI: 32 - 55) survived with the rTM and conventional therapies, respectively. Severe hemorrhagic events that led to discontinuation of anticoagulant therapy was significantly less in the rTM-treated group (3%; 95% CI, 0-7) compared with that in the conventional therapy group (15%; 95% CI: 5-23; P=0.02).

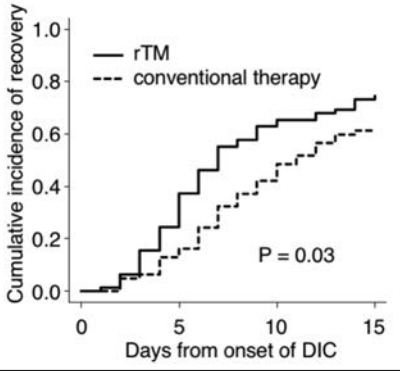

Figure 1. Recovery from h-DIC.

Summary/Conclusions: The recovery from h-DIC treated with rTM was more prompt compared to that with conventional anticoagulant therapy, although survival was not influenced by the therapy. We emphasize that rTM can be an effective anti-DIC agent without causing adverse hemorrhagic events even in DIC cases with preexisting bleeding tendency. However, the outcome was still significantly worse in i-DIC secondary to hematological malignancies even after introduction of rTM. Further development of anticoagulant therapy is required particularly for the control of i-DIC.

P772

EXTRACELLULAR HISTONES ENHANCE FACTOR XA MEDIATED PROTHROMBIN ACTIVATION WITHOUT PHOSPHOLIPID REQUIREMENT

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Background: Thrombin generation *in vivo* is central to coagulation activation and its dysregulation is pivotal to the development of disseminated intravascular coagulation (DIC) and multiple organ failure (MOF). Typically, thrombin is generated when the prothrombinase complex, composed of activated factor X (FXa), activated co-factor V (FVa) and phospholipids, cleaves prothrombin in the presence of calcium. Whilst histones form the basis of chromatin within cells, circulating histones released after extensive cellular injury are significantly elevated in critically ill patients with associations to enhanced thrombin generation. However, the underlying pathophysiological mechanism remains to be fully elucidated.
Aims: To characterise the mechanistic role of elevated extracellular histones in enhancing thrombin generation in critical illness.
Methods: Histone-prothrombin interactions were determined by binding assays. Thrombin generation was evaluated by *in vitro* prothrombin cleavage and thrombin generation assays.
Results: Here, we demonstrate that extracellular histones promote thrombin generation by acting as a co-factor to FXa. Unlike FVa which requires a phospholipid surface to form functional prothrombinase complexes, histones can substitute for FVa in the absence of phospholipids. The addition of histones to $FV^{-/-}$ deficient plasma restored thrombin generation, suggesting that histones can bypass FVa to induce thrombin generation. Mechanistically, histones directly bind to prothrombin (H3 [$Kd=6.8x10^{-7}$ M] and H4 [$Kd=7.0x10^{-7}$ M]), to facilitate FXa-induced prothrombin cleavage and thrombin generation (H4 [12.25±1.25 fold] and H3 [8.82±0.67 fold]). FXa levels are the limiting factor of histone-enhanced thrombin generation since this process was inhibited in $FX^{-/-}$ plasma unless exogenous FXa was added. Specifically, using either heparin or specific anti-histone antibodies to block histones, histone-prothrombin interactions, prothrombin cleavage and subsequent thrombin generation were significantly reduced.
Summary/Conclusions: This novel role for extracellular histones in increasing thrombin generation, independent of FVa, may offer insight into the underlying pathological mechanism for systemic dissemination of thrombin generation in critical illness. Importantly, it identifies circulating histones as a potential target for therapeutic intervention in reducing the development of DIC and subsequent MOF in critical illness.

P773

PHORBOL 12-MYRISTATE-13-ACETATE INDUCES VON WILLEBRAND FACTOR PROPEPTIDE RELEASE FROM ENDOTHELIAL CELLS FROM 2 DISTINCT COMPARTMENTS

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Background: Von Willebrand factor (VWF) and its propeptide (VWFpp) are stored for secretion within specialized endothelial secretory organelles called Weibel-Palade bodies (WPBs). Release of their content into the vascular lumen through a process called exocytosis enables the endothelium to actively participate in the arrest of bleeding and to slow down and direct leukocytes to areas of inflammation. WPBs undergo exocytosis upon stimulation with agonists that elevate intracellular free Ca^{2+} ($[Ca^{2+}]_i$) or cAMP concentrations.
Aims: Phorbol 12-myristate-13-acetate (PMA) is also a potent VWF secretagogue, and it is generally assumed that PMA-evoked VWF secretion arises solely from WPB exocytosis (the VWF storage compartment). Here we present evidence that PMA activated VWF release comprises an early (0-15 minutes) and a late (>15 minutes) component that originate from distinct intracellular compartments.
Methods: Using live cell imaging of WPB exocytosis in endothelial cells expressing fluorescently tagged WPB probes we have characterized PMA-evoked VWF secretion.
Results: A significant fraction of the early phase of PMA-evoked VWF secretion from human endothelial cells arises from a cycloheximide (CHX)-sensitive (*i.e.*, nascent or non-storage) compartment: stimulation for 15 minutes with either PMA (100 ng/ml) or histamine (100 µM) caused similar amounts of VWF secretion. However, prior inhibition of protein synthesis (10 µM CHX for 24 hours) reduced PMA-evoked secretion by ~70% while histamine-evoked secretion was unaffected. Optical analysis of live cells expressing VWFpp-EGFP targeted to WPBs showed that the first 15 minutes of histamine stimulation was associated with exocytosis of 41.4±4.0% (mean±sem, n=12) of fluorescent WPBs, compared to 8.4±2.1% (n=11) for PMA. The latter was independent of WPB matu-

ration state and for both stimuli, the percentage of fluorescent WPB exocytotic events was unaffected by CHX treatment. This suggests that the early phase of PMA-evoked VWF release originates from a compartment other than WPBs. Since VWFpp is proteolytically cleaved from proVWF in the *trans*-Golgi network (TGN), we focused on this compartment as the potential source of early PMA-evoked secretion. Phospholipase D (PLD) is a key enzyme regulating trafficking from the TGN. Inhibition of PLD with the pan-PLD inhibitor FIPI resulted in a reduction in the early phase of PMA-evoked VWFpp secretion, similar to that seen with CHX, although at later times VWFpp secretion was unaffected. Pre-incubation (10 minutes) with PMA (100 ng/ml) produced a profound shift in the Ca^{2+}-sensitivity of VWF secretion, to close to resting levels.
Summary/Conclusions: Together the data suggest that at early times PMA causes VWF release predominantly from a non-WPB compartment, most likely the TGN, while at later times WPB exocytosis accounts for the majority of secreted VWF. The slow onset in PMA-evoked WPB exocytosis could reflect a time-dependent shift in the Ca^{2+}-sensitivity of exocytosis. Our data help clarify the mechanisms by which PMA induces VWF secretion in endothelial cells, and highlight the need for care when interpreting PMA-evoked secretion data in terms of the underlying mechanisms regulating WPB exocytosis.

P774

NEWLY DIAGNOSED PLASMA CELL DYSCRASIAS ARE ASSOCIATED WITH ENHANCED TF PATHWAY ACTIVATION, THROMBIN GENERATION AND INCREASED CONCENTRATION OF PROCOAGULANT MICROPARTICLES
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Background: Multiple myeloma (MM) and other plasma cell dyscrasias (PCD) as well as the associated immunomodulatory treatments are linked to increased risk of venous thromboembolism (VTE). The identification of patients at VTE risk and the optimization of VTE prevention is an unmet medical need. Elaboration of a risk assessment model (RAM) specific for patients with PCD, which includes biomarkers of hypercoagulability, could improve the management of VTE risk.
Aims: We conducted a longitudinal observational study, to explore the relationship of MM with cellular and plasma hypercoagulability aiming to identify the most relevant biomarkers for use in a RAM for VTE in combination with clinical risk factors.
Methods: Newly diagnosed patients with PCD (n=186) were recruited from July 2014 to Dec 2015; including 27 with monoclonal gammopathy of undetermined significance (MGUS), 40 with asymptomatic multiple myeloma (AMM), 79 with multiple myeloma (MM), 30 with AL-amyloidosis, 8 with Waldenstrom's Macroglobulinemia (WM) and 2 with solitary plasmacytoma. They were compared against 30 healthy age and sex-matched individuals (CG). A systematic compression ultrasound was performed at baseline and at 6-12 months. Blood samples were obtained at diagnosis and at 6-12 months (n=89). Samples of platelet-poor plasma were assessed for thrombin generation (TG) with PPP-Reagent® (TF 5pM and 4 μM phospholipids), P-selectin, D-dimers (D-Di), activated FVII (FVIIa), Tissue Factor (TFa), fibrin monomers (FM), and procoagulant phospholipid-dependent clotting time (Procag-PPL). The upper and lower normal limits (UNL and LNL) were calculated by the mean±2SD.
Results: Median age was 67 years (37-89) and 49% of the population was male. Median time to follow up was 7 months (1-12 months). Among MM cases (n=79) symptomatic VTE rate was 10% (n=8) and mortality rate 7.5%. The events included 2 central venous catheter thromboses, 1 Pulmonary Embolism, 2 Deep vein thromboses, 2 superficial vein thromboses and 1 mesenteric vein thrombosis. Cases had significantly shorter PPL-ct, higher FTa and DDi, TG (increased Peak), heparanase and P-selectin compared to controls (p<0.001). Along the MGUS- AMM- MM continuum, D-Di concentration (p<0.0001), FM concentration (p<0.05), lag time (p<0.027) and MRI were highest in MM patients. Sixty percent of MM patients had Procoag-PPL: below the LNL, in 63% TFa levels were above the UNL, and 31% had MRI lower than LNL. After 6 months of treatment, FTa, DDi, MRI, peak thrombin and P-selectin levels decreased (p<0.05) and TM and ATIII levels increased (p<0.05).
Summary/Conclusions: In patients with PCD, increased procoagulant microparticles of cellular origin is a generalized phenomenon. In addition, patients with MM, are at high risk of VTE and present with significant TF pathway activation and increased TG. A significant fraction, but not all, of the patients present strong signs of plasma hypercoagulability. The finding of high inter-individual variability of TG underlines the heterogeneity of blood coagulation alterations in PCD patients. The data of the prospective part of this study will allow validation of the clinical significance of this finding.

P775

ROLE OF ROTATIONAL THROMBELASTOMETRY (ROTEM®) IN RAPID ESTIMATION OF RIVAROXABAN DRUG LEVEL
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Background: Rivaroxaban, a direct factor Xa inhibitor, allows the use of fixed dosing schemes and eliminates the need for routine coagulation monitoring. However, rapid assessment of the degree of rivaroxaban anticoagulation is required in certain scenarios, *e.g.* major haemorrhage, before thrombolysis or urgent surgery. Clinically significant Rivaroxaban drug levels have been proposed between 30-50ng/mL using a calibrated anti-Xa assay, but the turnaround time for the test is slow. Therefore, rapidly available results using a point-of-care haemostasis test like ROTEM® might be beneficial in these scenarios.
Aims: The aim of this study was to evaluate the role of conventional rotational thrombelastometry (ROTEM®) and modified low tissue factor activated ROTEM® (LowTF-ROTEM®) in estimating rivaroxaban drug level in in-vivo setting.
Methods: In this multicenter study, we analysed 77 patients receiving Rivaroxaban for therapeutic indications including treatment of venous thromboembolism, long-term prevention of recurrent venous thromboembolism and stroke prevention in non-valvular atrial fibrillation. Patients' citrated whole blood samples were tested on the ROTEM® delta analyser using commercially prepared EXTEM reagent according to manufacturer's instructions. Modified low tissue factor ROTEM® test was also performed, using a recombinant tissue factor reagent (Siemen's INNOVIN), used at a dilution of 1:1000 of the normal working concentration used for INR testing. Patients' Rivaroxaban levels (ng/mL) were determined in citrated platelet poor plasma by analysis on the Stago STA-R Evolution analyser, using a chromogenic anti-Xa method (Diagnostica Stago Liquid anti-Xa), paired with specific Rivaroxaban calibrators and controls (Diagnostica Stago).
Results: The relationship between ROTEM® clotting time (CT) and Rivaroxaban level is of moderate strength (R^2=0.461). Based on this relationship, we are 95% confident that for ROTEM® CT of 61 seconds or less, Rivaroxaban level will be less than 50 ng/ml. For prediction of clinically significant Rivaroxaban levels of >50ng/mL, ROTEM® CT has a positive predictive value of 81% and negative predictive value of 100%. The relationship between LowTF -ROTEM® clotting time (CT) and Rivaroxaban level is of a low strength (R^2=0.142) (Figure 1).

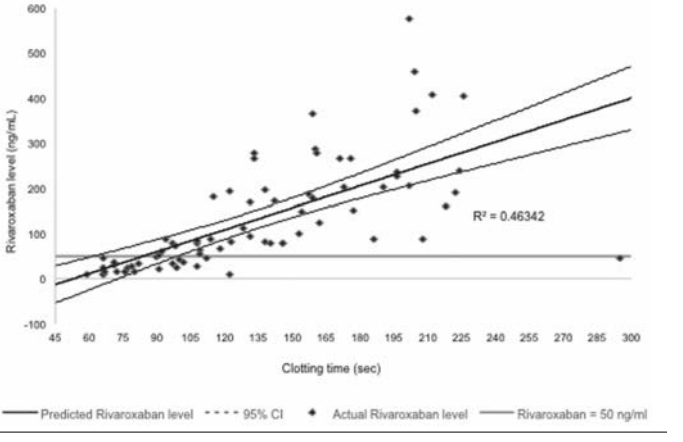

Figure 1.

Summary/Conclusions: ROTEM®, a point-of-care test, has utility in excluding clinically significant Rivaroxaban level, which may be critical for patients requiring urgent surgery or thrombolysis.

P776

PLATELET DERIVED MICROPARTICLES, AGGREGATES AND MARKERS OF IMMUNE ACTIVATION AND DISEASE PROGRESSION IN HIV INFECTED TREATMENT NAIVE ASYMPTOMATIC INDIVIDUALS
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Background: Chronic immune activation in human immunodeficiency virus (HIV) infection induces activation of leucocytes and platelets, resulting in the

formation of microparticles (MPs). Platelet microparticles (PMPs) are prothrombotic and have been described in inflammatory disease. Increased levels of platelet aggregation have been reported in HIV. PMP levels may be a more sensitive marker of platelet activation, compared to surface CD62P expression on activated platelets.
Aims: We aimed at measuring MPs, PMPs and platelet aggregates in HIV infected individuals, using flow cytometry based whole blood assay. Optimised to ensure artefactual platelet activation is kept minimal by omitting any centrifugation and platelet isolation steps. Moreover, we aimed at evaluating associations between MPs, PMPs, platelet aggregates and markers of immune activation and disease progression in asymptomatic treatment naïve HIV infected individuals.
Methods: Forty-Eight (48) antiretroviral therapy (ART) naïve HIV infected and 40 uninfected individuals were recruited in a clinic in Cape Town. Microparticles (MPs), Platelet microparticles (PMPs), platelet aggregates and platelet P-selectin CD62P were measured using flow cytometry. These were then correlated with CD4 count, viral load and %CD38 on CD4+ T cells.
Results: HIV infected individuals showed increased levels of median platelet %CD62P 2.93[1.23-12.88] *vs* control 1.15[0.19-3.59]; circulating MPs HIV group median 1.7[0.95-2.83] *vs* 1.12[0.63-1.57] Control group, p=0.0160; levels PMPs median %PMPs 26.64[11.33-36.62] *vs* control 20.02[18.08-26.08], p=0.0133. In the HIV group, levels of activated platelets correlated with; platelet aggregates (r=0.5530, p=0.011); activated PMPs (r=0.594, p=0.007). Baseline %platelet aggregates were similar between the groups. However the platelet aggregates in the HIV group showed increased levels of activation, platelet aggregates median %CD62P 14.10[5.49-39.94] *vs* 0.17[0-10.99] control group, p=0.0097. The HIV group showed increased levels of immune activation, median %CD38 on CD8+ cells 24.03[15.76-43.92] *vs* control 9.23[6.8-12.96], p<0.0001. In the HIV group levels of %CD38+ CD8+ T cells correlated with; %PMPs (r=0.4730, p=0.0196), %MPs (r=0.4386, p=0.025) and %PLT aggregates (r=0.321, p=0.135). In the HIV group %MP showed no correlation with; CD4+T cell count (r=-0.026, p=0.894); Viral load (r=0.207, p=0.355). %PMPs also showed no correlation with Viral load (r=0.152, p=0.511), CD4+ T cell count (r=0.-0.361, p=0.07).
Summary/Conclusions: We describe an optimized whole blood flow cytometry based assay for the evaluation of circulating MPs, PMPs and levels of activated platelets and aggregates. That mimics the *In vivo* physiological environment of MPs. Furthermore we report on increased levels of circulating MPs and PMPs in HIV infected asymptomatic individuals. We also describe increased levels of activated PMPs and platelet aggregates in HIV.

P777

Abstract withdrawn.

P778

INCIDENCE OF PERIPHERAL CIRCULATING CELLS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PHENOTYPE A FIRST EPISODE OF CEREBRAL SINUS VEIN THROMBOSIS: RESULTS FROM A PROSPECTIVE MULTICENTER STUDY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) clones have been rarely observed in association with atypical site venous thrombosis, mainly of the splanchnic veins, in absence of a known PNH diagnosis.
Aims: Primary aim of the current study was to evaluate the incidence of PNH clones in patients with a first episode of thrombosis localized at the cerebral sinus veins (CVT). Patients without known PNH were tested for PNH clone by high-sensitivity flow cytometric analysis on peripheral blood at CVT diagnosis.
Methods: All the enrolled patients had an objectively confirmed first episode of CVT. At enrollment, an accurate personal and familial clinical history was taken and a complete determination of known inherited and acquired risk factors for venous thromboembolism was performed. At CVT diagnosis, patients were assayed for PNH clone on peripheral blood at their enrolling institutions. In case of a positive PNH clone determination, a second sample was centrally assayed at the laboratory of the Study Coordinating Center at " Ospedale Ferrarotto", Catania. All PNH clone assays were performed using a high-sensitivity multiparameter flow cytometric analysis able to identify an abnormal population of >0.01% PNH cells. Enrolled patients underwent standard anticoagulant treatment for CVT and a two years clinical follow-up after diagnosis. If any sign or symptom of venous thrombosis recurrences occurred, a PNH clone was tested again. Descriptive statistic was adopted for the purpose of this study. The study was conducted in accordance with the Declaration of Helsinki and Ethics Committee approval was obtained at each enrolling center. Data were collected in a dedicate database
Results: Seventy seven subjects were enrolled (40 Females, 37 males; mean age: 41 years, range 19-87). Anticoagulant treatment was administered for a mean of six months (range:4-13 months); warfarin was administered in 64.9% of patients, Low Molecular Weight Heparin in 35.2% of patients. Adverse events related to anticoagulant therapy were not reported. CVT was associated with factor V Leiden gene mutation at heterozygous state in 5 patients (6.5%), FII (G20210A) gene mutation at heterozygous state in 6 patients (7.8%), JAK2 V6167F in 2 (2.5%) patients, Protein S deficiency in one case (1.2%), Lupus Anticoagulant and anti-phospholipid antibodies in five cases (6.5%), combined inherited and acquired risk factors in 3 cases (3.8%), recent surgery in 10.2%, oral contraceptives in 6.4% and pregnancy in 3.8% cases. Among screened patients, 38 (49.3%) did not have any of the known risk factor for venous thromboembolism. The incidence of PNH clones observed in our cohort was of 2.5%. PNH clones were detected in 2 cases: in one patient a small one (size 0.08%) was not further confirmed at the centralized determination, while in another case a clear demonstrable clone (size 1.5%) was confirmed on two consecutive independent determinations. The patient (a 27 years old Caucasian female) had a positive familial history of venous thromboembolism and an otherwise unremarkable personal thrombophilia screening, in absence of any symptom or other laboratory sign of PNH. Two venous thrombosis recurrences occurred at two years follow-up, both cases without any evidence of PNH clones.
Summary/Conclusions: Testing for PNH can be taken into account as part of the standard etiology-screening in atypical site venous thrombosis.However, at the present it is not clear whether detection of relatively small PNH clone, in absence of other signs and symptoms suggestive for PNH, may suggest specific therapy beyond anticoagulation.

P779

REGULATION OF WEIBEL-PALADE BODY EXOCYTOSIS FROM ENDOTHELIAL CELLS BY SYNTAXIN-3 AND STXBP5 CONTAINING SNARE COMPLEXES

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Background: Immediate release of a pre-stored cocktail of hemostatic, inflammatory and angiogenic mediators from endothelial secretory organelles, Weibel-Palade bodies (WPBs), forms the first line of defense of the vessel wall in response to vascular trauma. By releasing Von Willebrand factor (VWF), the main component of WPBs, the endothelium actively participates in the arrest of bleeding. This process also directs leukocytes to sites of inflammation. Dysregulation of VWF levels is associated with cardiovascular disease or can result in a bleeding tendency, such as Von Willebrand disease. Several components that are critical for regulated WPB exocytosis have been identified, including the small GTPase Rab27A and its effector Slp4-a, but the mechanism remains unclear. We have previously identified syntaxin binding protein 1 (STXBP1) as an endogenous Slp4-a binding partner involved in WPB release, along with the SNARE protein syntaxin-3.
Aims: In this study we investigated the possible role of syntaxin-3 in WPB trafficking and exocytosis.
Methods: The subcellular location of syntaxin-3 in endothelial cells was characterized using immunocytochemistry and density gradient ultracentrifugation. Iterative interactomics was performed to map the interacting proteome of syntaxin-3 and STXBP5.
Results: Interestingly, we found that the t-SNARE syntaxin-3 was primarily associated with WPBs. To further explore its role in WPB biology we mapped the endothelial interactome of syntaxin-3 through an unbiased label-free mass spectrometry approach. Among its interaction partners are various SNAREs and associated proteins such as syntaxin binding proteins 2 and 5 (STXBP2/5), N-ethylmaleimide-sensitive factor (NSF), SNAP23 and α-SNAP, suggesting we successfully pulled down a SNARE complex and its regulatory machinery that are involved in exocytosis. Taking forward one of the candidate, STXBP5, we found that silencing of STXBP5 expression using siRNA potentiated agonist-induced VWF release from endothelial cells, which suggests it acts as a negative regulator of WPB exocytosis. We narrowed down the inhibitory role of STXBP5 to the C-terminal Vamp-like domain (VLD), which interacts with syntaxins and attenuates histamine-induced VWF secretion upon lentiviral expression in endothelial cells. To further investigate the role of syntaxin-3 in endothelial cells we isolated blood outgrowth endothelial cells (BOECs) of a patient suffering from variant microvillous inclusion disease (MVID), a severe gastrointestinal disorder, caused by a homozygous mutation in *STX3* that leads to complete syntaxin-3 deficiency. WPBs of MVID BOECs are entirely devoid of syntaxin-3, display immature WPB morphology and have a reduced secretory potential when challenged with histamine.

Summary/Conclusions: Our data show that WPB exocytosis is regulated by a complex interplay between the WPB-associated SNARE syntaxin-3 and STXBP5.

Quality of life, palliative care, ethics and health economics 2

P780

COMPARISON OF QUALITY OF LIFE BETWEEN LONG-TERM SURVIVORS OF INDOLENT AND AGGRESSIVE LYMPHOMA: RESULTS OF A SINGLE CENTER PROSPECTIVE COHORT STUDY

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Background: Lymphoma is a basically curable disorder by chemotherapy and or radiotherapy. As clinical features and treatment outcomes are different according to subtypes of lymphoma, the various treatment strategies have been applied from intensified treatment such as high-dose chemotherapy followed by autologous stem cell transplantation to observation without any treatment. Thus, patients experience different treatments based on the nature of their diseases so-called indolent and aggressive lymphomas. However, there is limited ata about the health-related quality of life of long-term survivors of indolent and aggressive lymphoma.
Aims: The primary objective is to evaluate the health-related quality of life at diagnosis and after treatment in long-term survivors of indolent and aggressive lymphomas. The secondary objective is to evaluate psychosocial wellbeing in long-term survivors such as fear of recurrence, social support and spiritual well-being.
Methods: Patients were diagnosed with Hodgkin and non-Hodgkin lymphoma according to the diagnostic criteria of the World Health Organization between 2008 and 2011. Patients were enrolled into the Samsung Medical Center Prospective Cohort Study (NCT#01877109) and their health-related quality of life was assessed by the EORTC QLQ-C30. The follow-up evaluation of the health-related quality of life was done with long-term survivors in 2014. The quality of life at the time of diagnosis was compared with that of patients at the time of long-term follow-up, and they were compared according to indolent and aggressive lymphomas.
Results: Among 953 patients registered in the cohort study, 642 alive patients were requested to conduct the follow-up survey about their health-related quality of life. As we failed to get response from 242 patients due to refusal to respond, follow-up loss, and incomplete survey, we analyzed 400 patients' health-related quality of life and psychosocial wellbeing. 305 patients were diagnosed with aggressive lymphoma such as diffuse large B-cell and peripheral T-cell lymphoma whereas 95 patients were diagnosed with indolent lymphoma such as follicular and marginal zone lymphoma. The socioeconomic characteristics such as age, marital status, monthly family income, education and working status were not significantly different between indolent and aggressive lymphomas. Although the baseline health-related quality of life was significantly better in patients with indolent lymphoma that aggressive lymphoma, the follow-up survey showed the improvement of quality of life in patients with aggressive lymphoma. However, several aspects of psychosocial wellbeing such as fear of recurrence, social support, happiness, satisfaction and life purpose were impaired in patients with indolent and aggressive lymphoma (Figure 1).

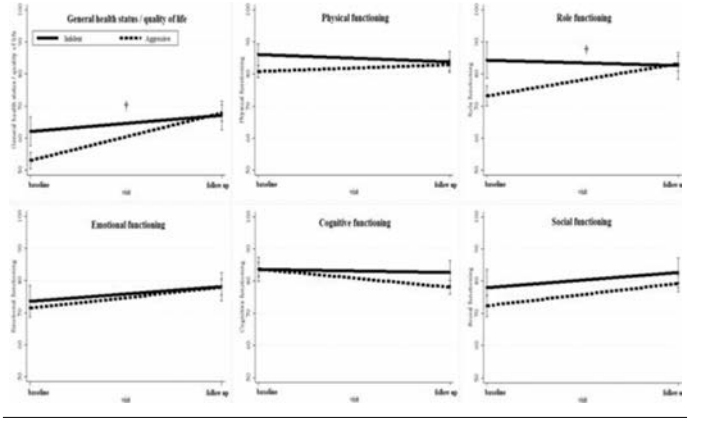

Figure 1.

Summary/Conclusions: The health-related quality of life could be recovered in long-term survivors with indolent and aggressive lymphoma after treatment. However, a majority of long-term survivors still suffer from impaired psychosocial wellbeing, especially fear of recurrence. Thus, more education about disease course and support for psychosocial wellbeing could be helpful for long-term survivors with lymphoma.

P781

IMPACT OF IRON DEFICIENCY ON QUALITY OF LIFE AND WORK CAPACITY OF WOMEN

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Background: The most severe stage of iron deficiency (ID), iron deficiency anemia (IDA)is a global problem, with the greatest prevalence among women and children.[1] IDA has been shown to negatively impact on cognitive performance and ability to concentrate and can result in fatigue.[1]
Aims: This survey aimed to gain an understanding of the awareness, prevalence and perceptions of ID/IDA within a European population.
Methods: An online, quantitative survey was carried out across Europe between in July and August 2015. The survey included respondents from the UK, France, Germany, Spain, Italy, Portugal and Sweden. In general the questionnaire was designed to take only 5 minutes. Respondents who reported a confirmed diagnosis of ID/IDA at some point in their lives completed a more in-depth questionnaire taking approximately 20 minutes.
Results: A total of 10,272 people completed the survey, with 7,025 aware of ID or IDA, and 934 of those having had a confirmed diagnosis of ID or IDA at some point in their lives.The awareness and prevalence of iron deficiency are higher amongst women than men (14% women *vs* 4% men). In general practice, heavy menstrual bleeding (HMB) is recognized as the primary cause of ID/IDA in women.[2] This was reflected in public perception, with 59% associating heavy periods or menstruation as the main cause of ID/IDA in women, and 50% of women ranking it among the top 5 causes. Results from the survey show that 28% of patients suffer from HMB in addition to their ID/IDA. The first symptom experienced most frequently was tiredness/fatigue, which was also the main driver for patients to seek medical help (54% reported it as their reason for visiting an HCP). On average, people experienced symptoms for 2.5 years before visiting a doctor. 63% of all patients felt that their ID/IDA had a negative impact on their ability to concentrate, affecting a greater proportion of women than men (64% of female *vs* 55% of male patients). Concern over skin pallor was also greater for women (49% of female patients *vs* 36% of male felt their ID/IDA made them look ill). Patients reported that their ID/IDA had the greatest impact on their personal well-being (79% citing a slight or severe negative impact). The second most significant impact was on working life, affecting 49% of patients.
Summary/Conclusions: This survey highlights the greater impact of ID/IDA among women than men, reflected in awareness, prevalence and effect on concentration and working life. Recent epidemiological studies suggest that the gender gap for anemia (primarily caused by ID) is widening further.[1]

References

1. Kassebaum NJ, Jasrasaria R, Naghavi M, *et al*. A systematic analysis of global anemia burden from 1990 to 2010. *Blood*. 2013:blood-2013-06-508325-. Available at: http://bloodjournal.hematologylibrary.org/content/ early/2013/11/26/blood-2013-06-508325.abstract. Accessed December 4, 2013.
2. Fraser IS, Mansour D, Breymann C, Hoffman C, Mezzacasa A, Petraglia F. Prevalence of heavy menstrual bleeding and experiences of affected women in a European patient survey. *Int J Gynecol Obstet*. 2015;128:196-200. doi:10.1016/j.ijgo.2014.09.027.

P782

IRON DEFICIENCY: UNDERSTANDING PERCEPTIONS OF SUFFERERS AND THE GENERAL PUBLIC

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Background: Iron deficiency anaemia (IDA) is the third-leading specific cause of global years lived with disability, impacting large portions of the global and European populations,[1] despite the availability of simple, effective treatment.
Aims: This survey aimed to gain an understanding of the level of awareness of iron deficiency (ID) and/or IDA amongst the general population, as well as perceptions of the disease and its symptoms.
Methods: A quantitative, online survey across Europe, UK, France, Germany, Spain, Italy, Portugal and Sweden was conducted in the period July 21st- August 21st, 2015. Approximately 1,000 participants from each nation, and representative of the population aged 18 years or over, were surveyed.
Results: A total of 10,272 people completed the survey, with 7,025 aware of ID or IDA and 934 of those reported having had a confirmed diagnosis of ID or IDA at some point in their lives. Tiredness/fatigue was the symptom most strongly associated with iron deficiency and IDA, followed by paleness. However, 36% of respondents who were aware of ID/IDA only knew the name and could not identify causes or symptoms. The main symptom leading patients to present to their General Practitioner (GP) was fatigue/tiredness (54%). 21% of patients did not experience symptoms before their diagnosis. In patients who experienced symptoms, these appeared an average of 9 months before they visited their GP, with a further 6 months required to receive a confirmed diagnosis. Despite having been diagnosed with ID/IDA, 11% of patients have never received a prescription for their ID/IDA. Patients felt that the most significant impact of their ID/IDA was on their personal well-being (79%), with 49% reporting a negative impact on their working life.
Summary/Conclusions: This survey revealed only low-level awareness of ID/IDA within the general European population. There is also a long delay between the appearance of symptoms and an appropriate diagnosis, despite the significant impact ID/IDA has on a patient's work and quality of life.

References

1. Vos T *et al*. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013 The Lancet Volume 386, No. 9995, p743–800, 22 August 2015.
2. Miller JL. Iron deficiency anemia: a common and curable disease. Cold Spring Harb Perspect Med. 2013;3(7):1-13. doi:10.1101/cshperspect. a011866.

P783

CIRS COMORBIDITY SCORE IS USEFUL FOR TREATMENT CHOICE AND PREDICT TREATMENT OUTCOME IN ELDERLY PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: The Cumulative Illness Rating Scale (CIRS) seems to be a reliable tool for the evaluation of the burden of comorbidity in elderly cancer patients (Extermann M, J Clin Oncol 1998). CIRS score is widely used in chronic lymphocytic leukemia clinical trials to identify fit and unfit patients and influence therapeutic decision-making.
Aims: The aim of our study was to evaluate CIRS impact in elderly patients with other hematological diseases treated in real life.
Methods: This was a longitudinal, prospective, monocentric study. We focused on patients aged 70 or older who received their first cycle of chemotherapy between September 1st 2014 and August 31th 2015. We recorded the multidisciplinary treatment decision taken: curative, symptomatic and palliative treatment. Geriatric tools recorded (G8 score, CIRS score, sheet A SEGA (Short Emergency Geriatric Assessment) frailty score, ADL, IADL) were not used during treatment decision. The first course of chemotherapy was considered as the standard dose. We analyzed during the following chemotherapy courses: dose reduction, chemotherapy delaying, early discontinuation. Impact of geriatric tools especially CIRS comorbidity score, SEGA frailty score on treatment choice (palliative *versus* curative treatment) was performed in all patients included in our study (n=141). Analysis of CIRS and other geriatric tools impact on treatment chemotherapy adaptation was performed in 118 patients (exclusion of palliative and symptomatic treatments).
Results: during the one year period analysis, 141 patients >70 years old (70-95) were discussed during our weekly multidisciplinary meeting. Average age was 80. The most frequent hematological diseases were distributed as follow: NHL (32%), multiple myeloma (29%), CLL (13%) and MDS (8%). Twenty one percent of patients and 18% were assigned to Bendamustine and Bortezomib based chemotherapy. Other chemotherapy regimens included (CHOP and CHOP like treatment +/- rituximab (10%), 5-azacytidine (8%), IMID (8%). Palliative and symptomatic treatments decisions were taken in 11 and 12 patients respectively. At diagnosis, the median scheet A SEGA score was 6 (0-18) indicating that the subjects included were mostly not frail. Median CIRS total rate excluding hematological malignancy was 6 (0-18). High CIRS score (median 10) and SEGA score (median 12) were significantly associated with palliative treatment, p=0,001 and p=0,01 respectively. Regarding patients assigned to curative approach, treatment was prematurely stopped in 32% of patients, dose reduction in 30% and chemotherapy was delayed in 18%. Only 33% did not experienced one of these events. CIRS grade 3 or 4 and CIRS score >6 were strongly associated with early chemotherapy discontinuation (p=0,0014 and 0,0008), respectively. In contrast no impact was observed regarding chemotherapy delaying or dose reduction. SEGA score was not associated with early discontinuation, nor dose reduction and chemotherapy delaying.
Summary/Conclusions: CIRS score and SEGA score could be helpful in treatment choice. Palliative treatment could be the best approach in patients with CIRS score >10 and or SEGA score >12. Patients CIRS score more than 6 are at higher risk of early chemotherapy discontinuation. A special attention is required for these patients to avoid such events. Reason of early discontinuation and impact on response will be presented at the EHA meeting.

P784

THE RELATIVE BURDEN OF AL AMYLOIDOSIS ON HEALTH-RELATED QUALITY OF LIFE

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Background: The SF-36v2® Health Survey (SF-36v2) is a widely used general patient-reported outcome survey that can describe and quantify the impact of disease and its treatment on health-related quality of life (HRQoL). Light chain (AL) amyloidosis is a rare disease characterized by misfolded amyloid protein deposits in tissues and vital organs, and little is known about the burden of AL amyloidosis on HRQoL.
Aims: To compare the HRQoL profile of patients with AL amyloidosis and key patient subgroups to a general US population (USP) sample.
Methods: The SF-36v2 was administered in an online, cross-sectional study of adults (≥18 years of age) with self-reported AL amyloidosis (n=341). The SF-36v2 measures eight domain scales (physical functioning (PF), role limitations due to physical health problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional health problems (RE), and mental health (MH)) and two component summary measures: physical (PCS) and mental (MCS). Analysis of variance was used to compare the norm-based SF-36v2 scores from patients with AL amyloidosis to samples representing the USP. The USP sample size ranged from 4,024 – 4,036 for specific SF-36v2 scores. The USP data were adjusted to the age and gender distribution of the patient sample using separate ordinary least-squares regression models, with each SF-36v2 scale or summary score as a dependent variable. Using the same method, we evaluated the burden of two key subgroups: 1) patients with recent diagnosis (within the past year) (n=52); and 2) patients with cardiac involvement (n=178).
Results: Relative to USP norms, HRQoL of patients with AL amyloidosis is significantly worse on scores from all eight SF-36v2 domain scales and both summary measures (p <0.05 for all). The largest decrement was in GH, where the AL amyloidosis patient mean was a full standard deviation worse than the USP (39.3 *vs* 49.0, respectively, Cohen's d -0.654; p <0.001). Large decrements (more than a half standard deviation) also were seen in PF (24.5 *vs* 36.9), RP (40.5 *vs* 47.6), VT (44.5 *vs* 50.2), SF (43.6 *vs* 49.6), and PCS (40.7 *vs* 46.7). Subgroup analysis showed that, relative to the USP, patients (1) recently diagnosed with AL amyloidosis and (2) with cardiac involvement had large decrements in each of the SF-36v2 domain scales and both summary measures (p <0.05 for all). The largest decrements for each subgroup, relative to USP, were:
• Recently diagnosed: All scores except BP were at least a half standard deviation lower than USP, with three domain scores at least one standard deviation lower (RP, GH, SF).• Cardiac involvement: Four domain scores and one summary measure score were at least a half standard deviation lower than USP (PF, RP, GH, VT, and PCS).
Summary/Conclusions: Results indicate that AL amyloidosis patients have broad HRQoL deficits across all areas of physical and mental functioning compared to the general USP, with greater impact evident among key AL amyloidosis subgroups – in particular, patients diagnosed within the past 12 months. Understanding the burden of AL amyloidosis can help physicians identify ancillary treatments and services that may ease their burden and ultimately improve patients' HRQoL.
Study supported by: Prothena Biosciences Inc.

P785

OUTCOME OF PATIENTS WITH RELAPSED/REFRACTORY LYMPHOMA IN A LARGE COHORT INSIDE A PHASE 1 CLINIC DEPARTMENT

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Background: Treating relapsed/refractory lymphoma remains a challenge. While phase 1 trials classically aim to determine the recommended phase 2 dose (RP2D), the search of anti-tumor activity is increasingly evaluated.
Aims: We aimed to assess the tolerance and efficacy of phase 1 trial and to determine a simple scoring system to identify patients who will prematurely discontinue phase 1 studies (before six weeks), in a large cohort of patients with relapsed/refractory (r/r) lymphoma.
Methods: Data from 137 consecutive patients with refractory/r lymphoma treated within a panel of 22 phase 1 trials were collected, between 2008 and 2016. At inclusion, median age was 66 years [range: 23-86]. Lymphoma histological patient's types were: 79 (55%) aggressive non-Hodgkin lymphoma (59 diffuse large B-cell lymphoma, 6 T-cell lymphoma and 14 Mantle cell lymphoma), 40 (29%) indolent non-Hodgkin lymphoma and 18 (13%) Hodgkin lymphoma. The predefined Gustave Roussy (GR) score combined two simple variables, PS and baseline serum albumin (+1 if PS=0, +1 if albumin≤35g/l).
Results: Grade 3 or 4 adverse events related to study drug were experienced by 39/137 (28%) patients, in 27/39 (69%) during first cycle and in 12/39 (31%) after first cycle. Dose-limiting toxicity was seen in 12/137 (9%) of patients. Any toxic death was recorded. With a median follow-up of 13 [0.3-85] months, median OS and progression free survival (PFS) were respectively 22 ($CI_{95\%}$: 17-38) and 4 ($CI_{95\%}$: 2-5) months. Best overall response rate Cheson 2007 analysis shown overall response rate and disease control rate at 30% and 63%, respectively. Median overall survival was better in responder (undefined) compare to disease controllers (31 months) and non-responder patients (12 months) (p=0.0003). At inclusion WHO performance status (PS) >0, baseline albumin ≤35 g/l and baseline LDH ≥250 UI/L were significantly associated with poorer OS. Patients with a GR score=0 experienced significantly better OS compared to patients with a score=1 and a score=2 (37 months *vs* 17 months *vs* 9 months; p=0.007). A premature study discontinuation was recorded in 46/137 patients (34%). The GR score distinguishes patients most likely to remain on study for more than 6 weeks. A molecular portrait was performing in 40 patients to improve the patient orientation in the trials.

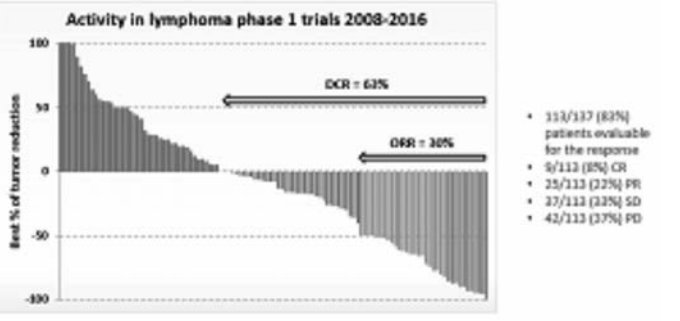

Figure 1.

Summary/Conclusions: The three parameters WHO performance status (PS) >0, baseline albumin ≤35 g/l and baseline LDH ≥250 UI/L are associated with OS and premature withdrew of study and could serve as a basis to better select patient in hematological phase 1 trials. Survival was dramatically better in responder's patients, pledging to accelerate the search for efficacy signals from phase 1.

P786

QUALITY OF LIFE IN SURVIVORS OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: CHILDREN'S AND PARENTS' PERCEPTION

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Background: Improved outcome and predicted long survival for children with acute lymphoblastic leukemia (ALL) make the early and efficient therapy, quality of life, and psychosocial intervention more important. It is well known that quality of life is negatively affected in ALL survivors who are cured.
Aims: The aim of this study is to investigate the social, physical, emotional, family, and functional factors that may affect the quality of life in children with ALL to find out how we can support their lives after treatment to provide better quality of life in our country.
Methods: The study population consisted of 46 children who were diagnosed with ALL and had been treated at both centers with ALL-BFM 95 chemotherapy protocol. We included children who had been completed therapy at least two years ago and were 7-17 years-old. The control group consisted their siblings at closest age and gender. The sociodemographic data of patients were recorded from hospital files. Patients and their siblings were evaluated with KINDL questionnaire for measuring health-related quality of life in children and adolescents. The perception of child about quality of life also evaluated by the parents.
Results: No significant differences could be found among life quality scores with respect to variables as gender, therapy type, risk group, time after the end of therapy, income status, and having chronic illness. Mean quality of life scores of patients were as follows: Physical well-being 75.27±19.9, emotional well-being 76.77±16, social functioning 79.21±18, family 82.74±19.7, self-esteem 60.73±24.4, and school 63.99±19.3. Mean quality of life scores of siblings (between 7-17 years) were as follows: Physical well-being 61.36±20.12, emotional well-being 85.22±11.27, social functioning 86.36±15, family 79.54±27.96, self-esteem 69.88±24.97, and school 64.20±14.54. Total mean quality of life score of patients and siblings were 73.12±13.4 and 74.43±11.28, respectively. Mean quality of life scores of parents questionnaires were as follows: Physical well-being 78.06±20.80, emotional well-being 77.13±15.76, social functioning 81.38±14.64, family 82.74±15.74, self-esteem 71.28±19.44, and school

63.70±14.60; total mean quality of life score was 75.51±11.60. When we investigated the questionnaires of patients and parents, we found that the self-esteem scores of the patients were significantly lower than the parents evaluation (p=0.037); we found no correlation in school subscales between patients' and parents' evaluation. There was good positive correlation between physical well-being, emotional well-being, and social functioning. When the results of patients and their siblings were compared, mean physical well-being scores of siblings were lower than the patients (p=0.049).
Summary/Conclusions: The quality of life scores of 7-17 years-old ALL survivors were not affected by gender, therapy type, risk group, time after the end of therapy, income status, and having chronic illness. Physical well-being scores were higher in patients than their siblings. Our findings demonstrated that the parent evaluated self-esteem and school scores differed from their child; we think that the parents should follow-up their children more closely for their self-esteem development and school performances.

P787

HEALTH-RELATED QUALITY OF LIFE ASSESSMENT IN LONGITUDINAL STUDIES INCLUDING MULTIPLE MYELOMA PATIENTS: A CRITICAL REVIEW OF INTERPRETING CHANGES OF SCORES OBTAINED BY EORTC QLQ-C30

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Background: Multiple myeloma (MM) patients report more symptoms and more severe symptoms than patients with other hematological malignancies, resulting in reduced health-related quality of life (HRQoL). The goal of MM treatment and care is to prolong progression free survival and overall survival, and to reduce symptoms with least possible side effects to therapy. Because survival is improving in MM patients there is increasing focus on HRQoL.
Aims: The aim of the review is to evaluate longitudinal studies including MM patients according to minimal important difference (MID) for physical function, global QoL, fatigue and/or pain, according to the two available guidelines by Cocks (Eur J Cancer 2012, 48:1713-21) and Kvam (Eur J Haematol 2010, 84:345-53).
Methods: A literature search was performed 14 December 2015 in PubMed and Embase and 29 December 2015 in PsycINFO and CINAHL. Publications with longitudinal follow-up using EORTC QLQ-C30 for HRQoL measurement of physical function, global QoL, fatigue or pain were included, if the mean score at baseline and minimum one follow-up time point were presented in the text, a table or a figure, or the change in mean score from baseline over a given time point was specified. Publications concerning patients with mixed hematological diagnoses were excluded, if the results were not presented for MM patients separately. Articles in other languages than English were excluded.
Results: A total of 25 publications were identified, which relates to 15 primary and 5 relapse studies, one population-based and one mixed population study. Evaluation of change in score concluded by guidelines of Cocks compared to Kvam, showed diversity in the interpretation of changes of HRQoL scores over time. Large improvement (only possible for pain) and medium improvement in one or more of the evaluated HRQoL parameters were only achieved in studies including newly diagnosed MM patients. During primary treatment with autologous hematopoietic stem cell transplantation (ASCT), temporary large deteriorations are seen, but three months after ASCT equalization occurs with even large improvements in physical function, global QoL, fatigue and pain. In the studies of MM patients undergoing relapsed treatment regimes, no MID or variable degrees of deteriorations are generally documented. Only two relapse studies documented improvement during relapse treatment, which both were for pain, one with small and one with medium improvement. The population-based study demonstrated deterioration in global QoL, fatigue and pain at one year follow-up.
Summary/Conclusions: Several longitudinal studies of changes in HRQoL scores during MM treatments have been published over the last two decades, and guidelines for interpreting HRQoL changes with thresholds of MID have been developed. In return, these methods are not definite, and further research is required. It is still unresolved, which is the optimal duration between the two measuring points for interpretation of MID and possible different thresholds for MID in younger *vs* frail patients, and in newly *vs* relapsed MM patients, respectively. Based on our review, it is clear that improvements in HRQoL are far more likely during treatment of newly diagnosed patients compared to relapsed patients. Whether this is due to the trajectory of the MM disease, differences in the type and/or efficacy of anti-myeloma regimens used in the primary or relapsed settings, or individual factors such as comorbidity or response shift/calibration, should be in focus in future studies.

SIMULTANEOUS SESSIONS III

Aggressive lymphoma with emphasis on novel agents

S788

CLINICAL CHARACTERISTICS AND PROGNOSTIC FACTORS OF IMMUNOCOMPROMISED AND NON IMMUNOCOMPROMISED PLASMABLASTIC LYMPHOMA PATIENTS: ANALYSIS OF 135 PATIENTS TREATED IN THE LYSA GROUP

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Background: Plasmablastic lymphoma (PBL), initially described in 1997 in the oral cavity of HIV positive patients, is now recognized as a distinct aggressive and rare entity of diffuse large B-cells lymphoma by the World Health Organization classification. Others cases have been reported since the original description, both in immunocompromised and immunocompetent patients. However, these latter are largely derived from case reports or small series, and PBL remains a diagnostic and therapeutic challenge with an aggressive clinical course.
Aims: The aim of this study was to specify the clinical, biological, pathological features and outcome of patients with PBL.
Methods: The clinical, biological, pathological features and outcome of a cohort including 135 patients with PBL diagnosed after 2000 in the LYSA centers were reported and analyzed.
Results: The median age was 58 years, with a male predominance. The cohort was divided into 56 HIV-positive patients, 17 post-transplant patients, and 62 non immunocompromised patients including 11 patients with local or systemic inflammatory disease, 13 patients with history of cancer and 31 elderly patients. Despite PBL in immunocompetent patients has already been described in the literature, this study highlights that this subtype of patients finally presents some degree of immunodepression, confirming a strong relationship between immunodeficiency and PBL. The most frequent primary involved site was the oral cavity in 19% of cases. Immunophenotype showed CD138 positivity in 88% of cases and CD20 negativity in 90% of cases. EBER expression was observed in 62% of cases. Chemotherapy was administered to 108 of 135 patients, with a complete response rate of 55%. The use of rituximab, combined with chemotherapy, seems to be interesting with a trend towards improved complete response rate and event free survival. The median overall survival was 32 months. In the multivariate prognostic analysis, International Prognostic Index score, chemotherapy and complete response rate were associated with survival benefit. HIV positive status showed better overall survival when compared with HIV negative status, but only in univariate analysis.
Summary/Conclusions: This cohort, the largest reported to date, lead to a better understanding and increase the spectrum of knowledge on PBL. However specific guidelines to clarify all the treatment options are lacking, and may improve the poor prognosis of this rare disease.

S789

SUBGROUP ANALYSES OF DIFFUSE LARGE B-CELL LYMPHOMA AND INDOLENT LYMPHOMA COHORTS FROM A PHASE IIA STUDY OF SINGLE-AGENT MOR208 IN PATIENTS WITH RELAPSED OR REFRACTORY NON-HODGKIN'S LYMPHOMA

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Background: There is a high unmet medical need for new efficacious and tolerable regimens for patients with relapsed or refractory (R-R) non-Hodgkin's lymphoma (NHL). CD19 is expressed by most B-cell NHLs; in contrast to other targets, CD19 expression is maintained at a stable level during the course of the disease. MOR208, an Fc-engineered, humanized monoclonal CD19 antibody demonstrates activity in certain leukemia and lymphoma model systems through enhanced antigen-dependent cell-mediated cytotoxicity and antigen-dependent cell-mediated phagocytosis. A phase I study showed MOR208 to be safe and well-tolerated with encouraging single-agent activity in patients with chronic lymphocytic leukemia. Therefore MOR208 may have clinical use as a new therapeutic agent in this setting.
Aims: This analysis focused on the preliminary efficacy and safety of MOR208 in diffuse large B-cell lymphoma (DLBCL) and indolent (i) NHL cohorts. The primary endpoint was investigator assessed overall response rate (ORR).
Methods: This is an open-label, multicenter, phase IIa study of MOR208 in R-R NHL patients progressing after at least one prior rituximab-containing therapy. Patients were enrolled into 4 cohorts of aggressive (DLBCL and mantle cell lymphoma) and iNHLs (follicular lymphoma and other iNHLs). Patients received single-agent MOR208, 12 mg/kg IV, weekly, for 8 weeks (2 cycles); treatment could continue for 4 additional weeks in patients with at least stable disease after 2 cycles. MOR208 maintenance could be given (every 2 or 4 weeks) until progression in patients with complete (CR) or partial response (PR) at 12 weeks.
Results: In total, 92 patients were enrolled including 35 (38%) with DLBCL and 45 (49%) with iNHL. ORR was 26% in DLBCL (9/35 patients; 2 CRs and 7 PRs), and 27% in iNHL (12/45 patients; 5 CRs and 7 PRs). In evaluable patients (those who completed at least 2 cycles and had a response assessment) the ORR was 36% in DLBCL (25 evaluable patients), and 30% in iNHL (40 evaluable patients), respectively. Median duration of response was 13.7 months for DLBCL patients (1.2-26, 3 ongoing and >20 months in remission) and 8.4 months for iNHL patients (2.5-20.3, 6 ongoing, including 1 patient >20.3 months in remission). In DLBCL responders, 5/9 were rituximab-refractory (either no response or response lasting<6 months to a previous rituximab-containing therapy), of which 2 patients had a response duration of >19.8 months and 1 patient of >1 year under MOR208 treatment. The incidence of grade ≥3 hematologic treatment emergent adverse events was low (DLBCL: 9/35, 26%; iNHL: 4/45, 9%), including neutropenia, anemia and thrombocytopenia in 14%, 9% and 6% of DLBCL patients and 4%, 0% and 0% of iNHL patients. Infusion-related reactions were seen in 3/35 (9%) and 4/45 (9%) patients in the DLBCL and iNHL cohorts, all of grade 1-2 except for one grade 4 dyspnea. There were no treatment-related deaths and no trend towards late toxicity.
Summary/Conclusions: Single-agent MOR208 was associated with a promising ORR including CRs and long-lasting responses in both DLBCL and iNHL, including in patients with rituximab-refractory disease. These efficacy data, and the excellent safety and tolerability profile of MOR208 further justify the development of MOR208 as part of a combination therapy in B-cell malignancies.

S790

HAPLOTRANSPLANTS *VERSUS* HLA-IDENTICAL DONOR (HID) FOR ALLOGENEIC STEM-CELL TRANSPLANTATION (ALLOSCT) IN REFRACTORY NON-HODGKIN LYMPHOMA (NHL)

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Background: AlloSCT is an effective therapeutic option for patients with refractory lymphoma. However, the donor source is the biggest problem for alloSCT. The feasibility of haploSCT has dramatically expanded the donor pool, making allogeneic transplantation available for the vast majority of patients, especially for patients with rapidly progressing disease in whom unrelated donor search cannot be awaited.
Aims: In this study, we aim to compare outcome of haplotransplants with conventional related and matched unrelated donor transplants for refractory relapsed or high risk NHL.
Methods: Seventy-eight patients who had undergone alloSCT between January 2006 and October 2015 in our center were analyzed retrospectively. The median age was 30 years (range, 12 to 58 years). The histological subtypes included diffuse large B cell lymphoma (13), mantle cell lymphoma (3), Burkett's lymphoma (1), B-cell lymphoblastic lymphoma (8), other type mature B cell lymphoma (10), T-cell lymphoblastic lymphoma (18), peripheral T cell lymphoma (18) and NK/T cell lymphoma (7). Bone marrow was involved in 65 patients, 9 patients had experienced treatment failure with prior autologous SCT. All of the patients received a myeloablative conditioning regimen which is TBI/Cy or Bu/Cy. 33 patients received Haplo Donors, 45 patients received HID, including sibling and matched unrelated donors. Before alloSCT, 49 patients were in CR, 12 patients were in PR, 18 patients were in NR. Primary refractory or progression was more common in the HAPLO group (p=0.001), other variables were balanced.
Results: Seventy-eight patients were engrafted successfully and all patients achieved full donor chimerism. There is no difference for hematopoietic recovery between two groups. In HAPLO group, the median time of neutrophils and platelets recovery were 12 days (range, 9d to 15d) and 17 days (range, 11d to 60d). In HID group, the median time of neutrophils and platelets recovery were 11 days (range, 9d to 16d) and 17days (range, 12d to 45d), respectively. After transplantation, 75 patients acquired complete response, another 3 patients

acquired partial response. In HAPLO group, 6 patients had recurrent disease, and 5 patients died from transplantation-related (TRM) including infection and other organ failure at 1-year. In HID group, 6 patients had recurrent disease and 8 patients died of TRM. The median follow-up duration was 23 months (range, 2-139) in HAPLO group, 29 months (range, 2-139) in HID group. Among HAPLO and HID recipients, the 3-year progress free survival rate was 56.8% and 60.0% (P =0.45), respectively; overall survival rate was 58.9% and 62.1% (P=0.48), respectively; the 3-year cumulative incidences of relapse were 48.4% and 47.7% (P =0.36), and those of the 2-year-non-relapse-mortality were 25.2% and 27.8% (P =0.86), respectively. The cumulative incidences of grade 3–4 acute GVHD were 28.4% (95% CI =7.2-28.1%) and 20.5% (95% CI =5.7–22.2%)(P =0.43), respectively. Univariate analysis showed that disease status before alloSCT may be a factor affecting OS and PFS (p=0.001).

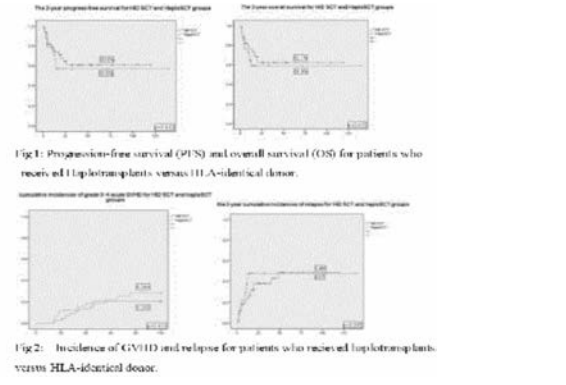

Fig 1: Progression-free survival (PFS) and overall survival (OS) for patients who receive d Haploiransplants versus HLA-identical donor.

Fig 2: Incidence of GVHD and relapse for patients who received haploiransplants versus HLA-identical donor.

Figure 1.

Summary/Conclusions: This retrospective study showed haploSCT resulted in similar clinical efficacy compared to HID in patients with refractory/relapsed or high risk lymphoma. Importantly, TRM and acute GVHD were acceptable without obvious difference between two groups. HaploSCT provides a chance of long-term disease control even for patients without HID.

S791

UPDATED RESULTS FROM ZUMA-1: A PHASE 1-2 MULTICENTER STUDY EVALUATING THE SAFETY AND EFFICACY OF KTE-C19 (ANTI-CD19 CAR T CELLS) IN REFRACTORY AGGRESSIVE B-CELL NON-HODGKIN LYMPHOMA (NHL)

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Background: Non-Hodgkin lymphoma (NHL) is the most prevalent hematologic malignancy in the US and the 5th most deadly cancer with nearly 19,790 deaths/year. Diffuse large B-cell lymphoma (DLBCL) is the most common NHL subtype, representing 25-35% of new cases annually. Approximately 1 in 3 patients treated in the first line will be refractory to treatment or relapse post-treatment and represent a significant unmet medical need. Patients with relapsed/refractory B-cell malignancies demonstrated durable remissions when treated with CD28/CD3zeta anti-CD19 CAR T cells at the National Cancer Institute (NCI) (Kochenderfer, JCO 2015; NCT00924326). KTE-C19 includes the same CAR construct as that used in the NCI trial, but is centrally manufactured in a streamlined 6- to 8-day process. Updated data from the phase 1 portion of the ZUMA-1 clinical trial are reported.
Aims: The primary goal was to determine the safety of KTE-C19. Secondary aims included evaluation of overall response rate, duration of response, blood concentrations of CAR T cells, and serum cytokine levels.
Methods: Patients with relapsed/refractory aggressive B-cell NHL were treated with a single target dose of 2×10^6 anti-CD19 CAR T cells (KTE-C19)/kg following a fixed dose of cyclophosphamide/fludarabine conditioning chemotherapy. Key inclusion criteria were ECOG 0-1 and chemo-refractory disease, which was defined as progressive or stable disease as best response to last line of therapy or disease progression ≤12 months after autologous stem cell transplantation. All patients provided informed consent.
Results: Seven patients received KTE-C19 as of January 15, 2016. A previously reported dose-limiting toxicity of grade 4 cytokine release syndrome and neurotoxicity occurred in one patient. All other grade ≥2 cytokine release syndrome and neurotoxicity adverse events resolved within one month. There were no other adverse events due to KTE-C19 after one month post-dosing. The overall response rate was 71%, with a complete response rate of 57%. Ongoing complete responses of 3 to 6+ months were present in 3 patients who previously demonstrated disease progression within 6 months of autologous stem cell transplantation. Levels of CAR T cells peaked within 2 weeks and remained detectable 1 to 6+ months after infusion. Updated results will be presented.
Summary/Conclusions: Additional follow up of patients enrolled in the ZUMA-1 trial demonstrated durable complete remissions after a single dose of KTE-C19, with no new KTE-C19-related adverse events. The KTE-C19 regimen and central, streamlined manufacturing process were safe and feasible for future studies. The phase 2 portion of the ZUMA-1 clinical trial (NCT02348216) is ongoing.

S792

LOW-DOSE CHEMOTHERAPY FOLLOWED BY ANTI-CD19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS INDUCES REMISSIONS IN PATIENTS WITH ADVANCED LYMPHOMA

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Background: Non-Hodgkin lymphoma (NHL) is the most common hematologic malignancy in the US and the 5th deadliest cancer with almost 20,000 deaths per year. Anti-CD19 CAR T cells achieve durable remissions in patients with relapsed/refractory B-cell malignancies. Pre-treatment with chemotherapy allows depletion of recipient leukocytes, which improves the anti-cancer effects of adoptively transferred T cells. Increased serum concentrations of interleukin (IL)-15 is believed to contribute to this beneficial effect of chemotherapy. We (Kochenderfer et al. *Journal of Clinical Oncology*, 2015) and others previously reported effects of high-dose chemotherapy administered before anti-CD19 CAR T-cell therapy. We now report the effects of low-dose conditioning chemotherapy followed by anti-CD19 CAR T-cell infusion.
Aims: The main goals of the study were to evaluate efficacy and safety of low-dose conditioning chemotherapy followed by anti-CD19 CAR T cell therapy in patients with advanced lymphomas.
Methods: Twenty-two patients with advanced lymphoma were included in the study. Eighteen patients were treated with cyclophosphamide 300 mg/m^2 daily for 3 days; the remaining 4 patients received cyclophosphamide 500 mg/m^2 on this same schedule. Fludarabine 30 mg/m^2 was provided to all patients on the same schedule and days as cyclophosphamide. A single dose of CAR T cells was administered 2 days after chemotherapy was completed. CAR T cell concentrations and cytokine levels were examined in blood samples collected from patients. Informed consent was received from all patients.
Results: Among 19 patients with diffuse large B-cell lymphoma of various subtypes, 8 demonstrated complete response, 5 partial response, 2 stable disease, and 4 progressive disease. A complete response was also noted in one patient with mantle cell lymphoma. Two patients with follicular lymphoma both obtained complete response. Duration of response ranged from 1 to 20 months, with 10 remissions that remain ongoing. All but 4 patients had either chemo-refractory lymphoma or relapsed lymphoma following autologous stem cell transplantation. Neurological toxicities were the most commonly observed adverse events. Fever and hypotension were also observed. The median peak blood CAR+ cell concentration was 47/μL (range 4-1217/μL), with higher levels found in patients who demonstrated complete or partial response than in those with stable or progressive disease. The mean serum IL-15 level was 4 pg/mL prior to administration of conditioning chemotherapy and 32 pg/mL after chemotherapy (P<0.0001).
Summary/Conclusions: Low-dose conditioning chemotherapy followed by anti-CD19 CAR T cells induced remissions in patients with advanced B-cell lymphomas.

Relapsed Hodgkin Lymphoma & Primary Mediastinal Large B-Cell Lymphoma (PM-DLBCL)

S793

CHECKMATE 205: A PHASE 2 STUDY OF NIVOLUMAB IN PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION AND BRENTUXIMAB VEDOTIN

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Background: Classical Hodgkin lymphoma (cHL) is characterized by Hodgkin Reed–Sternberg cells, which contain genetic aberrations at 9p24.1 leading to overexpression of the programmed death receptor-1 (PD-1) ligands PD-L1 and PD-L2. Thus, cHL may be uniquely sensitive to PD-1 blockade. Nivolumab (Nivo) is a fully human IgG4 immune checkpoint inhibitor antibody targeting PD-1 that showed promising results in a phase 1b study (NCT01592370) in patients (pts) with relapsed/refractory cHL (Ansell SM et al. NEJM 2015;372:311-9) who currently have limited treatment options.
Aims: This study, Cohort B of the phase 2 Checkmate 205 study (NCT02181738), evaluated the efficacy and safety of nivo in pts with cHL who had received brentuximab vedotin (BV) after failed autologous stem cell transplantation (ASCT).
Methods: Nivo was given at 3 mg/kg IV every 2 weeks (wk). Response was assessed using 2007 IWG criteria, by both an independent radiologic review committee (IRRC) and investigators (Inv). The primary endpoint was IRRC-assessed objective response rate (ORR). Quality of life was assessed by EQ-5D VAS (0–100 scale) and EORTC QLQ-C30. Written informed consent was obtained for all pts. Results of the primary ORR analysis after approximately 6 months (mo) minimum follow-up are reported.

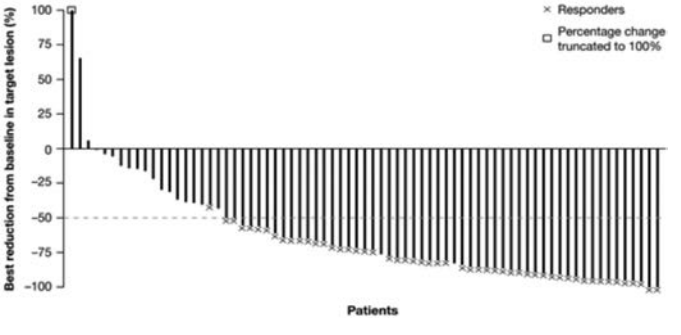

Figure 1. Best change from baseline in target lesions for all response-evaluable patients (IRRC assessment).

Results: Among 80 pts enrolled and treated, the main characteristics included median (range) age, 37 years (18–72) and median (range), 4 prior regimens (3–15). 33 pts enrolled from Europe; 47 were from the US or Canada. At data cut-off (October 2015), 51 pts (64%) remained on therapy. Of 29 pts (36%) who discontinued, the most common reason was disease progression (n=13). Only 4 pts discontinued due to an AE. 90% of pts had drug-related AEs, including 25% Grade 3–4 AEs and 1% Grade 5 (multi-organ failure). The most common drug-related AEs were fatigue (25%), infusion reaction (20%), and rash (16%). The most common serious AEs were pyrexia, tumor progression, arrhythmia, infusion reaction, septic meningitis, and pneumonia (≤4% each). Most common immune-mediated AEs were hypothyroidism/thyroiditis (14%), rash (10%), and hypersensitivity (6%). All 6 pts who stopped nivo and received subsequent stem cell transplant were alive at data cut-off. After median (range) follow-up of 8.9 mo (1.9–11.7), IRRC ORR (95% CI) was 66% (54.8%, 76.4%); CR and PR rates were 8.8% (3.6%, 17.2%) and 57.5% (45.9%, 68.5%), respectively. Almost all pts had some degree of tumor regression (Figure). All but one responder had tumor reduction of ≥50% from baseline; the other had response determined by negative FDG-PET scan. In 43 pts who had no response (SD or PD) to BV, nivo treatment resulted in IRRC ORR of 72% (31/43). Median (range) time to response was 2.1 mo (1.6–5.7). Inv ORR (95% CI) was 73% (61.4%, 81.9%); CR and PR rates were 27.5% (18.1%, 38.6%) and 45.0% (33.8%, 56.5%), respectively. At data cut-off, 62% (33/53) of IRRC responders remained in response. IRRC 6-mo PFS was 77%; OS was 99%. Mean EQ-5D VAS score increased over time, from 62 at baseline to 80 at Wk 33, with a clinically meaningful improvement in health state by Wk 9 (>7-point change). EORTC QLQ-C30 findings suggested a trend towards improvement in functional, symptom, and global health scores.
Summary/Conclusions: In pts with cHL who progressed following ASCT and BV, nivo is associated with a high response rate, long-lasting responses, and an acceptable safety profile. PFS and OS are encouraging in this heavily pretreated population. Patient reported outcomes suggest improvement in quality of life while on nivo treatment.

S794

MULTICOHORT PHASE 2 STUDY OF PEMBROLIZUMAB FOR RELAPSED/REFRACTORY CLASSICAL HODGKIN LYMPHOMA (R/R CHL): KEYNOTE-087

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Background: Patients with cHL who relapse after autologous stem cell transplantation (ASCT) or progress after brentuximab vedotin (BV) have a poor prognosis. In cHL, nearly universal 9p24.1 amplification, as well as less likely mechanisms such as Epstein-Barr virus infection and chromosomal rearrangements, induce PD-L1 and PD-L2 expression on the tumor cell surface, suggesting that cHL may have a genetically determined dependence on the PD-1 pathway. Pembrolizumab is a humanized monoclonal antibody against PD-1 that blocks interaction with PD-L1 and PD-L2. In the phase 1b KEYNOTE-013 study, pembrolizumab demonstrated high antitumor activity (objective response rate [ORR] of 65%) in heavily pretreated patients with cHL. KEYNOTE-087 is a phase 2 study designed to further evaluate the clinical activity of pembrolizumab in this patient population.
Aims: To determine the ORR, safety, and tolerability of pembrolizumab in patients with R/R cHL.
Methods: KEYNOTE-087 (NCT02453594) is a multicenter, single-arm, multicohort phase 2 study designed to evaluate the clinical activity of pembrolizumab in 3 cohorts: R/R cHL after ASCT and subsequent BV therapy (cohort 1); ineligible for ASCT due to chemo-resistance (no response to salvage chemotherapy) and BV therapy failure (cohort 2); and R/R cHL after ASCT but not treated with BV after ASCT (cohort 3). Patients received pembrolizumab at a fixed dose of 200 mg intravenously every 3 weeks. Primary end point was ORR, with response assessed every 12 weeks according to Revised Response Criteria for Malignant Lymphomas. A prespecified interim analysis, based on investigator-assessed response, was performed after 30 patients in cohorts 1 and 2 reached the first response assessment. Informed consent was obtained for all patients.
Results: At the time of data cutoff (Feb 1, 2016), 60 patients were evaluable for cohorts 1 and 2. Median (range) age was 36 (19 - 64) years in cohort 1 and 33 (20 - 71) in cohort 2. 35% had primary refractory disease (no response to frontline therapy), 67% received ≥4 prior lines of therapy, and by design 100% failed prior BV. ORR among 30 patients in cohort 1 was 70% (95% CI, 51% - 85%). 6 patients (20%) achieved complete response (CR; residual mass permitted if PET negative), 15 (50%) partial response (PR), and 6 (20%) stable disease as best response. ORR among 30 patients in cohort 2 was 80% (95% CI, 61% - 92%). 8 patients (27%) achieved CR, 16 (53%) PR, and 4 (13%) stable disease as best response. With a median of 6 treatment cycles, the most common treatment-related AEs (TRAEs) were pyrexia (13%), diarrhea (8%), and fatigue, platelet count decrease, dry skin, and cough (7% each). There were 7 grade 3 TRAEs in 3 patients: neutropenia, colitis, diarrhea, cytokine release syndrome, herpes zoster infection, increased amylase, and lichen planus; and only 1 grade 4 TRAE, increased lipase. There were no treatment-related deaths.
Summary/Conclusions: PD-1 blockade with pembrolizumab shows frequent responses in heavily pretreated patients with cHL. Of note, pembrolizumab provided a high ORR (80%) in patients who were not candidates for ASCT and failed previous BV therapy. Cohort 3 continues accrual and interim analysis results will be presented.

S795

VERY LATE RELAPSE >5 YEARS AFTER FIRST DIAGNOSIS OF HODGKIN LYMPHOMA: AN ANALYSIS OF THE GERMAN HODGKIN STUDY GROUP HD7-HD12 TRIALS

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Background: Patients who are free of Hodgkin lymphoma (HL) for >5 years after first diagnosis are usually considered cured. Nevertheless, late relapses occur and biology, clinical characteristics, therapeutic approaches and prognosis are currently poorly understood.
Aims: Our study hence aimed at a comprehensive analysis of very late relapse (VLR) of HL.
Methods: We retrospectively analyzed 5149 patients of the GHSG HD7-HD12 trials, who were observed and relapse-free for >5 years after first diagnosis to estimate the incidence of VLR of HL. Cumulative incidence of VLR was calculated with death without preceding relapse considered as competing risk and compared between groups using Gray's test. To investigate, whether VLR can be considered as new cases of HL, standardized incidence ratio (SIR) was estimated using age- and sex-specific reference values for the German population. Overall survival (OS) was estimated according to Kaplan-Meier from first diagnosis to death from any cause. Additionally, OS from date of relapse was estimated for patients with VLR and compared with a group of 487 patients from the respective trials having early relapse (≤5 years from first diagnosis). Patient characteristics and therapy at relapse were analyzed descriptively.
Results: With a median observation time of 10.3 years, a total of 169 relapses >5 years after first diagnosis were observed. In patients relapse-free for >5 years, cumulative incidences at 10, 15 and 20 years rose in a linear fashion and were 2.8%, 5.1% and 8.6%, respectively, with an SIR of 97.1 (95% CI: 83.0-112.9). VLR were more frequently observed in patients with early-stage favorable than early-stage unfavorable or advanced-stage disease at first diagnosis (15-year cumulative incidence 8.0% *vs* 4.4% and 4.2%, respectively, p<0.001). OS was significantly worse in patients with VLR when compared to non-relapse survivors (10-year OS: 95.9% *vs* 88.5%, HR: 2.4, 95% CI: 1.7-3.4, log-rank p<0.001). Compared to patients with early relapse, fewer patients with VLR were treated with autologous stem-cell transplantation (ASCT, 35% *vs* 49%). Instead, they often received polychemotherapies such as BEACOPP (18% *vs* 13%) or ABVD (15% *vs* 11%). Compared to early relapse, we observed superior OS after VLR (HR: 0.5, 95% CI: 0.3-0.8, p<0.01) after adjustment for type of therapy and risk group at first diagnosis. In addition, relapse characteristics, changes in histologic subtype and risk factors for VLR will be presented.
Summary/Conclusions: Besides therapy-associated side effects, survivors after initially successful HL-therapy are at a 100-fold increased risk of re-occurrence of disease compared to German reference values. After modern risk-adapted treatment strategies especially in early-stage favorable HL, thorough regular follow-up is hence needed for timely detection. With adequate treatment, prognosis of VLR seems favorable when compared to early relapses.

S796

ALLOGENEIC STEM CELL TRANSPLANTATION AND BRENTUXIMAB VEDOTIN IN RELAPSED/REFRACTORY HODGKIN LYMPHOMA: A MULTICENTER EXPERIENCE

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Background: Patients with relapsed/refractory (RR) Hodgkin lymphoma (HL) have a very poor prognosis. Allogeneic stem cell transplantation (allo-HCT) has been used in this setting with controversial results.
Aims: Aim of our study is to investigate the role of allo-HCT in RR HL.
Methods: This study reported on 69 patients with RR HL, median age 34 (range, 18 - 64), consecutively transplanted between 2000 and 2015 in 3 transplant center in Northwest Italy. Coordinator of the project was the Division of Hematology of the University of Torino. Patients were heavily pre-treated with a median of 4 therapy lines (range, 2 - 6). Sixty-four patients (93%) received previous high-dose chemotherapy and autologous transplantation. At the time of allo-HCT, 52 patients (75%) were at least in partial remission (PR) and were defined responsive. The remaining 16 patients (23%) had progressive disease (non-responsive). The disease status of 1 patient was not available. Brentuximab Vedotin (BV) was given as bridge to transplant in 11 patients. Moreover, 7 patients received BV after allo-HCT as salvage. The majority of patients underwent reduced intensity allo-HCT, 64 patients (93%). Donors were HLA-matched unrelated in 39 cases (57%), HLA-matched siblings in 28 cases (41%), HLA-haploidentical in 2 cases (3%). The stem cells source was peripheral blood in 61 patients (88%), bone marrow in 7 patients (10%) and cord blood in 1 patient (1%).
Results: Overall, 32 patients (46%) have died at the time of last contact, including 20 patients who had relapsed. This resulted in a median overall survival (OS) [Figure 1], and relapse-free survival (RFS) of 5.1 years (range, 0 - 13.8), and 1.3 years (range, 0-12.6), respectively. Median follow-up among the 37 survivors was 7.2 years (range, 0.1-13.8). The 5-year cumulative incidence of treatment related mortality (TRM) and relapse were 17.7% and 43.4%, respectively. As expected, patients younger than 35 had a lower 5-year TRM compared to older patients (8.7% *versus* 27.1%). The day-100 cumulative incidence of grades II-IV acute graft-*versus*-host disease (GvHD) was 36.7% (60 evaluable patients). Only 8 patients had grade III disease. No grade IV acute GvHD was reported. The cumulative incidence of limited or extensive chronic GvHD was 45.6% (57 evaluable patients). The 5-year estimated of RFS was significantly higher in responsive compared to non-responsive patients, 46.9% *versus* 12.5%, respectively, p= 0.009. There was a trend to improved 5-year OS in responsive patients, 54.6% *versus* 37.5%, respectively, p= 0.19. The RFS advantage of responsive patients was confirmed by multivariable Cox regression (HR= 3, 95% CI 1.4 - 6.4, p= 0.005). The 5-year relapse incidence was 35.3% and 68.8% in responsive and non-responsive patients, respectively. Eleven patients received BV as bridge to allo-HCT for a median of 6 cycles. Only 3 patients failed to achieved at least PR. Then, BV was used as salvage for post-alloHCT relapse in 7 patients. All patients achieved at least PR. None of patients treated with BV had unexpected toxicity or GVHD worsening.

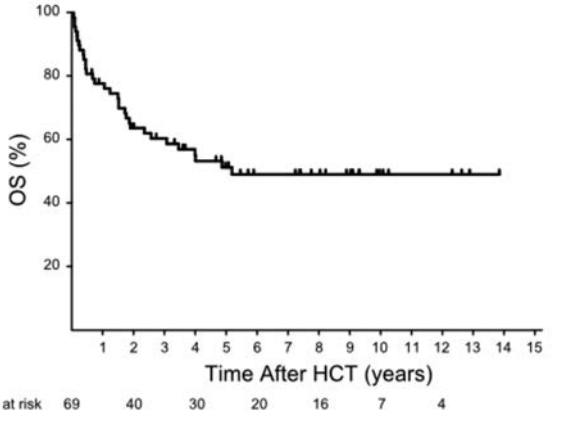

Figure 1.

Summary/Conclusions: Allo-HCT is a feasible and effective option for RR HL. In our series, the disease status at HCT was the main predictor of outcomes, primarily relapse. Furthermore, BV showed efficacy as a bridge to allo-HCT as well as post allo-HCT rescue.

S797

PHASE 1B STUDY OF PEMBROLIZUMAB IN PATIENTS WITH RELAPSED/REFRACTORY PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: KEYNOTE-013

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Background: Primary mediastinal large B-cell lymphoma (PMBCL) frequently exhibits 9p24.1 alterations, leading to overexpression of the PD-1 ligands, PD-L1 and PD-L2. This provides a possible mechanism of immune evasion and suggests that PMBCL could have a genetically determined vulnerability to PD-1 blockade. Pembrolizumab is a humanized monoclonal antibody that blocks the interaction between PD-1 and its ligands. Pembrolizumab has already demonstrated robust antitumor activity in advanced solid tumors and in classical Hodgkin lymphoma. KEYNOTE-013 (NCT01953692) is a multicenter, multicohort phase 1b study designed to evaluate the safety and preliminary efficacy of pembrolizumab in patients with hematologic malignancies. Preliminary results of the PMBCL cohort of KEYNOTE-013 are reported.
Aims: To determine the safety, tolerability, and antitumor activity of pembrolizumab in patients with relapsed/refractory (R/R) PMBCL.
Methods: Patients with R/R disease who have relapsed after or are ineligible for autologous stem cell transplantation (ASCT) received pembrolizumab IV 10 mg/kg Q2W or 200 mg Q3W for up to 2 years or until confirmed disease progression or unacceptable toxicity. Primary end points were safety and ORR.

Response was evaluated using computed tomography (CT) and positron emission tomography (PET) at weeks 6 and 12, and every 8 weeks thereafter, using IHP 2007 criteria. Other end points included complete remission rate and duration of response (DOR). Informed consent was obtained for all patients.
Results: As of February 1, 2016, 16 patients were enrolled and treated in this cohort. The first 11 patients received pembrolizumab 10 mg/kg every 2 weeks; the next 5 patients received a fixed dose of 200 mg every 3 weeks, which has demonstrated equivalence based on PK/PD studies. Median age was 30 years (range, 22-62), 44% had ≥4 prior lines of therapy, 31% had prior ASCT, and 75% had prior radiation. 10 patients (62%) experienced treatment-related AEs, most commonly grade 1-2 decreased appetite, diarrhea, fatigue, hypothyroidism, nausea, and pyrexia (2 patients each). Only 1 patient experienced a grade 3 treatment-related AE (neutropenia), and there were no grade 4 treatment-related AEs or treatment-related deaths. 4 patients experienced serious AEs, including grade 3 pneumonia in 3 patients and grade 3 cough in 1 patient; all were unrelated to study drug. No patients discontinued for toxicity. Among the 16 treated patients, the median follow-up was 5.0 months (range, 0.8-22 months) and the objective response rate (ORR) was 37.5% (6/16), with 1 patient (6.25%) achieving a complete remission and 5 patients (31.25%) achieving a partial response. One subject discontinued treatment based on clinical progression before the first assessment and was considered a non-responder. The median DOR has not been reached (range, 0.03+-17+ months), with 5 responses ongoing at the time of data cutoff. 9 have discontinued treatment: 5 because of progressive disease based on imaging, 3 because of clinical progressive disease, and 1 because of physician decision.
Summary/Conclusions: The preliminary results of KEYNOTE-013 indicate that PD-1 blockade with pembrolizumab is associated with a tolerable safety profile and a promising response rate in heavily pretreated patients with R/R PMBCL. These patients often have a very poor outcome with conventional therapy, justifying further studies of pembrolizumab in this population.

ALL Biology - Transcriptional dysregulation

S798

TRANSCRIPTION-COUPLED GENETIC INSTABILITY MARKS ACUTE LYMPHOBLASTIC LEUKEMIA STRUCTURAL VARIATION HOTSPOTS

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Background: Next generation sequencing technologies have drawn attention to the analysis of genetic alterations across different cancer genomes. Precursor leukemias are unique in that they often harbor structural variations (SV) and have relatively few mutations. Instead, the maturing lymphoid cells are vulnerable to off-target effects downstream of the recombination activating genes (RAG) and activation-induced deaminase (AID, encoded by the *AICDA* gene) activity that is required for immune gene rearrangement.
Aims: Earlier *in vitro* studies on DNA binding and genome-wide binding profiles of RAGs have shed light on the mechanisms how this complex is recruited to DNA; however these studies have been carried out using normal cells or mouse models that limit their integration with patient WGS data. More recently, a functional role of transcription in genomic instability has begun to emerge: AID can generate off-target DNA breaks at loci that harbor highly active enhancers and display convergent transcription (transcription from both strands) in lymphomas. The present study represents a systematic investigation of SVs detected in acute pre-B-cell leukemia (pre-B-ALL) in the context of global transcriptional activity in leukemic cells.
Methods: RNA polymerases engaged into primary transcription across the genome can be measured using Global-Run-On sequencing (GRO-seq). Therefore, this method is ideally suited to distinguish features of transcription at SV sites. Transcriptional activity was assayed using GRO-seq from ALL cells representing seven different pre-B-ALL cytogenetic subtypes, and jointly analyzed with public WGS data from the ETV6-RUNX1 (51 cases), high hyperdiploid (16 cases), hypodiploid (20 cases) and MLL-rearranged (22 cases at diagnosis and 2 relapses) subtypes of precursor B-ALL. Pre-B-ALL patient expression profiles (N=1,382), B-lymphoid cell chromatin conformation capture (HiC), RNA polymerase ChIP-seq and DNAse hypersensitivity (DNAse-seq) data were used as additional data.
Results: The active transcriptional profiles from ALL patients revealed striking similarity at SV sites to AID off-target lesions reported in lymphomas. Based on pre-B-ALL transcriptomes, high *AICDA* levels were particularly prevalent in sample clusters that corresponded to high-risk ALL cases and 62% were cytogenetically classified to the subtype "other". The highest level of *AICDA* expression was presented by a relapsed ALL case with high expression already at diagnosis. SV with RAG recognition motifs (RSS motif) also associated strongly with features of transcriptional activity. We could show that elevated levels of convergent transcription that typically occurs at intragenic enhancers positively correlated with detected R-loop levels (needed for AID recruitment) and number of RNA polymerase stalling events at breakpoints. The wide stalling regions identified correlated with highly accessible DNA regions. Accordingly, RNA polymerase stalling sites detected at TSS regions with breakpoints harboring the RAG recognition motif were significantly wider than stalling sites found at TSS regions with no breakpoints, indicating that RNA polymerase stalling could mediate RAG access to its cleavage sites.
Summary/Conclusions: The results support a model in which transcriptional features (convergence of transcription and Pol2 stalling), through altering access to DNA, could act to guide secondary DNA lesions and therefore leukemia progression. Our results position RAG among complexes involved in transcription-coupled genomic instability. Moreover, we present evidence of expression of *AICDA* in high-risk / cytogenetic class "other" pre-B-ALL cases.

S799

TRIGGERING THE TCR DEVELOPMENTAL CHECKPOINT ACTIVATES A THERAPEUTICALLY TARGETABLE TUMOR SUPPRESSIVE PATHWAY IN T-CELL LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) results from the leukemic transformation of thymic cell precursors caused by a multistep pathogenesis involving numerous genetic and epigenetic abnormalities. They are characterized by high relapse rates and poor prognosis, calling for the search of novel therapeutic options. T lymphocytes differentiate in the thymus according to a highly orchestrated process involving developmental checkpoints. Among these, negative selection is a major process by which thymocytes recognizing self-Major Histocompatibility complex with high affinity are eliminated by TCR-mediated apoptosis.
Aims: In this study, we hypothesized that tissue homeostatic regulators may be amenable to reactivation in tumor cells. We inferred that experimentally chronic/persistent induced TCR signaling in T-ALL could initiate a molecular program similar to negative selection of thymic T-cell progenitors and induce cell death.
Methods: We used the transgenic Marilyn TCR-HY in a human T-ALL cell line (ALL-SIL) and in a mouse model of TEL-JAK2-induced T-ALL, to test TCR activation by its cognate peptide/MHC. We evaluated chronic/persistent TCR activation by anti-CD3ε monoclonal antibody *in vitro* (in TCR-HY ALL-SIL and in a panel of 36 primary T-ALL patients cases) and *in vivo* (in TJK2/CD3ε+ and TJK2/CD3ε- mouse T-ALL model and in xenotransplanted mice with 7 human T-ALL).
Results: Using the Marilyn TCR-HY transgene, we showed that TCR activation by its cognate peptide/MHC induced apoptosis *in vitro* (ALL-SIL TLX+ cell line) and dramatically impaired development/maintenance of leukemias *in vivo* (murine TEL-JAK2-induced T-ALL) exclusively in DBY-expressing male mice. Anti-CD3ε monoclonal antibody stimulation mimicked high affinity self-peptide/MHC-induced TCR signaling (human OKT3; murine, 145-2C11): this led to massive leukemic cell death and remarkably induced a gene expression program similar to thymic negative selection. In addition, *in vitro* stimulation of CD3/TCR complex with an anti-CD3ε antibody resulted in cell death of primary CD3/TCR-expressing T-ALL (n=36) regardless their underlying oncogenetic characteristics. Keeping with this, *in vivo* treatment by anti-CD3ε monoclonal antibody prevented from the emergence of the leukemic process in several TCR+ T-ALL murine models, whereas it had virtually no impact on the survival of mice engrafted with murine CD3ε$^{-/-}$ T-ALL cells. Importantly, anti-CD3ε treatment hampered leukemogenesis in xenotransplanted mice with human TCR/CD3+ T-ALL (n=7) in both preventative and curative settings.
Summary/Conclusions: In our study, we found that reactivation in T-ALL blasts of the lineage-specific checkpoint control normally set by TCR signaling during T-cell development, displays anti-tumoral functions. Importantly, despite the multiple and complex oncogenic mechanisms driving T-ALL, this TCR-dependent checkpoint remains switchable to induce massive tumor cell apoptosis. These data provide a strong rationale for targeted therapy based on anti-CD3 treatment of TCR-expressing T-ALL patients and demonstrate that endogenous developmental checkpoint pathways are amenable to therapeutic intervention in cancer cells.

S800

FAST GENERATION OF CONDITIONAL ROSA26-BASED MOUSE MODELS THAT RECAPITULATE GENOMIC EVENTS IN HUMAN T-ALL

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Background: T-cell acute lymphoblastic leukemias (T-ALLs) are aggressive hematologic tumors that result from the malignant transformation of T-cell progenitors. Due to intensified chemotherapy, the prognosis of T-ALL has gradually improved. Nevertheless, this clinical improvement is most pronounced in pediatric treatment protocols, whereas adult patients more often present with primary resistant or relapsed disease.
Aims: Our Aim is to develop new mouse models that mimic oncogenic lesions identified in human disease. These mouse models are critically required to further enhance our knowledge on the molecular mechanisms that drive T-cell leukemogenesis. Moreover, they serve as important pre-clinical models to evaluate new therapeutic strategies for the treatment of human T-ALL.
Methods: Here, we generated a fast method for embryonic stem cell (ESC) targeting and mouse chimera production. We constructed gateway-compatible vectors that allow tailor-made targeting vector design, including conditional expression of a transgene combined with an eGFP/luciferase reporter from the ROSA26(R26)-promoter or from (inducible) exogenous promoters. The final targeting vector is recombination-mediated cassette exchange (RMCE)-compatible and can be inserted in a genetically engineered R26-locus via RMCE. Correct integration of the incoming construct reactivates the NeoR gene and results in up to 100% ESC targeting efficiencies.
Results: Using our technology, we generated R26-based conditional knock-in mouse models for putative oncogenes that have previously been implicated in T-ALL disease biology. More specifically, the MYB leucine zipper transcription factor is aberrantly activated in a subset of T-ALL patients through T-cell receptor driven translocations (t(6;7)(q23;q34)) or genomic duplications of the MYB locus itself. Moreover, Myb was found to be crucial for the initiation of oncogenic superenhancers in T-ALL. In addition, another subset of T-ALLs show specific TCR-mediated translocations that drive aberrant expression of the cell cycle regulator Cyclin D2 (CCND2). To study the *in vivo* roles of cMyb and Ccnd2 in the pathogenesis of T-ALL, we used the above-mentioned genomic engineering technology to generate cMyb and Ccnd2 conditional knockin mouse models. These animals were crossed with Lck-Cre mice to obtain T-cell specific expression of the oncogenes of interest at moderate pathophysiological relevant levels. Currently, we are monitoring tumor development in T-cell specific homozygous knockin mice. In addition, Myb and Ccnd2 transgenic animals are crossed into a Pten null background to accelerate disease onset. Preliminary data shows that T-cell specific Ccnd2 overexpression cooperates with Pten loss to decrease T-ALL latency.

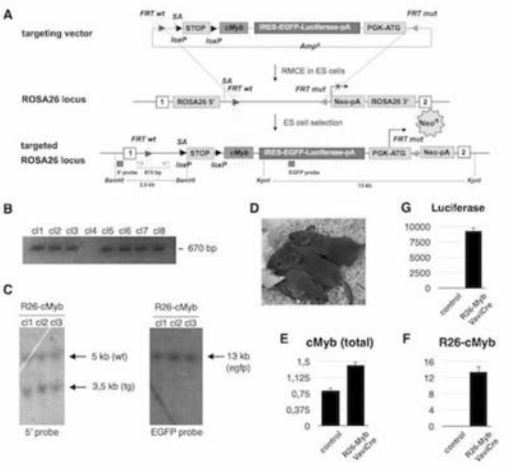

Figure 1.

Summary/Conclusions: All together, we have used a fast genomic engineering pipeline to develop new and pathophysiological relevant conditional overexpression mouse models for human T-ALL.

S801

ACTIVATED JAK-STAT SIGNALING COOPERATES WITH HOXA9 AND DRIVES LEUKEMIA DEVELOPMENT

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Background: The JAK-STAT signaling pathway is critical for the normal development and proliferation of hematopoietic cells. Mutations in JAK1 or JAK3 occur within ~20% of T-cell acute lymphoblastic leukemia (T-ALL) cases and have also been described in other malignancies. We have previously shown that JAK3 mutations are able to transform hematopoietic cells *in vitro* and cause long latency T-ALL in a mouse model.
Aims: To determine mutations which are significantly associated with ectopic activation of the JAK-STAT signaling pathway and determine whether they cooperate in leukemia development *in vivo*.
Methods: Targeted-resequencing of 115 genes across 155 diagnostic T-ALL samples and qPCR on patient derived xenograft (PDX) samples was used to evaluate candidate genes significantly associated with mutations within the JAK-STAT5 pathway. Oncogenic cooperation and transformation to cytokine independent growth was evaluated using primary hematopoietic progenitor cells *ex vivo*. *In vivo* leukemia cooperation was assayed using a modified bone marrow transplant assay using either wild type or CD4-Cre donor mice in conjunction with either a retroviral vector for constitutive expression in all hematopoietic lineages or a novel antiparallel lox66/71 retroviral vector for restricted expression within developing T-cells.
Results: Mutations in JAK3 were found to frequently co-occur with HOXA cluster rearrangement and our qPCR analysis of PDX samples identified HOXA9 as the most upregulated HOXA gene in cases with mutant JAK3. Constitutive expression of the JAK3(M511I) mutant together with HOXA9 transformed murine hematopoietic progenitors to cytokine independent growth *ex vivo*, while JAK3 mutant or HOXA9 alone did not transform cells *ex vivo*. To model the possible cooperation of JAK3(M511I) and HOXA9 in vivo we initially used the bone marrow transplant model with constitutive expression of JAK3(M511I) and HOXA9. This led to the rapid development of a mixed myeloid-lymphoid leukemia in vivo (median survival=82 days) compared to JAK3(M511I) alone (median survival=164 days) or HOXA9 alone (no disease). To study the cooperation during T-ALL development specifically, we modified the bone marrow

transplant model by using a novel inducible retroviral vector in combination with CD2-Cre or CD4-Cre donor bone marrow cells so that JAK3(M511I) and HOXA9 expression is delayed to lymphoid progenitors or T-cells. All models confirmed a clear cooperation between JAK3(M511I) and HOXA9, and ChIP-seq, ATAC-seq, and RNA-seq on the leukemic cells confirmed cooperation at the transcriptional level between STAT5 (downstream of JAK3) and HOXA9
Summary/Conclusions: JAK3 mutations and ectopic expression of HOXA9 cooperate to transform hematopoietic cells and cause rapid leukemia development *in vivo.*

S802

BET INHIBITOR AS A NOVEL THERAPEUTIC APPROACH FOR THE TREATMENT OF MLL-REARRANGED INFANT ACUTE LEUKEMIA
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Background: Despite the improvements in the field of pediatric leukemia achieved in the last decades, some groups of patients still suffer from a low cure rate. Acute lymphoblastic leukemia occurring in infants carrying the *MLL* gene translocation (MLL+ infant ALL) has an overall poor outcome due to drug resistance and high relapse rate. Numerous studies have shown that the perturbation of the chromatin structure and the transcriptional deregulation induced by the MLL translocation represent the main mechanism of leukemogenesis. Therefore, new therapeutic strategies targeting the chromatin structure and/or the recruitment of the transcriptional complex represent nowadays the most promising approaches.
Aims: In this study we aimed to investigate the effect of Bromodomain and Extra Terminal (BET) inhibition by using the inhibitor I-BET151. BET is a family of adaptor proteins, including BRD2, BRD3 and BRD4, which bind to the acetylated residues of the histone core, and function as epigenetic readers.
Methods: The anti-leukemic effects of I-BET151 administration was tested *in vivo* in a mouse model of human MLL+ infant ALL. A human MLL+ cell line or primary samples derived from infant patients were transplanted into immunedeficient mice, in order to recapitulate the disease in vivo. Subsequently, the animals were treated with I-BET151 or with the vehicle only, via intraperitoneal injection. Additionally, the biological mechanism of the compound was further elucidated at the molecular lever, both *in vitro* using several human MLL+ ALL cell lines, as well as *ex vivo* in patients-derived xenograft (PDX) samples from mice.
Results: Herein we observed that I-BET151 administration reduces the engraftment and the disease burden of MLL+ infant ALL *in vivo* and prolongs the survival in mice. Importantly, the anti-leukemic effect of I-BET151 was also confirmed in a "curative setting" experiment, where the treatment was started when the leukemia was already consolidated in mice. Furthermore we elucidated the biological mechanism of the compound and demonstrated that I-BET151 is able to block cell proliferation (G0/G1-phase arrest) and induce apoptosis through the downregulation of known BRD4 target genes (i.e. *c-MYC*, *BCL2* and *CDK6*) and the impairment of the IL7Ra/STAT5 signaling pathway. Moreover, the analysis of transcriptional profile changes of PDX samples exposed to I-BET151 *ex vivo* allowed us to identify a specific "I-BET core signature" of genes deregulated upon treatment and belonging to the BRD4 and HOXA gene network. The function of these newly identified genes and their potential role in MLL leukemogenesis is currently under investigation. Finally, we have observed that I-BET151 in combination with HDAC inhibitors is even more efficient compared to the single therapy, as these two compounds have a synergic activity.
Summary/Conclusions: Taken together our data show that I-BET151, alone or in combination with HDAC inhibitors, exerts a potent anti-leukemic effect on MLL+ infant ALL. Notably, our study demonstrated for the first time the efficacy of I-BET151 in primary patients' samples. In conclusion, given the aggressiveness of the disease and the lack of a cure for infant patients with MLL leukemia, this study is extremely novel and particularly relevant in the field of hematology, as I-BET151 may represent in the future a promising novel approach for the treatment of this high-risk leukemia.

AML Biology - Novel targeted therapies

S803

TARGETING CHROMATIN REGULATORS INHIBITS LEUKEMOGENIC GENE EXPRESSION IN NPM1 MUTANT LEUKEMIA
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Background: Homeobox (HOX) proteins and the receptor tyrosine kinase FLT3 are frequently highly expressed and mutated in acute myeloid leukemia (AML). Aberrant *HOX* expression is found in nearly all AMLs that harbor a mutation in the *NPM1* gene (*NPM1^{mut}*), and *FLT3* is concomitantly mutated in ~50% of these cases. Little is known how these cells maintain aberrant gene expression. Since specific chromatin regulatory complexes control *HOX* expression in normal hematopoiesis and in leukemias expressing an oncogenic MLL-fusion protein, we hypothesized that they might also regulate *HOX* expression in other settings, and that inhibition of *HOX* and *FLT3* expression would produce an antiproliferative effect in *NPM1^{mut}* AML.
Aims: To investigate dependencies of *NPM1^{mut}* AML on MLL and DOT1L complexes and to delineate their potential role in control of leukemogenic gene expression.
Methods: CRISPR-Cas9 genome editing across exons encoding specific protein domains was used in a negative selection screen to assess potential dependencies of *NPM1*^{mut} AML on specific MLL protein domains, and this concept was extended with inhibitors of the menin-MLL interaction (MI2-2 and MI503). Dependencies on DOT1L were explored using the inhibitor EPZ4777. *In vitro* and *in vivo* target validation was performed in two conditional murine knock-in models (*Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$; *Npm1*$^{CA/+}$*Rosa*$^{SB/+}$) and in a disseminated human xenograft model of *Npm1^{mut}* AML.
Results: Using a CRISPR-Cas9 negative selection screen, we discovered the menin binding site of MLL as a dependency in *NPM1^{mut}* OCI-AML3 cells, a region critically involved in chromatin binding of the MLL complex. We next sought to assess treatment effects of MI2-2 and MI503 and observed dramatic suppression of *HOX* and *MEIS1* expression, differentiation induction, and profound growth inhibition in human OCI-AML3 cells and murine *Npm1^{mut}* AMLs. Of interest, ectopic expression of *Meis1, Hoxb4,* or *Hoxa9-Meis1* rescued the antiproliferative effects of menin-MLL inhibition. *In vivo* MI503 treatment of leukemic OCI-AML3 xenografts resulted in profound *HOX* and *MEIS1* suppression, significant reduction of leukemia burden, and in a significant survival advantage of both, human xenografts and secondarily transplanted *Npm1*$^{CA/+}$*Rosa*$^{SB/+}$ mice compared to vehicle controls. As *MEIS1* was most dramatically suppressed across all three models, we determined drug effects on *FLT3* expression, a reported target gene of *MEIS1* and found consistent suppression of *FLT3* to almost undetectable levels. MI503 treatment depleted menin and H3K4me3 on *HOX* and *MEIS1* loci. We also observed a significant decrease of H3K79me2 at those loci, pointing to a potential role for DOT1L, the sole H3K79 methyltransferase. Treatment with EPZ4777 resulted in *HOX, MEIS1,* and *FLT3* down regulation, cell growth inhibition, and profound differentiation of *NPM1^{mut}* AML blasts just as we found with inhibition of the menin-MLL interaction. Next, we investigated effects of combinatorial menin-MLL and DOT1L inhibition and found superior suppression of *HOX* and *FLT3* expression, dramatically more pronounced differentiation, and synergistic growth inhibition. Combinatorial drug treatment further reduced leukemia initiating potential of *NPM1^{mut}* AML cells dramatically compared to single drug treatment *in vivo.*
Summary/Conclusions: MLL and DOT1L are chromatin regulators that control *HOX*, *MEIS1* and *FLT3* expression in *NPM1^{mut}* AML. Combinatorial small-molecule inhibition has synergistic on target activity and represents a potential therapeutic opportunity that should soon be ready for clinical assessment.

S804

CHEMO-GENOMIC INTERROGATION OF PRIMARY ACUTE MYELOID LEUKEMIA WITH BIALLELIC CEBPA MUTATIONS REVEALS RECURRENT CSF3R MUTATIONS AND SUBGROUP SENSITIVITY TO JAK INHIBITORS
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Background: Acute myeloid leukemias (AML) with *CEBPA* mutations define a provisional entity in the WHO 2008 classification. Patients with biallelic *CEBPA* (*CEBPA*^{bi}) mutations comprising N-terminal frameshift and C-terminal in frame mutations (hereafter termed typical *CEBPA*^{bi} AML) characteristically present a normal karyotype and have a favorable clinical outcome. In contrast, characteristics of samples with other combinations of mutations (atypical *CEBPA*^{bi} AML) are less well established.
Aims: Using the RNA-sequencing data from 415 primary AMLs in Leucegene cohort, we aimed to refine the transcriptomic and mutational landscape of 14 *CEBPA*^{bi} AML samples (7 typical and 7 atypical) present in this collection, and to interrogate the novel mutations identified in this subgroup in a targeted chemical inhibitor screen.
Methods: We performed RNA-sequencing, comparative transcriptomic analysis, mutation detection, cell culture and chemical screening using the methods previously reported (Lavallée *et al*, Nature Genetics, 2015).
Results: The 7 typical *CEBPA*^{bi} specimens are best characterized by 95 differentially expressed genes. Using this gene expression profile (GEP), we next performed a principal component analysis and determined that 4/7 atypical *CEBPA*^{bi} samples clustered with typical *CEBPA*^{bi} (hereafter termed GEP+), while 3/7 atypical *CEBPA*^{bi} samples did not (GEP-). Identification of low *HOXA9* expression alone was sufficient to discriminate between GEP+ and GEP- atypical *CEBPA* AML (>300 fold median *HOXA9* expression ratios). In our cohort of *CEBPA*^{bi} AML we identified 23 mutated genes, including the previously reported mutations in *WT1* and *GATA2* in 3/14 (21% each). The most frequent mutations in *CEBPA*^{bi} AML was the activating *CSF3R* T618I mutation present in 29% (4/14) of this subgroup compared to only 3/401 *CSF3R* mutated samples in other AML subtypes (p<0.0001). *CSF3R* encodes the granulocyte colony stimulating factor receptor (G-CSFR) and is a direct target of CEBPA, suggesting a selective pressure for acquisition of these mutations in *CEBPA*^{bi} AML cells. Interestingly, an additional mutation in G-CSFR pathway (STAT5B N642H) was present in a fifth *CEBPA*^{bi} AML sample. Considering the high frequency of *CSF3R* mutations in *CEBPA*^{bi} AML we conducted a targeted chemical screen employing a collection of compounds (n=11) enriched for JAK inhibitors. Inhibitors were tested in a dose response assays using *CEBPA*^{bi} (n=14) and control normal karyotype *CEBPA* wild-type (NK *CEBPA*^{wt}, n=14) primary AML cells. Results showed that all *CSF3R* T618I mutated samples were sensitive to JAK inhibitors (*e.g.* median ruxolitinib IC_{50}=66nM (range: 48 - 94)). Most interestingly, *CEBPA*^{bi} GEP+ samples (n=11), irrespective of their *CSF3R* mutation status, were uniformly and significantly more sensitive than NK *CEBPA*^{wt} specimens to JAK inhibitors (*e.g.* ruxolitinib median IC_{50}: 62 *vs* 181 nM, p=0.01), but not to other inhibitors such as dasatinib or sorafenib, or to cytotoxic agents daunorubicin and cytarabine. This may suggest that networks upstream of JAK-STAT are aberrantly activated in a majority of these specimens. In contrast, *CEBPA*^{bi} GEP- were less sensitive to JAK inhibitors (*e.g.* ruxolitinib median IC_{50}: 285, range: 48 to >10,000 nM), with the exception of a single sample carrying *CSF3R* T618I mutation. These results indicate that the transcriptionally distinct *CEBPA*^{bi} GEP- AML are also distinguishable from *CEBPA*^{bi} GEP+ AML in their responses to various chemical compounds.
Summary/Conclusions: Our study reports a novel co-occurrence of mutations within the *CEBPA*/*CSF3R* pathway in *CEBPA*^{bi} AML and reveals a uniform sensitivity to JAK inhibitors in the transcriptionally uniform *CEBPA*^{bi} GEP+ AML cells. Altogether, it paves the way to personalized clinical trials repositioning JAK inhibitors for *CEBPA*^{bi} AML.

S805

AGONISTIC TARGETING OF TLR1/TLR2 FORCES ACUTE MYELOID LEUKEMIA CELLS INTO DIFFERENTIATION

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Background: Acute myeloid leukemia (AML) is a fatal disease that is characterized by a rapid expansion of myeloid leukemic blasts with impaired differentiation that accumulate in the bone marrow. Because the prognosis for AML is poor and the treatment for most AML subgroups has remained similar for almost four decades, demand is strong for new types of therapies in this disorder. It is thought that one of the major obstacles in successfully treating AML is to efficiently target the AML cells with leukemia-initiating capacity, which often evade standard chemotherapy treatments, leading to disease relapse.
Aims: The aim of this project has been to characterize Toll-like receptor 1 (TLR1) as a therapeutic target on primitive AML cells.
Methods: A murine AML model driven by retroviral *MLL-AF9* fusion expression was used for the mouse studies. Flow cytometry was used to measure Tlr1 expression, for cell cycle analysis, and to measure activated AKT and NFκB. For studies of human AML, *MLL-AF9* transformed cord blood cells were used, and also AML patient samples. Global gene expression profiling was performed using RNA-sequencing.
Results: In search for candidate therapeutic cell surface targets in AML, we here identified TLR1 as upregulated in primitive AML cells relative to corresponding normal bone marrow cells. Stimulating primitive leukemic cells with Pam3CSK4, a Tlr1/Tlr2 agonist, resulted in an increase in actively cycling cells and cell expansion. Morphologic analysis and enhanced CD11b expression indicated rapid myeloid differentiation of the cells. Moreover, Pam3CSK4 strongly suppressed their leukemia-initiating capacity as assessed in *ex vivo* treatment experiments followed by transplantations into sublethally irradiated recipient mice. In contrast, *ex vivo* Pam3CSK4-stimulation of normal hematopoietic stem and progenitor cells only mildly suppressed their long-term repopulating ability in competitive transplants. In human leukemia, Pam3CSK4-stimulation of *MLL-AF9*-transformed cord blood cells pushed them into differentiation and eradicated their colony-forming capacity accompanied by AKT and NFκB activation. In AML patient samples, Pam3CSK4 caused cell differentiation, evidenced by morphological changes consistent with macrophage outgrowth and an increase in CD15-expressing cells, whereas minor effects were observed in normal bone marrow cells. The Pam3CSK4-induced differentiation showed strong correlated (r=0.98) with the level of TLR1 expression in AML $CD34^+CD38^-$ cells. Hence, our data suggest that AML patients with high TLR1 expression would be more susceptible to treatments targeting TLR1.
Summary/Conclusions: In summary, these findings demonstrate that TLR1 is upregulated in primitive AML cells and that agonistic targeting of the TLR1/TLR2 complex forces AML cells into differentiation, revealing a new putative strategy for therapeutically differentiating AML cells.

S806

GPR56 CONTRIBUTES TO THE DEVELOPMENT OF ACUTE MYELOID LEUKEMIA IN MICE

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Background: One of the major goals in AML research is to identify key characteristics of leukemic stem cells (LSCs) in patients with AML. Gene expression profiling in functionally validated primary AML LSCs has established a gene signature, which consists of 42 genes associated with LSC properties and inferior treatment outcome in patients with AML (Eppert et al., Nature Medicine 2011). Among these 42 genes the G-protein coupled receptor 56 (GPR56) was highly expressed in the LSC enriched fraction compared to the CD34-negative leukemic bulk.
Aims: The aim of this study was to determine the functional role of Gpr56 in AML and to test its accessibility for antibody-mediated targeting of human AML LSCs.
Methods: For this retrovirally engineered overexpression of Gpr56 and the murine bone marrow transplantation model was used. For analyses in the human system NSG xenografting was employed.
Results: 43 clinically and molecularly annotated AML samples of different genotypes were analysed by RQ-PCR, confirming high expression in NPM1c mutated AML and lower expression in the CBF INV16 AML subtype with a 4.7fold difference between the two genotypes. Of note, expression of GPR56 was highest in normal $CD34^+$ hematopoietic stem and progenitor cells, indicating that there is substantial, but not aberrantly high GPR56 expression in human AML cells. To test whether expression levels of GPR56 correlated with treatment outcome, microarray based GPR56 expression of 423 clinically and molecularly annotated newly diagnosed patients treated in two independent prospective clinical trials was correlated with event free and overall survival: when the median expression level of GPR56 was taken as cut-off, high GPR56 expression was associated with inferior event free and overall survival in the total cohort of patients (n=423) as well as in the patients with normal karyotype (n=184) Functional relevance of GPR56 expression was validated in mice, in which co-expression of Gpr56 and Hoxa9 induced leukemia in transplanted mice after a median time of 148 days post transplantation (range 93-264) in contrast to the Hoxa9 and Gpr56 controls, which did not develop any signs of disease in the observation period of up to 380 days post transplant. Vice versa, the onset of leukemia was significantly delayed by shRNA-mediated knockdown of GPR56 in Hoxa9/Meis1 transduced cells when transplanted into mice. Overexpression of Gpr56 grossly changed the molecular phenotype of Hoxa9 transduced cells affecting pathways involved in G-coupled protein receptors and associated intracellular signaling. As GPR56 is part of the LSC signature in human AML, associated with inferior prognosis and as we had shown that GPR56 in collaboration with the oncogene Hoxa9 contributes to myeloid leukemogenesis, it was tested whether antibody-mediated blockage of the surface receptor GPR56 impairs leukemic engraftment in the NSG mouse model. Blockage of GPR56 with a monoclonal naked antibody significantly impaired engraftment into NSG mice. Importantly, also $FLT3\text{-}ITD3^+$ $NPM1c^-$ primary patient samples showed an up to 68% decrease in BM engraftment at 12 weeks (p<0.04).

Summary/Conclusions: Taken together, these data demonstrate that high expression of GPR56 is able to contribute to AML development and characterize the GPR56 as a potential novel target for antibody-mediated anti-leukemic strategies.

S807

SUPER-ENHANCER ANALYSIS DEFINES NOVEL AML AND MDS SUB-TYPES SENSITIVE TO SY-1425, A POTENT AND SELECTIVE RARA AGONIST

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Background: Super-enhancers (SEs) are exceptionally large, highly active chromatin regions that are densely occupied by transcription factors. They have been implicated in directing gene expression programs that define cell identity and also regulate oncogenes critical to the pathogenesis of cancer. One such target, the retinoic acid receptor alpha (RARα) was identified by the asymmetric distribution of its enhancer in a cohort of 60 non-APL AML patient samples profiled by our gene control platform.
Aims: This study sought to characterize the relationship between a *RARA* super-enhancer and response to SY-1425, a potent and selective RARα agonist, in AML patient samples, cell lines, and mouse models. MDS patient samples were also tested for comparable *RARA* enhancer levels and elevated biomarker.
Methods: ChIP-seq and RNA-seq were used to study the presence of SEs and correlated mRNA in patient samples. ChIP-seq, RNA-seq, and expression arrays were used in AML cell lines for the same profiling in addition to characterization of the transcriptional and chromatin level response to SY-1425. The effect of SY-1425 was studied in proliferation assays for AML cell lines and in patient-derived murine xenograft models of AML.
Results: The presence of a SE at the *RARA* gene locus was found to predict anti-proliferative sensitivity to the potent, selective RARα agonist, SY-1425, both *in vitro* and *in vivo.* SY-1425 is a clinical stage RARα agonist with greater potency, selectivity, and improved PK over the pan-RAR agonist, ATRA. SY-1425 is not susceptible to auto-induced metabolism and markedly inhibited growth of *RARA* SE-selected patient-derived murine xenograft models in which ATRA had no significant benefit. Normal bone marrow blast cells have low levels of *RARA* enhancer activity. However, the formation of the SE at the *RARA* gene locus drives RARα transcription in excess of endogenous ligand level, favoring the unliganded, repressive state of the transcription factor that leads to myeloid differentiation block. SY-1425 treatment of SE-containing AML cells strongly induces activation of these formerly repressed genes, halting proliferation and promoting differentiation. To examine transcriptional response to SY-1425, ChIP-seq for enhancer (H3K27ac) and RARα binding was paired with mRNA profiling before and after treatment. Following treatment, loci with strong RARα peaks showed increased acetylation and there was formation of new enhancers where little acetylation was previously observed, consistent with RARα serving as a repressor when unliganded and as a transcriptional activator with SY-1425 bound. Moreover, the genes near the SY-1425-induced enhancers are found to be strongly upregulated. This effect is exemplified by enhancer and mRNA upregulation of *TGM2*, a canonical ATRA differentiation response gene in APL. This mechanism is similar to PML-RARα differentiation blockade and SY-1425-induced gene expression changes are very similar to those observed with retinoid treatment of APL cells. In addition to AML, a sub-population of MDS patient samples also contain elevated levels of RARA SE and associated biomarker, suggesting the potential utility of SY-1425 in this closely related disease. Furthermore, ChIP-seq analysis has established that SEs exist in MDS which are comparable to those in *RARA* SE-driven AML.
Summary/Conclusions: In summary, our gene control platform has identified novel subsets of non-APL AML and MDS patients who may respond to the selective RARa agonist, SY-1425. Based on these findings, a phase 2 clinical investigation of SY-1425 in AML and MDS patients, using an SE-derived biomarker to enrich for patients likely to respond, is planned for later this year.

Treatment in specific AML subgroups

S808

HIGHER DOSE DAUNORUBICIN APPEARS BENEFICIAL IN PATIENTS HARBOURING A FLT3-ITD MUTATION: UPDATED RESULTS OF THE UK NCRI AML17 TRIAL

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Background: The use of anthracycline and ara-C has been standard induction therapy for Acute Myeloid Leukaemia for at least 30 years. Several trials have compared 90mg/m^2 of daunorubicin with 45mg/m^2 and found benefit either overall or in subgroups. In the NCRI AML17 trial we compared 90mg/m^2 with the more commonly used control arm of 60mg/m^2 in the first course of chemotherapy. Our preliminary findings showed no benefit for increasing the dose of daunorubicin, with increased early mortality (Burnett et al, Blood 2015,125, 3878).However, follow-up was short at only 14.8 months, and there was a suggestion of heterogeneity, with FLT3-ITD mutant patients potentially benefitting. We present here an updated analysis with a median follow up of 28 months.
Aims: To establish whether high dose daunorubicin in induction therapy for AML patients predominantly aged 18-60 years improved survival overall or in any cytogenetic or genetic subgroup.
Methods: Between 09/2011 and 10/2013, 1206 patients were randomised in 1:1 to daunorubicin 90mg/m^2 or 60mg/m^2 (d1,3,5) in course 1, then 50mg/ m^2 (d1,3,5) in course 2, with Ara-C 100mg/m^2 12-hourly d1-10 (course 1) and d1-8 (course 2). The median age was 53yrs (16-72); 54% were male; 84% had de novo AML, 10% secondary, and 6% high risk MDS; median presenting WBC was 8.5 (0.3-430); 10% had favourable cytogenetics; 72% intermediate and 18% adverse.
Results: Overall, 60-day mortality remained higher with DA90 (10% *vs* 5%, p=0.002), and 3-year overall survival did not differ (50% *vs* 47%, p=0.7) suggesting that no overall survival benefit has emerged for DA90. However, analysis stratified by FLT3-ITD showed significant heterogeneity (p=0.02). In ITD WT patients, DA90 did not improve outcome (51% *vs* 49%, p=0.3), but in ITD mutant patients a survival significant benefit (54% *vs* 34%, p=0.03) has emerged with longer follow up. 60-day mortality was not different (7% *vs* 6%, p=0.8). Although numbers were small the benefit appeared greater with higher allelic ratio (p=0.01 for trend). We did not observe a benefit related to age, cytogenetic group, NPM1 mutation status, nor in patients with a FLT3 TKD mutation.

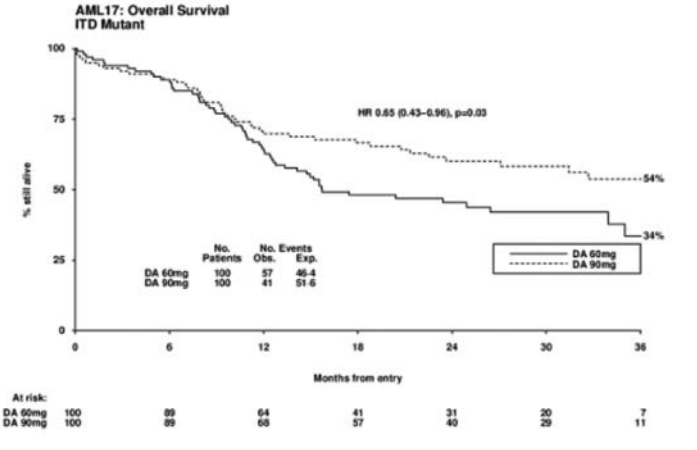

Figure 1.

Summary/Conclusions: These results indicate a potential role for higher doses of daunorubicin in patients with FLT3-ITD mutations. Such therapy could complement the use of FLT3-inhibitors, but its delivery is dependent on early knowledge of mutation status or possibly delivery of the escalated dose in the second course of induction therapy.

S809

OUTCOME OF PATIENTS WITH REFRACTORY OR RELAPSED AML WITH IDH1 AND IDH2 MUTATIONS AFTER CONVENTIONAL SALVAGE THERAPY: A STUDY OF THE GERMAN-AUSTRIAN AML STUDY GROUP (AMLSG)

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Background: Somatic mutations affecting isocitrate dehydrogenase 1 (*IDH1*) or 2 (*IDH2*) genes are found in 15% to 20% of patients (pts) with acute myeloid leukemia (AML). The incidence of these mutations increases with age, which is mainly attributed to *IDH2* mutations. The mutated enzymes emerge as promising therapeutic targets given their abnormal function to produce the oncometabolite 2-hydroxyglutarate. First selective inhibitors of mutant IDH1 and IDH2 proteins are in early clinical development, however, it is currently unknown how results from single-agent inhibitor therapy compare to those of conventional salvage therapy.
Aims: The primary objective of this study was to assess the outcome of patients with refractory or relapsed AML with *IDH1/2* mutations.
Methods: A total of 2825 adults with AML [median age: 55 years (yrs), range 18-84 yrs] who entered eight prospective first-line AMLSG trials of intensive conventional induction and consolidation therapy were included in the study. Refractory disease (RD) was defined as failure to achieve complete remission (CR) after two cycles of induction. Mutational status of *IDH1* and *IDH2* was assessed at the time of initial diagnosis using a combination of denaturing-high-performance liquid chromatography followed by direct DNA-sequencing.
Results: Overall, *IDH1/2* mutations were identified in 530 (18.8%) of 2825 pts; *IDH1* mutations in 7.5% (212/2822) and *IDH2* mutations in 11.5% (326/2825) of pts; 8 pts had a mutation in both *IDH1* and *IDH2*. All but one *IDH1* mutation involved codon R132. Among the *IDH2* mutated cases, *IDH2*^{R140} and *IDH2*^{R172} mutations were detected in 246 (75%) and 75 (23%) pts, respectively; there were 5 cases with *IDH2* mutations involving other codons. In *IDH1/2* mutated AML the CR rate after induction therapy was 62% (327/530) (*IDH1*: 64%; *IDH2*: 61%). A total of 138 pts, including those who only received one induction cycle, were refractory to the primary induction; among the pts receiving two induction cycles 82 had RD (*IDH1*, n=31; *IDH2*, n=50; *IDH1+IDH2*, n=1). Two hundred and eight *IDH1/2* mutated pts relapsed (*IDH1*, n=112; *IDH2*, n=92; *IDH1+IDH2*, n=4). Median overall survival (OS) in pts with RD (measured from the date of RD) and relapse (RE; measured from the time of RE) was 1.03 yrs and 0.79 yr, respectively; in *IDH1* mutated AML 0.53 yr and 0.63 yr, respectively, and in *IDH2* mutated AML 1.22 yrs and 1.09 yrs, respectively. The median OS in all *IDH1/2* mutated pts with RD/RE was 0.81 yr; pts with *IDH2* mutations had a longer (P=.003) survival, which was mainly attributed to the cases carrying *IDH2*^{R172} mutations. Of the 82 pts with RD, 39 (48%) received allogeneic hematopoietic-cell transplantation (alloHCT); the median survival time with alloHCT was 1.80 yrs compared to 0.53 yr in those who did not undergo alloHCT. Of the 208 relapsed patients, 153 received intensive chemotherapy or alloHCT, and 89 (58%) of the 153 pts subsequently achieved a CR/CRi. Seventy-four relapsed pts received alloHCT. Median survival after relapse of pts receiving alloHCT was 1.50 yrs compared to 0.63 yr in those not receiving an alloHCT.
Summary/Conclusions: *IDH1/2* mutations represent a frequent (in this study ~19%) molecular alteration in AML. A substantial proportion of AML pts with *IDH1/2* mutations have RD or relapse. Median survival with conventional salvage treatment in these pts was 0.81 yr, with *IDH2* mutated pts and those receiving alloHCT having significantly longer survival. Our results represent a benchmark for clinical trials evaluating IDH inhibitors in this clinical setting.

S810

LONG NON-CODING RNA PROFILING PROVIDES IMPORTANT PROGNOSTIC INFORMATION AND BIOLOGIC INSIGHTS IN YOUNGER PATIENTS WITH CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA (ALLIANCE/CALGB 8461, 9665, 20202)

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Background: Aberrant expression levels of mRNA and microRNA (miR) transcripts have been shown to associate with clinical outcome of cytogenetically normal acute myeloid leukemia (CN-AML) patients (pts). Recently, long non-coding RNA (lncRNA) expression was found to be an independent prognostic marker in older CN-AML pts (Garzon et al. PNAS 2014;111:18679-84). However, the prognostic value and biological implications of lncRNA expression in younger CN-AML patients are unknown.
Aims: The aims were to determine whether lncRNA expression is associated with clinical features, recurrent mutations and prognosis of younger CN-AML patients, and to obtain biological insights into the function of lncRNAs in this disease setting.
Methods: We performed whole transcriptome profiling (RNA-seq) in 377 younger (<60 years) *de novo* CN-AML adult pts and investigated the associations of lncRNA expression with recurrent, prognostically-relevant gene mutations and clinical features. All patients were treated on frontline Alliance/Cancer and Leukemia Group B (CALGB) protocols. Analysis of gene mutations was performed using targeted amplicon sequencing (*NPM1*, *DNMT3A*, *TET2*, *WT1*, *FLT3*-TKD, *IDH2*, *IDH1*, *RUNX1* and *ASXL1*) and Sanger sequencing (*FLT3*-ITD, double *CEBPA*). Small RNA sequencing was performed for miR profiling. The 377 patients were randomly divided into a training set for exploratory analyses (n=263) and a validation set (n=114).
Results: We derived gene mutation-related lncRNA signatures in the training set and tested their accuracy in detecting the pts' mutational status in the validation set. Of the mutations tested, only double *CEBPA* mutations, *NPM1* mutations and *FLT3*-ITD associated with distinct lncRNA signatures. These signatures were able to predict mutated or wild-type gene status in the validation set with high accuracy (sensitivity and specificity >72% in all cases). Using the training set, we identified 24 lncRNAs associated with event-free survival ($P<10^{-6}$). A small number of these lncRNAs associated with prognostic gene mutations (1 with *FLT3*-ITD, 4 with double *CEBPA* mutations). Linear combination of the weighted expression values of the 24 lncRNAs yielded a prognostic lncRNA score. Using the median value as a cut-off, the lncRNA score divided the training set into two groups with very different prognoses; pts with high lncRNA scores had shorter disease-free (DFS, *P*<.001) and overall survival (OS, *P*<.001) than pts with low lncRNA scores. In the validation set, pts with high lncRNA scores were more likely to have higher white blood cell counts (*P*=.009), *FLT3*-ITD (*P*=.007) and high miR-155 expression (*P*<.001) at diagnosis. There was no significant difference in complete remission rates between pts with high and low lncRNA scores (84% v 89%). After a median follow-up of 6.2 years, pts with high lncRNA scores had shorter DFS (*P*<.001; 5-year DFS, 17% v 51%) and OS (*P*=.002; 5-year OS, 26% v 52%) than those with low lncRNA scores. In multivariable analyses, the association of high lncRNA score with shorter DFS (HR: 2.16, *P*=.01) and OS (HR: 1.75, *P*=.04) remained significant after adjusting for other covariates. We also correlated the lncRNA score with mRNA and miR expression and identified several oncogenes (*FOSB*, *JUN*, *ETS2*, *SRC*, *RET*) and cell-cycle regulators (*PLK2*, *PLK3*), which were highly expressed in pts with high lncRNA scores. Gene Ontology analysis revealed enrichment for genes implicated in apoptosis, programmed cell death and inflammation in the high lncRNA score pts. Similarly, miRs that associate with aggressive malignant phenotype (e.g., miR-155, miR-500, miR-362) were overexpressed in pts with high lncRNA scores.
Summary/Conclusions: We conclude that lncRNA profiling provides meaningful prognostic information and identifies potentially targetable oncogenic pathways in younger CN-AML adult pts.

S811

RESPONSE-ADAPTED SEQUENTIAL AZACITIDINE AND INDUCTION CHEMOTHERAPY IN PATIENTS >60 YEARS OLD WITH NEWLY DIAGNOSED AML ELIGIBLE FOR CHEMOTHERAPY (RAS-AZIC): INTERIM ANALYSIS OF THE DRKS00004519 STUDY

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Background: Treatment options for AML in patients (pts) >60 years (y) include intensive chemotherapy (IC) and/or azacitidine (AZA). Yet, AZA and IC need not automatically be mutually exclusive.
Aims: In the multicenter DRKS00004519 (RAS-AZIC) study of the East German Study Group (OSHO), first-line treatment with AZA followed by AZA or IC in pts >60 y with AML was evaluated. Data of a planned interim analysis per protocol of the first 40 consecutive pts are presented.
Methods: Patients >60 y with newly diagnosed AML and eligible for IC were included irrespective of white blood count (WBC). In the phase I part of the trial, safety of upfront AZA (75 mg/m2/day s.c.) for 7 days followed by IC (mitoxantrone 10 mg/ m²/day on day (d) 1-3 and cytarabine 1g/ m²/BID on d 1, 3, 5, 7) on d 17 was established through a 3+3 design. In the multicenter phase II part (figure), upfront AZA was sequentially followed by AZA or IC based on d 15 bone marrow (BM) blast count (<45 *versus* ≥45%) and CR, CRi on d 56 previously identified as early predictors for long-term response to AZA in AML (Al-Ali et al., *Leuk Lymph* 2012). The primary endpoint was response (OR) [CR, CRi, and PR] at d 90 according to the International Working Group criteria. Safety, OS, and TRM were main secondary end points. On the basis of an optimal two-stage design (Simon, *Control Clin Trials* 1989), an expected OR of 61% at the end of the trial was estimated. Thus, in an intention-to-treat analysis, if ≤19 of the first 40 pts did not achieve the primary endpoint, protocol treatment would be considered inferior to standard IC. The trial is supervised by an independent Data Monitoring Committee. Adverse events (AEs) are reported according to the NCI CTCAE 4.03. All pts gave written informed consent.
Results: The median age of the 40 pts was 70y (55% males). *de novo* AML was present in 63% of pts. Median BM blasts and WBC were 39% and 6.7x10^9/L respectively. Cytogenetics was high- in 29% and intermediate-risk in 71% (normal cytogenetics 50%) of 38 pts. *FLT3* and *NPM1* were mutated in 11% and 17% of 36 pts respectively. All pts received the upfront AZA. 37 (92.5%) pts received protocol assigned treatment based on BM blasts on d 15 (54% continued with AZA; 46% received IC). Overall, in the 33 (82.5%) pts who continued therapy per protocol until d90, an OR was achieved in 27 (82%) pts [CR/CRi (n=17/7); PR (n=3)]. In the intention-to-treat cohort (n=40), this represents an OR of 67.5% [CR/CRi (60%); PR (7.5%)] with AZA+one IC cycle (n=28) or two IC cycles (n=3). TRM on d 30 and d 90 was 0% and 5% respectively. After a median follow-up of 202 days, OS for the entire cohort was 84.5%. OS for responding pts was 95.5%. Except for one patient, upfront AZA was not complicated by leukocytosis even in pts with WBC >15x10^9/L and was generally well tolerated. Constipation grade 1+2 was the most frequently reported AE under AZA (45%). Overall, the most frequent grade 3+4 non-hematologic AE was infection [11 times in 31 IC-cycles (35%); 7 times in 60 AZA-cycles (12%)].

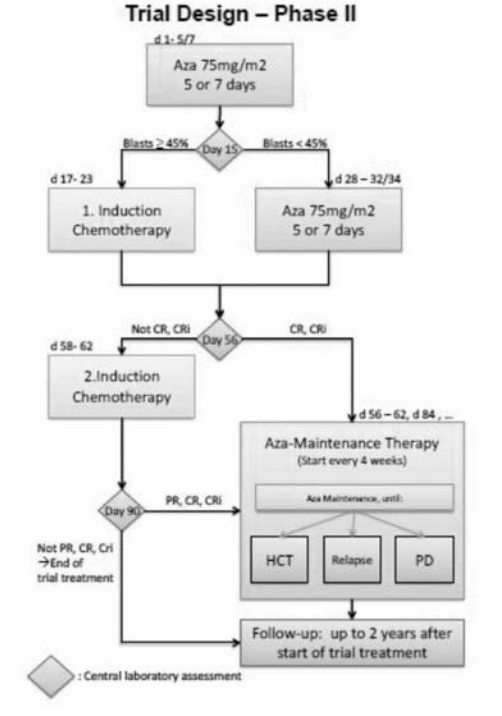

Figure 1.

Summary/Conclusions: These data imply that sequential epigenetic therapy and intensive chemotherapy in elderly pts with AML in an individualized response-based approach is feasible with a very low treatment-related mortality and yields responses that are at least comparable to those achieved after repeated cycles of standard chemotherapy. The final results of this trial will substantiate these data and scrutinize the impact of this approach on long-term survival.

S812

RESULTS FROM ONGOING PHASE 2 REGISTRATION STUDY OF SL-401 IN PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM (BPDCN)

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Background: SL-401 is a novel targeted therapy directed to the interleukin-3 receptor (IL-3R; CD123). IL-3R is overexpressed by many hematologic cancers, including blastic plasmacytoid dendritic cell neoplasm (BPDCN), acute myeloid leukemia (AML), myeloproliferative neoplasms (MPN) and multiple myeloma. In a prior Phase 1/2 trial, SL-401 demonstrated clinical activity, including multiple complete responses (CRs), in patients with BPDCN, a highly aggressive malignancy of unmet medical need and poor prognosis. Building on this prior experience, we report herein updated efficacy and safety data from our ongoing SL-401 registration trial in BPDCN.
Aims: This Phase 2 trial is a single-arm, open-label, two-stage study consisting of a lead-in dose escalation (stage 1) and subsequent expansion stage (stage 2) designed to generate safety and efficacy data to support potential registration in BPDCN.
Methods: In stage 1, pts with BPDCN or relapsed/refractory (r/r) AML received SL-401 as a daily IV infusion for up to 5 days (7, 9, 12 or 16 µg/kg/day) every 21 days. In stage 2, pts with BPDCN receive SL-401 at the optimal stage 1 dose and schedule. Response criteria include assessment of skin by modified severity weighted assessment tool (mSWAT), and bone marrow, lymph node and viscera by standard measures. Clinical CR (CRc) is defined as no detectable disease in bone marrow, lymph nodes or viscera with microscopic-only skin disease.
Results: At data cut-off (1/20/16), 48 pts (18 BPDCN; 30 r/r AML) were treated with SL-401. 18 BPDCN pts (9+9 in stages 1&2) received SL-401 (7 µg/kg, n=3 [stage 1]; 12 µg/kg, n=15 [6+9 in stages 1&2]); median age 70 yrs (range 45-82 and 1 compassionate use pt age 15); data on 30 r/r AML pts (14+16 in stages 1&2) to be reported separately. Most common treatment-related AEs (all grades) are transient transaminase elevation (57%) and hypoalbuminemia (40%). Transient thrombocytopenia was also noted (15%). Two stage 1 pts had capillary leak syndrome (CLS): gr 5 (7 µg/kg) and gr 4 (12 µg/kg). Safety precautions were developed and successfully implemented to minimize risk of severe CLS. Since adoption, severe CLS has not been observed at doses up to 12 µg/kg. No cumulative side effects were observed over multiple cycles. In stage 1, 12 µg/kg was the maximum tested and recommended stage 2 dose for BPDCN and MTD in r/r AML; MTD was not reached in BPDCN. An 87% (13/15) overall response rate (ORR) was observed in 15 evaluable BPDCN pts, with marked disease reductions in skin, bone marrow, lymph node and viscera. A 100% (10/10) ORR was observed in evaluable first-line BPDCN pts, with 90% (9/10) of pts achieving CR (n=7) or CRc (n=2). A 100% (8/8) ORR was observed in evaluable first-line BPDCN pts treated at the optimal dose (12 µg/kg), and all 8 pts achieved either CR (n=6) or CRc (n=2). 6 of the 8 pts either remain on SL-401 in CR (n=4; duration ongoing at 3+ to 8+ cycles) or were bridged to stem cell transplant (n=2; 1 CR, 1 CRc after 7, 4 cycles).
Summary/Conclusions: SL-401 demonstrated robust single-agent activity in BPDCN, including 100% ORR in first-line, and 87% in all-lines, with multiple CRs; response duration data are maturing and encouraging. This trial (NCT02113982) is designed to support registration in BPDCN and updated data will be presented. SL-401 is also being developed in additional IL-3R/CD123+ hematologic malignancies, including AML in CR with minimal residual disease and high-risk MPN.

Experimental approaches for plasma cell disorders

S813

RICOLINOSTAT (ACY-1215), THE FIRST SELECTIVE HDAC6 INHIBITOR, COMBINED WITH POMALIDOMIDE AND DEXAMETHASONE SHOWS PROMISING EARLY PHASE 2 RESULTS IN RELAPSED-AND-REFRACTORY MULTIPLE MYELOMA: ACE-MM-102

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Background: Ricolinostat (Rico, ACY-1215), an oral selective HDAC6 inhibitor, is well tolerated as monotherapy (Raje *Blood* 2012) and demonstrates potent synergistic activity with lenalidomide (Len) and pomalidomide (Pom) in preclinical models (Quayle *Blood* 2013). Pan-HDAC inhibitors vorinostat and panobinostat are active in MM in combo with bortezomib (Btz) and Len, but toxicities limit dosing and treatment exposure, with resulting diminished efficacy. Rico selectively targets HDAC6 while retaining reduced and tolerable levels of HDAC1, 2, 3 inhibition that down-regulates expression of critical transcription factors regulating cancer cell proliferation, such as c-Myc and IRF4.
Aims: This trial explored activity of Rico in combo with Pom and dexamethasone (Dex) in a population comparable to both the MM-002 and MM-003 trials for Pom/Dex (Richardson *Blood* 2015; San Miguel *Lancet Oncol* 2013). At the primary endpoint of PFS in MM-003, the ORR was 16.6% at 18.1 wks med follow-up, and was 31% at 10 mos. In our trial, 7 pts were treated and no DLTs were observed in Phase 1b (Raje *Blood* 2015), and Phase 2 results are now presented.
Methods: Pts had measurable paraprotein, adequate BM reserve and hepatic function with CrCl ≥45 mL/min. Refractory disease was defined as progression on or within 60 days of last therapy (RRMM). Pts with non-secretory MM, prior Pom or HDAC inhibitor therapy were excluded. Blood samples were obtained for PK/PD assessment of acetylated tubulin and histones. A sample size of 66 was determined to be adequate to detect an ORR of 44% against a historical rate of 29%.
Results: Phase 2 opened at a dose of 160 mg BID, with a predetermined SRC review to occur after 6 pts had completed 1 cycle of therapy. No DLTs were observed with 160 mg BID dosing; however, diarrhea or fatigue leading to dose reductions prompted SRC recommendation for QD dosing in ongoing Phase 2 to maintain maximum therapeutic exposure. Enrollment was closed in Nov 2015 after 96 pts were enrolled in Phase 2. Med age was 67 (39-87) yrs and med number of prior regimens was 2 (2-7). 93% were refractory to Len, 64% were refractory to Btz, and 49% were refractory to both Len and Btz as defined in the entry criteria. Treatment was very well tolerated and common toxicities were predominantly low grade and included neutropenia (44%), fatigue (39%), anemia (30%), diarrhea (28%), thrombocytopenia (19%) and upper respiratory tract infection (16%). Important related grade 3/4 toxicities included neutropenia (22%), thrombocytopenia (7%), anemia (5%), diarrhea (4%), and fatigue (3%). Serious related adverse events reported included bradycardia (n=1), bronchitis (n=1), chronic cardiac failure (n=1), dehydration (n=1), dyspnea (n=1), febrile neutropenia (n=1), general physical health deterioration (n=1), hypoxia (n=1), pneumonia (n=2), renal failure (n=1), and thrombocytopenia (n=1). PK/PD relationship demonstrates selective inhibition of HDAC6 (increasing acetylated tubulin) *vs* nonselective HDAC inhibition (increasing acetylated histones) at therapeutic doses. There was no evidence of Rico accumulation or drug-drug interaction with Pom. 84 efficacy evaluable pts at 4 mos med follow-up demonstrated: PR or better (ORR) of 36%, MR or better (clinical benefit rate, CBR) of 52%, SD or better (disease control rate, DCR) of 89%, med PFS of 5.5 mos, and med DOR of 9 mos. Of these pts, 51 were enrolled at least 6 mos prior to the data cut, with a 5 mos med follow-up and demonstrated an ORR of 47%, CBR of 63%, DCR of 90%, med PFS of 7 mos, and med DOR of 13 mos. This compares favorably to the MM-003 trial of 16.6% ORR at time of primary PFS analysis (med follow-up of 18.1 wks) and ORR of 31% (10 mos med follow-up), as well as similar data from MM -002, with efficacy data continuing to mature.
Summary/Conclusions: These results establish proof of concept and demonstrate that the addition of ricolinostat (a potent oral selective HDAC6 inhibitor) to Pom/Dex is an active regimen in RRMM, leading to superior and durable responses compared to the historical experience with Pom/Dex alone in RRMM.

S814

PHASE 1 STUDY OF VENETOCLAX MONOTHERAPY FOR RELAPSED/ REFRACTORY MULTIPLE MYELOMA

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Background: The anti-apoptotic protein BCL-2 has been implicated in the survival of multiple myeloma (MM) cells. Venetoclax (VEN) is an oral, highly selective BCL-2 inhibitor, which induces cell death in MM cell lines and primary samples, particularly those with t(11;14), and a high BCL-2 and low BCL-$_{XL}$ profile.
Aims: The objectives of the study are to evaluate safety, PK, recommended phase two dose, and preliminary efficacy of VEN monotherapy in relapsed/refractory (R/R) MM.
Methods: After 2-week dose-ramp-up, VEN was given daily at 300, 600, 900, or 1200 mg in dose escalation cohorts and 1200 mg in the safety expansion cohort.
Results: As of 9/17/15, 48 patients were enrolled: 30 in the dose escalation and 18 in the safety expansion. Median age was 65 and 28 patients had ISS stage II/III. Median (range) number of prior therapies was 5 (1–15), and 45 patients had prior bortezomib (32 refractory), 46 lenalidomide (37 refractory), and 40 had SCT. 18 patients had t(11;14)-positive MM. Adverse events (AEs) in ≥30% of patients were diarrhea (40%), nausea (40%), and thrombocytopenia (31%). Grade 3/4 AEs in ≥10% were thrombocytopenia (29%), anemia and neutropenia (17% each). SAEs in ≥2 patients were sepsis (n=3), cough, malignant neoplasm progression, and pyrexia (2 each). Median (range) time on VEN was 1.9 (0.2–13.8) months. 33 (69%) patients discontinued (DC): 26 related to disease progression, 4 due to AEs (worsening shortness of breath, hypokalemia, nausea, lung disorder), 2 withdrew consent, 1 due to death (brain hemorrhage following injury). 5 deaths occurred (3 disease progression, 1 brain hemorrhage, 1 lung disorder). 2 DLTs were seen at 600 mg (cohort was expanded): epigastric pain,and nausea with abdominal pain. Steady state mean C_{max} and AUC_{24} were ~dose proportional at all doses except 900 mg (n=21). 43 of 48 patients were evaluable for efficacy (Table). In the patients with complete responses, these were maintained for 9.7 (600 mg) and 9.0 months (900 mg, ongoing). Very good partial responses were reported for 3 patients, all in the 1200 mg dose cohort.

Table 1.

Best response, n (%)	With t(11;14) (n=17)	Without t(11;14) (n= 26)	All (n= 43)
ORR	4 (24)	1 (4)	5 (12)
CR	2 (12)	0	2 (5)
VGPR	2 (12)	1 (4)*	3 (7)
MR	1 (6)	0	1 (2)
SD	5 (29)	12 (46)	17 (40)
PD	6 (35)	12 (46)	18 (42)
DC before assessment	1 (6)	1 (4)	2 (5)

**Patient has translocation of chromosome 14 with unknown partner*

Summary/Conclusions: Venetoclax monotherapy has a tolerable safety profile in R/R MM. Preliminary efficacy, including complete responses, and very good partial responses, support venetoclax single agent activity in this population, primarily in patients with MM harboring the t(11;14) chromosomal translocation.

S815

A PHASE 2, OPEN-LABEL, MULTICENTER STUDY OF ELOTUZUMAB MONOTHERAPY IN PATIENTS WITH HIGH-RISK SMOLDERING MULTIPLE MYELOMA

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Background: Smoldering multiple myeloma (SMM) is an asymptomatic precursor of active multiple myeloma (MM), with no approved therapies. Patients (pts) with MM have compromised natural killer (NK) cell function; during early disease, pts may have better immune activity. Elotuzumab is an IgG1 immunostimulatory antibody targeted against SLAMF7, a glycoprotein expressed by myeloma and NK cells.[1] Progression from SMM to MM, and association between NK cell status and tumor burden, is incompletely understood. SMM provides a disease setting to assess elotuzumab monotherapy in less immune compromised pts.

Aims: To explore the association between baseline (BL) CD56^{dim} NK cells in bone marrow and maximal change in serum M protein (measure of tumor burden) in pts with high-risk SMM treated with elotuzumab. Efficacy and safety of elotuzumab monotherapy were also assessed.
Methods: Pts with high-risk SMM participated in this Ph 2, open-label study (NCT01441973). High risk was defined as serum M protein ≥3 g/dL and bone marrow plasma cells (BMPCs) ≥10% (criteria 1); serum M protein 1–3 g/dL, BMPCs ≥10%, and an abnormal free light chain (FLC) ratio (<0.125 or >8.0) (criteria 2); or urine M protein >200 mg/24 h, BMPC ≥10%, and an abnormal FLC (≤0.125 or ≥8.0; criteria 3).[2] Two high-risk cohorts, enrolled serially, were examined to explore different dosing schedules: pts in cohort 1 (C1) received elotuzumab 20 mg/kg IV in 28-day cycles (cycle 1: Days 1 and 8; cycle 2+: once monthly); in cohort 2 (C2), pts received elotuzumab 10 mg/kg IV in 28-day cycles (cycles 1 and 2: wkly; cycle 3+: twice monthly). Tumor response was assessed every 4 wks. Pts received elotuzumab until disease progression per modified International Myeloma Working Group (IMWG) criteria (including progressive disease or progression to symptomatic active myeloma). Primary endpoint was association between BL percent of bone marrow CD56^{dim} NK cells (CD45^{+}/CD3^{-}/CD56^{dim}/CD16^{+}) and maximal change in serum M protein. Secondary endpoints were progression-free survival (PFS) and overall response rate (ORR). Safety was an exploratory endpoint. Informed written consent was obtained for all pts.
Results: 31 pts were treated (C1, n=15; C2, n=16). Across both cohorts, 32% of pts met criteria 1 for high-risk SMM, 61% met criteria 2, 13% met criteria 3. At data cut-off (January 26, 2016), 27% and 38% of pts from C1 and C2, respectively, were still on treatment. Main reason for discontinuation was disease progression (C1, 47%; C2, 31%). A clear association between BL percent CD56^{dim} cells and maximal change in serum M protein (p=0.779) and between BL CD56^{dim} cells and response (≥minimal response; p=0.988) was not established. PFS and ORR are reported (Table). AEs were reported in all pts in both cohorts. No grade 5 AEs were reported. Most common AEs of any grade were upper respiratory tract infection (C1, 53%; C2, 63%) and fatigue (C1, 47%; C2, 38%). Infusion reactions were reported in 13% of pts; all grade 1–2. SAEs were reported in 53% of pts in C1 and 44% in C2.

Table 1. Progression-free survival and overall response rate.

	Cohort 1 (n=15) Elotuzumab 20 mg/kg	Cohort 2 (n=16) Elotuzumab 10 mg/kg	Total (N=31)
IMWG progression-free survival (PFS)*			
Number of events, n (%)	7 (47)	7 (44)	14 (45)
1-year PFS rate, % (90% CI)	79 (54, 91)	81 (58, 92)	80 (65, 89)
2-year PFS rate, % (90% CI)	71 (45, 86)	54 (31, 72)	62 (45, 75)
Median PFS, months (95% CI)	38 (12, NE)	NE (12, NE)	38 (15, NE)
Best overall response (BOR), n (%)			
Partial response (PR)	2 (13)	1 (6)	3 (10)
Minimal response (MR)	1 (7)	5 (31)	6 (19)
Stable disease (SD)	12 (80)	10 (63)	22 (71)
Progressive disease (PD)	0	0	0

*Progression based on modified IMWG criteria. NE, not estimable.

Summary/Conclusions: An association between BL percent CD56^{dim} NK cells in bone marrow and changes in serum M protein with elotuzumab treatment was not established. Elotuzumab monotherapy may delay SMM progression to MM, as most pts achieved a best overall response of SD or MR, with ≥MR in 29% of pts, including PR in 10%, and favorable PFS. Treatment was generally well tolerated; the AE profile was consistent with prior elotuzumab experience.
Funding: BMS. Medical writing: S Addison, Caudex, funded by BMS.

References
1. Hsi E et al. *Clin Cancer Res* 2008;14:2775–84;
2. Kyle R et. al. *Leukemia* 2010;24:1121–27.

S816

DELETION OF CDKN2C DRIVES THE PROGNOSTIC IMPACT OF DELETION 1P IN MULTIPLE MYELOMA
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Background: Survival in multiple myeloma is highly variable and predicting high risk behaviour is key for implementation of risk-adapted therapies. Ultimately, drivers of high risk myeloma need to be identified and directly targeted to turn risk markers into predictive markers for precision medicine.
Aims: To identify the prognostic impact of different genetic lesions affecting chromosome 1p.
Methods: CD138 immunomagnetically selected bone marrow tumour material of 1,036 newly diagnosed myeloma patients enrolled in the NCRI Myeloma XI trial were studied. The trial investigated CTD *vs* CRD induction therapy as well as lenalidomide maintenance *vs* no maintenance and enrolled both young and fit patients who received intensive chemotherapy (n=598 in this analysis), as well as elderly and less fit patients (n=438) who were treated on the non-intensive arm. Cases were molecularly profiled using a combination of MLPA and qRT-PCR (Boyle EM, et al. Gen Chrom Canc 2015; Kaiser MF, et al. Leukemia 2014). These high-throughput methods run on standard molecular biology equipment and allow for robust parallel assessment of multiple genetic lesions in one molecular reaction, including different lesions at chromosome 1p.
Results: Of the 1,036 patients, 10% carried deletion of CDKN2C at 1p32, 18% deletion DPYP (1p21.3), 15% deletion COL11A1 (1p21.1) and 25% deletion FAM46C (1p12), consistent with previous reports. In 7% CDKN2C and COL11A1 co-occurred and also in 7% CDKN2C and FAM46C co-segregated. In 8% of COL11A1 and 18% of FAM46C deleted cases CDKN2C copy number was normal. Only CDKN2C was affected by recurrent homozygous deletions, which was seen in 2% of cases. Deletion of CDKN2C (1p32) was associated with inferior outcome in the 598 intensively treated, younger patients with median progression free survival (PFS) of 21.8 *vs* 33.8 months (P=0.028) and 24-months median overall survival (OS) of 80% *vs* 88% (P=0.003). Deletion of CDKN2C was not associated with shorter survival in the elderly group of patients. Deletions of DPYP, COL11A1 and FAM46C were not associated with shorter PFS in the intensively treated group. Deletion of COL11A1 was associated with inferior OS (P=0.02). However, this was dependent on co-occurrence of deletion CDKN2C and was not significant when looking at cases with isolated COL11A1 deletion (P=0.6). No significant impact of deletion FAM46C on outcome was observed.
Summary/Conclusions: Deletion of chromosome 1p is an established risk factor in intensively treated patients. Our analysis suggests that CDKN2C at 1p32, rather than DPYP (1p21.3), COL11A1 (1p21.1) or FAM46C (1q12) is the relevant gene conferring poor prognosis. This has important implications for the potential development of therapeutic strategies for del(1p) myeloma.

S817

CARDIAC UPTAKE IN AL AMYLOIDOSIS OF 99MTC-DPD AS A NOVEL MARKER OF POOR PROGNOSIS IN SYSTEMIC AL AMYLOIDOSIS
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Background: The prognosis and outcomes in systemic AL amyloidosis are determined by cardiac involvement. The standard method for assessing degree of cardiac impairment in AL amyloidosis has relied on cardiac biomarkers like NT-proBNP and cardiac troponin-T. However, these markers are not specific for cardiac amyloidosis and other factors like renal failure or fluid overload may affect the levels and limit their utility in determining the prognosis. Thus far, no imaging modality has accurately predicted outcomes in AL amyloidosis. In recent years, the role of bone scintigraphy tracers like technetium-99m-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) has been evaluated. They are very sensitive in non-AL amyloidosis. The utility of ^{99m}Tc-DPD scintigraphy in AL amyloidosis has not been studied.
Aims: We report here in a series of 189 patients in which cardiac uptake using ^{99m}Tc-DPD scintigraphy is a marker of very poor outcome in AL amyloidosis.
Methods: All patients who underwent ^{99m}Tc-DPD scintigraphy with a confirmed diagnosis of AL amyloidosis were identified from the database of the UK National Amyloidosis Centre. 189 patients with systemic AL cardiac amyloidosis had ^{99m}Tc-DPD scans at diagnosis from June 2010-November 2015. All patients also had a full protocolized amyloid assessment including cardiac biomarkers, haematology, biochemistry, echocardiography and SAP scintigraphy. All patients are followed up every 3-6 months for prospective protocolized follow up. The characteristics of patients with a positive scan were compared with those patients in whom the scan did not show cardiac uptake. The survival outcomes were assessed by the method of Kaplan and Meier. Cox regression was used to determine univariate and multivariate variables impacting survival.
Results: A total of 189 patients were identified. 114 (60%) had no cardiac uptake on ^{99m}Tc-DPD scans (DPD-negative) and 75 (40%) had cardiac uptake on ^{99m}Tc-DPD scans (DPD-positive); 57 (76%) had grade 1 cardiac uptake, 13 (17%) had grade 2 and 5 (7%) grade 3. There was no significant difference between the DPD-positives and DPD-negatives in terms of NYHA class I/II *versus* III/IV (64% and 36% in the DPD-positives *vs* 67% and 33% in the DPD-negatives, p=0.75); Mayo staging 1/2 *vs* 3 (17% and 83% *vs* 25% and 75%, p=0.20); supine systolic blood pressure (112 *vs* 116, p=0.26). The echocardiographic parameters were also similar in the positive and negative groups: LVEF (50% *vs* 55%, p=0.1); mean LV wall (15mm *vs* 15mm, p=0.39); e/e' (18 *vs* 17, p=0.58). Organ involvement did not show a significant difference between the DPD-positives and DPD-negatives (renal: 52% *vs* 48%, p=0.66; autonomic nervous system: 20% *vs* 14%, p=0.32). The serum free light chains showed no

significant difference (median dFLC 343mg/L *vs* 316mg/L, p=0.40). However, the cardiac biomarkers were significantly greater in the DPD-positives compared to the DPD-negatives: N-terminal fragment of brain natriuretic peptide (NT-proBNP) (8296 ng/L *vs* 4411 ng/L, p=0.005) and high sensitivity troponin (hsTNT) (113 ug/L *vs* 83 ug/L, p=0.001). The overall median survival of the whole cohort was 7 months (95% CI 3 to 11 months). In the DPD-positive group the median survival was 4.3 months compared to 11.8 months in those with cardiac involvement by conventional criteria but DPD-negative (p=0.045). On univariate analysis factors impacting survival were: cardiac involvement, Mayo disease stage, presenting dFLC levels, NT-proBNP, hsTNT and cardiac uptake on ^{99m}Tc-DPD scintigraphy. Multivariate models will be presented.
Summary/Conclusions: ^{99m}Tc-DPD scintigraphy is a useful imaging modality in cardiac AL amyloidosis showing cardiac uptake in 40% of patients with cardiac involvement by conventional criteria. Patients with positive scans for cardiac uptake have significantly higher NT-proBNP and hsTNT compared to those with negative scans. Cardiac uptake on ^{99m}Tc-DPD scan is associated with very poor outcomes. We believe that uptake on a ^{99m}Tc-DPD scan shows advanced cardiac disease independent of all other markers of cardiac involvement in AL amyloidosis. These findings suggest that ^{99m}Tc-DPD scintigraphy should be considered as a simple baseline prognostic investigation in all patients with cardiac AL amyloidosis.

Stem cell transplantation - Clinical 2

S818

PRETRANSPLANT ANTI-CCR4 ANTIBODY AGAINST ADULT T CELL LEUKEMIA/LYMPHOMA WAS ASSOCIATED WITH SIGNIFICANTLY INCREASED RISKS OF SEVERE/STEROID-REFRACTORY GVHD, NON-RELAPSE MORTALITY AND OVERALL MORTALITY

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is one important treatment option for patients with aggressive adult T cell leukemia/lymphoma (ATLL). Mogamulizumab (anti-CCR4 monoclonal antibody; Mog) was recently approved as a treatment for ATLL. There are major concerns regarding the possible adverse effects of pretransplant Mog, as Mog depletes regulatory T cells (Tregs) for several months.
Aims: Here, we aimed to assess the impact of pretransplant Mog on the clinical outcomes after allo-HSCT.
Methods: We included 996 allo-HSCT recipients aged 70 years old or younger with aggressive ATLL who were diagnosed between 2000 to 2013, and received intensive chemotherapy using multiple chemotherapeutic drugs as first-line therapy. Before allo-HSCT, 82 patients received Mog with a median interval 45 days from the last Mog to allo-HSCT.
Results: Pretransplant Mog was associated with an increased risk of grade III-IV acute GVHD (30.9% *vs* 17.2%, P<0.01) and refractoriness to systemic steroid for acute GVHD (48.9% *vs* 23.5%, P<0.01). The cumulative incidence of 1-year non-relapse mortality was significantly higher in patients with pretransplant Mog compared to those without Mog (43.7% *vs* 25.1%, P<0.01). The probability of 1-year overall survival was also significantly inferior in patients with pretransplant Mog compared to those without Mog (49.4% *vs* 32.3%, P<0.01). In particular, use of Mog with intervals<50 days to allo-HSCT was associated with a dismal clinical outcome.
Summary/Conclusions: Our study clearly showed that pretransplant Mog significantly worsened the clinical outcome, which strongly supports the important relevance of Tregs in allo-HSCT in humans as in animal models. In clinical practice, Mog should be cautiously used for patients with ATLL who are eligible for allo-HSCT.

S819

REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADULT DE NOVO ACUTE LYMPHOBLASTIC LEUKEMIA: A PROSPECTIVE STUDY FROM THE UKALL14 TRIAL (ISRCTN 66541317)

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Background: Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a potentially curable treatment modality for adults with acute lymphoblastic leukaemia (ALL) with significant improvement in overall survival (OS) and reduction in relapse incidence. However, in the donor *versus* no donor analysis of the UKALL12/E2993 trial in adult ALL, the OS benefit did not extend to older patients in whom myeloablative allogeneic hematopoietic cell transplant related mortality (TRM) was 35% at 2 years and outweighed the reduction in relapse risk. To address this elevated TRM, reduced intensity conditioning (RIC) has been introduced. In our current UK National Cancer Research Institute

UKALL14 study all patients ≥41 years in CR, regardless of Philadelphia (Ph+) status and other high risk factors, are considered "high risk" and recommended a RIC alloHSCT with a matched sibling (sib) or matched unrelated donor (MUD) after a 2 course induction and high dose methotrexate.

Aims: The primary endpoint is event free survival (EFS). We report here the early outcome of 120 patients with at least day 100 follow up (FU) who received RIC alloHSCT on UKALL14 trial.

Methods: Standard reduced intensity conditioning was with fludarabine 30mg/m^2 d -6 to -2, melphalan 140 mg/m2 d -2 and alemtuzumab 30mg d -2 to-1 (MUD) or d-1 (sib). Graft *versus* host disease (GVHD) prophylaxis was ciclosporin A only. A small group of patients did not receive the standard conditioning protocol. Multilineage chimerism (MC) and minimal residual disease (MRD) were assessed 3 monthly post alloHSCT. Escalating doses of donor lymphocyte infusions (DLI) were given for T-cell mixed chimerism or MRD, starting dose 1 x 10^6 CD3 cells/kg, escalating by half log increments every 3 months.

Results: Five hundred and ninety seven patients were registered on the trial to date, 173 were registered for a RIC alloHSCT, 122 have completed the transplant, 120 of whom have sufficient follow up to report. Median age was 51 years (range 29 to 64). Donor was sib in 39 and MUD in 81 patients, respectively. Median WBC at diagnosis was 8.7 x 10^9/L (0.6-557.23). 57 of 106 (54%) evaluable patients had high-risk cytogenetics, 31 (26%) were Ph+. 18 of 74 (24%) with MRD data were MRD +ve pre-alloHSCT. Post-alloHSCT, myeloid engraftment occurred in 111 patients at a median of 14 days, 9 had missing data. No graft failures were reported. Acute graft *versus* host disease (GVHD) occurred at grade 1 in 34 patients (28%) and grade 2-3 in 12 patients (10%). 47 patients developed chronic GVHD (40% of 117 patients surviving beyond D100), 20 limited and 27 extensive. Of 15 patients who suffered transplant-related mortality (TRM), 8 died of infection (one post-transplant lymphoproliferative disease). Other causes of TRM included organ toxicity (2) and GVHD (2). TRM was not associated with age or donor type. 27 patients relapsed at a median time of 230 days (range 97-1034) with a relapse risk of 27.5% (18.9-38.1) at 22 months post alloHSCT. Of those, 16 (of 25 with data) had high-risk cytogenetics (EFS, p=0.11) and 10 (of 19 with data) were MRD +ve pre-alloHSCT (EFS, HR 2.41, p=0.025). 37 patients in total received 84 DLI (median maximum dose 1 x 10^6 CD3 cells/kg) including 20 for mixed chimerism, 6 for rising MRD and 9 for both. 5 patients (14%) developed post-DLI GVHD at grade 1 (n=4) and grade 2 (n=1). Serial MC data is available for 43 patients. By 6 months post transplant, 34 out of 43 patients (79%) achieved full donor total peripheral blood chimerism whereas only 15 (35%) achieved full donor chimerism in the T-cell compartment. With longer follow up, 26 patients (60%) achieved full donor T-Cell chimerism (median 9 months), 10 after receiving DLI (38%). Figure 1 shows Kaplan Meier curves of OS 66.3% (55.0-75.5, 95% CI) and EFS 59.4% (48.6-68.7) at 22 months (median follow-up).

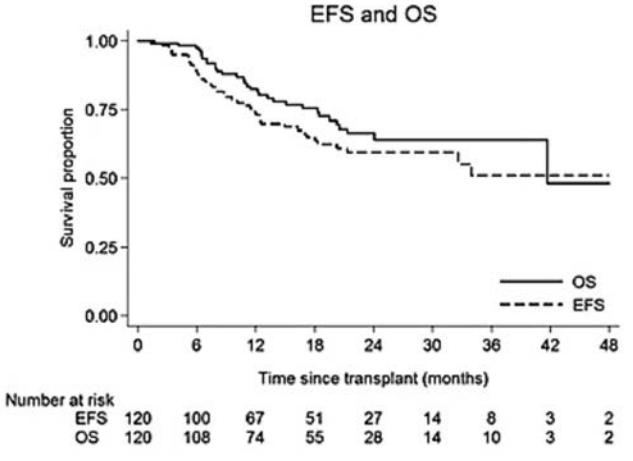

Figure 1.

Summary/Conclusions: This is early follow up from the first prospective data of RIC alloHSCT in older adults with ALL from a large, multicenter trial and the early results are promising. Severe GVHD and TRM were relatively low leading to a higher EFS than expected for this age group, however, longer follow up is needed. T-cell mixed chimerism was common at first MC assessment but early data indicate that conversion to full donor chimerism is achievable and safe with DLI. The impact of mixed chimerism and pre-emptive DLI on relapse remain to be evaluated.

S820

HIGH EXPRESSION OF THE STEM CELL MARKER GPR56 IS ASSOCIATED WITH AN INCREASED RELAPSE INCIDENCE IN AML AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: In acute myeloid leukemia (AML) leukemia initiating cells (LICs) are believed to exist within the CD34+/CD38- cell compartment. Recently, the G protein-coupled receptor 56 (GPR56) was shown to define a novel LIC compartment independent of the CD34+/CD38- phenotype. LICs are assumed to be responsible for AML maintenance & relapse & may be less immunogenic than AML bulk cells. Hematopoietic stem cell transplantation (HSCT) is a consolidating approach mainly based on immunological graft-*versus*-leukemia (GvL) effects & its therapeutic success is associated with the elimination of AML LICs by GvL effects.

Aims: To evaluate the clinical relevance of aberrant *GPR56* expression in the context of the CD34+/CD38- burden at diagnosis in AML patients (pts) receiving HSCT.

Methods: We analyzed 148 AML pts (median age 61, range 14-75 years [y]) for whom diagnostic bone marrow aspirate material was available & who received a non-myeloablative (3x30mg Fludarabine+2Gy total body irradiation [TBI]) or myeloablative (2x60mg Cyclophosphamide+12Gy TBI) HSCT in 1st (69%) or 2nd (19%) complete remission (CR) or in CR with incomplete peripheral recovery (12%). At diagnosis, the mutation (mut) status of *CEBPA*, *NPM1*, presence of *FLT3*-ITD & expression levels of *BAALC* & *EVI1* were evaluated. For 68 pts *RUNX1*mut status was available. The CD34+/CD38- burden & common surface markers expressions were determined by flow cytometry. A 5% cut-off defined pts with a high & low CD34+/CD38- burden. *GPR56* was measured by qRT-PCR, normalized to *ABL1* & the median normalized gene expression was used to define high & low expressers. Median follow up was 4.9y.

Results: European LeukemiaNet (ELN) classification was 24% favorable, 24% intermediate-I, 17% intermediate-II, 28% adverse & 7% unknown. At diagnosis high *GPR56* expressers had lower white blood cell counts (*P*=.04), were less likely to have *de novo* AML (*P*=.02), core-binding factor AML (*P*=.01), to be *NPM1*mut (*P*=.02) & were more likely to have a del7 (*P*=.004), to be *RUNX1*mut (*P*=.09) by trend, to be *EVI1* positive (*P*=.04) & to have higher *BAALC* (*P*<.001) expression. BM blasts in the high *GPR56* group were less likely to be positive for myeloid markers (CD33, *P*=.009; CD38, *P*=.04; CD64, *P*=.04; CD15, *P*=.002; CD65, *P*=.001) & the pan-leukocyte marker CD45 (*P*=.03) & more likely to be positive for T-cell (CD7, *P*=.03; by trend CD2, *P*=.1), thrombocytic & erythroid (CD61, *P*=.005; Glykophorin A, *P*=.003) & immature (by trend CD34, *P*=.07) markers. High *GPR56* expression was associated with high CD34+/CD38- cell burden at diagnosis (*P*=.002) & with a higher cumulative incidence of relapse (CIR, *P*=.04, Figure 1A) in AML pts receiving HSCT. Since *GPR56* defined a novel LIC compartment independent of the CD34+/CD38- phenotype, we analyzed the impact of aberrant *GPR56* expression in the low CD34+/CD38- burden pts. Similar clinical, cytogenetic & molecular associations were observed as in the entire set of pts. In the low CD34+/CD38- burden group, high *GPR56* expression identified a group of AML pts with a higher CIR as compared to pts with low *GPR56* expression (*P*=.05, Figure 1B).

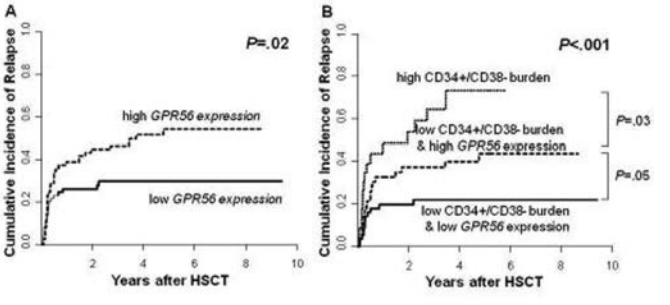

Figure 1.

Summary/Conclusions: High *GPR56* expression was associated with a higher relapse rate & worse outcome predictors. Among pts with low CD34+/CD38- burden high *GPR56* expression defined a subgroup with higher CIR after HSCT possibly due to an independent *GPR56*-defined LIC population. HSCT-associated therapeutic GvL effects might be insufficient to control the disease in pts with high LIC burden defined by the CD34+/CD38-phenotype & *GPR56* expression status at diagnosis.

S821

COMPARISON OF OUTCOMES AFTER SINGLE OR DOUBLE UNIT UCBT FOLLOWING RIC IN ADULTS WITH ACUTE LEUKEMIA: A REPORT FROM EUROCORD, THE ALWP & THE CORD BLOOD COMMITTEE OF THE CTIWP OF THE EBMT

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Background: The feasibility of cord blood transplantation (CBT) in adults is limited by the relatively low number of hematopoietic stem/progenitor cells contained in one single CB unit. The infusion of two CB units from different partially HLA-matched donors (double CBT) is frequently performed in patients who lack a sufficiently rich single CB unit. In patients given CBT following myeloablative conditioning, previous studies have demonstrated that, although double CBT extended the use of CBT for patients lacking a single unit with adequate cells (>2.5 x 10^7 total nucleated cells (TNC)/kg), it failed to improve engraftment and outcomes.
Aims: Here we investigated whether these observations remained true in the setting of reduced-intensity conditioning (RIC) CBT.
Methods: Inclusion criteria included adult (>18 yrs) patients, acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL), complete remission (CR) at the time of transplantation, first single (with a cryopreserved TNC ≥2.5 x 10E7/kg) or double CBT between 2004 and 2014, and RIC conditioning.
Results: Data from 534 patients with AML (n=408) or ALL (n=126) receiving a first single (n=172) or double (n=362) CBT were included in the analyses. In comparison to patients transplanted with a single CB, double CB recipients had a shorter follow-up (34 *vs* 54 months, P=0.0005), were more frequently male (56 *vs* 41%, P=0.002), received more frequently a conditioning combining TBI, cyclophosphamide and Flu (TCF regimen, 83 *vs* 66%, P<0.001), and received less frequently ATG (16% *vs* 37%, P<0.001). In univariate analysis, in comparison to patients transplanted with a single CB, double CB recipients had a suggestion for a higher incidence of grade II-IV acute GVHD (36 *vs* 28%, P=0.08) but a similar incidence of neutrophil engraftment (83 *vs* 77%, P=0.3). Further, at 3-year, in comparison to single CB recipients, double CB recipients had a similar incidence of chronic GVHD (36 *vs* 28%, P= 0.17), a similar incidence of relapse (32 *vs* 35%, P=0.4), a similar incidence of nonrelapse mortality (22 *vs* 29%, P=0.17) but a better OS (51 *vs* 41%, P=0.03) and LFS (46 *vs* 36%, P=0.06) (Figure 1). In multivariate analyses OS (HR=0.8, 95% CI 0.7-1.1, P=0.20) and LFS (HR=0.8, 95% CI 0.6-1.1, P=0.17) were no longer significantly better in double CB than in single CB recipients, although there remained a trend in that direction.

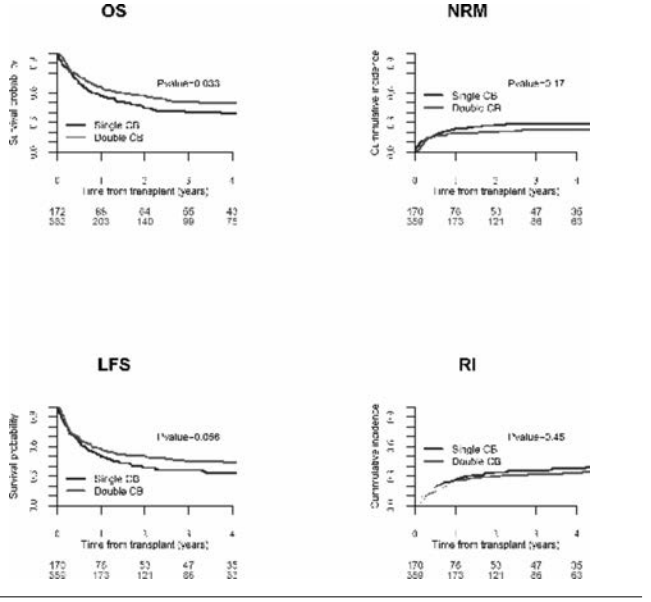

Figure 1. Unadjusted CBT outcomes in patients transplanted following RIC with a single or a double CB.

Summary/Conclusions: These data suggest that LFS and OS are not different for double than single UCB with an adequate TNC dose in the RIC setting. These observations should serve as basis for future prospective randomized studies.

S822

DONOR AND RECIPIENT TOLL-LIKE RECEPTOR 1 VARIATIONS COMPARABLY PREDICT TRANSPLANT-RELATED MORTALITY AFTER UNRELATED BONE MARROW TRANSPLANTATION

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Background: Toll-like receptor 1 (TLR1), the most ubiquitous among the TLR family, plays an essential role in the innate immunity system through initiating the production of inflammatory cytokines. Its genetic variant (rs5743551, -7202 A>G), which resides in the promoter region, has been reported to be associated with susceptibility to various infectious diseases and the mortality and morbidity.
Aims: To investigate the impact of *TLR1* variation on transplant outcomes of bone marrow transplantation (BMT).
Methods: *TLR1* genotyping was performed on 333 patients who underwent unrelated HLA-matched BMT for hematologic malignancies through the Japan Marrow Donor Program between May 2006 and April 2009 and their donors, and its association with the transplant outcomes was retrospectively examined.
Results: The genotype frequencies of A/A, A/G, and G/G were 10%, 39% and 50% in the recipients and 11%, 36% and 51% in the donors (*P*=0.82), respectively. The A/A genotype *vs* A/G or G/G genotype both in the donors (37% *vs*. 18%, respectively; *P*=0.0042) and the recipients (39% *vs* 17%, respectively; *P*=0.021) was associated with a significantly higher 3-year transplant-related mortality (TRM). The A/A genotype in the donors (hazard ratio [HR], 3.4; 95% confidence interval [CI], 1.6-7.2; *P*=0.0017) and the recipients (HR, 2.6; 95% CI, 1.3-5.4; *P*=0.0093) remained statistically significant in the multivariate analysis for 3-year TRM.

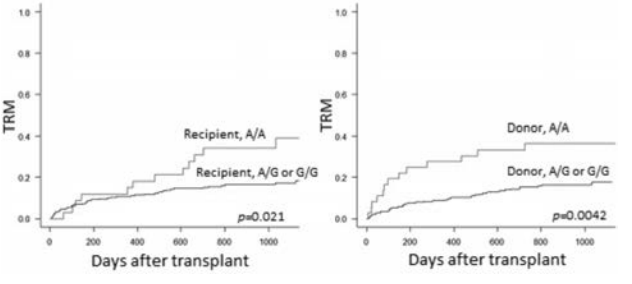

Figure 1.

Summary/Conclusions: These results suggest an association of the donor and recipient *TLR1* A/A genotype with increased TRM after unrelated BMT. An analysis of the *TLR1* genotype could therefore be useful for selecting the donor, managing patients in a risk-adapted manner, and creating therapeutic strategies to prevent TRM after hematopoietic stem cell transplantation.

Platelet disorders 2

S823

PLATELET PROPERTIES IN A PATIENT WITH STORMORKEN SYNDROME (STIM1 MUTATION) - ADHESION RECEPTOR LEVELS, CALCIUM HOMEOSTASIS AND PHOSPHATIDYLSERINE EXPOSURE

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Background: Stormorken syndrome is a rare monogenic disorder, recently attributed to a gain-of-function mutation of the stromal interaction molecule 1 (STIM1) gene, which is involved in calcium homeostasis. While this syndrome is associated with macrothrombocytopenia and a bleeding phenotype of variable severity, the pathophysiology underlying the platelet abnormalities is not fully described.
Aims: Evaluate parameters in platelets from a patient with Stormorken syndrome and assess the value of platelet flow cytometry in aiding differential diagnosis of Stormorken syndrome in patients with bleeding not fully explained by macrothrombocytopenia.
Methods: The presence of a p.R304W mutation in STIM1 exon 7 was confirmed after a Stormorken syndrome-like phenotype was recognised in a 21-year old male with long-standing thrombocytopenia, hyposplenism, pupillary miosis and tubular aggregate myopathy. Citrated blood was analysed by flow cytometry for platelet adhesion receptor levels, intracellular calcium levels (both at rest and flux in response to agonists, using Fluo-3-AM), microparticles and platelet phosphatidylserine (PS) exposure (annexin-V binding). Testing on two occasions compared samples from the patient, his unaffected mother, and healthy donors.
Results: Flow cytometry of platelet-rich plasma from the patient confirmed the morphological finding of macrothrombocytopenia, with altered forward and side scatter properties, and also increased numbers of CD41-positive microparticles. In comparison with the control samples, levels of metalloproteinase-sensitive adhesion receptors GPVI and GPIbα were present at the lowest end of normal ranges. The ratio of GPIbα:CD41 was significantly reduced, indicating that GPIbα receptor density was independent of mean platelet volume. Levels of sGPVI were within the normal range suggesting that aberrant metalloproteinase-mediated shedding was not responsible for reduction in GPVI levels. Resting intracellular calcium levels were elevated ~5-fold, and calcium flux in response to ionophore was significantly enhanced. Resting platelet PS exposure was at the upper end of the normal range.

Table 1.

	Microparticles		Calcium ratio		PS exposure	
	Total (%)	CD41+ (%)	Resting	Activated	Resting (%)	Activated (%)
Healthy donor	0.08	6.22	1.94	29	0.69	97.9
Patient	30.6	20.35	8.91	45.8	2.9	94.3

Summary/Conclusions: Using flow cytometry, platelets from a patient with a confirmed gain-of-function mutation of STIM1 demonstrated altered physical properties, reduced adhesion receptor levels, enhanced PS exposure, significantly increased intracellular calcium at rest and an exaggerated calcium flux response to platelet agonists. Blood samples contained elevated levels of platelet-derived microparticles. Flow cytometric analyses of Stormorken syndrome platelets demonstrate reproducible abnormalities, which begin to inform the structural and functional differences in platelets that may contribute to the bleeding phenotype of this rare monogenic disorder, in particular with regards to surface receptor expression and calcium homeostasis.

S824

IMPAIRED FUNCTION OF MESENCHYMAL STEM CELLS FROM IMMUNE THROMBOCYTOPENIA PATIENTS IN INDUCING REGULATORY DENDRITIC CELL DIFFERENTIATION VIA NOTCH2/JAGGED-1 SIGNALLING PATHWAY

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Background: Immune thrombocytopenia(ITP) is characterized by platelet destruction and megakaryocyte dysfunction due to the breakdown of self-tolerance. Mesenchymal stem cells(MSCs) play a pivotal role in immune tolerance. Bone marrow MSCs(BMMSCs) residing in bone marrow stroma (BMS)have been shown to induce regulatory dendritic cell(regDC) differentiation from CD34+ haematopoietic progenitor cells (HPCs). MSCs from ITP patients (ITP-MSCs) exhibit increased senescence and apoptosis, but whether ITP-MSCs have an impaired ability to instruct regDCs remains unknown. Notch is a major factor mediating the interaction between HPCs and BMS.BMS has been reported to prevent the terminal differentiation of DCs through the Notch ligand Jagged-1. Moreover, Jagged-1 has been shown to be involved in the immunosuppressive effects of MSCs on T cells and DCs. It has shown that Notch signalling is important for the modulation of MSCs.
Aims: To investigate whether ITP-MSCs displayed impaired function in inducing regulatory dendritic cell differentiation via notch2/jagged-1 signalling pathway.
Methods: CD34+HPCs were differentiated in the absence or presence of ITP-MSCs or control-MSCs, and then immature DCs were harvested and induced to mature with LPS in the absence of MSCs. MGG staining, flow cytometry and ELISA were performed to characterize the DCs. Mature DCs were then cocultured with CD4+T cells, and proliferation, differentiation, and the anergic and regulatory features of T cells were assessed by flow cytometry. The differential expression of notch receptors, notch intracellular domain(NICD),hes-1, and Jagged-1, was assessed by RT-PCR, flow cytometry and western blot analysis.NICD2-expressing lentivirus,Notch2-targeting shRNA lentivirus, exogenous Jagged-1, and anti-Jagged-1 Ab were used to analyse the underlying mechanism(s).
Results: ITP-MSCs showed impaired function in inhibiting morphological and phenotypic development, proliferation, interleukin-12(IL-12) secretion, and in enhancing endocytosis and interleukin-10(IL-10) production of DCs. DCs that were differentiated in the presence of ITP-MSCs(ITP-MSC-DCs) exhibited a dramatically impaired ability to inhibit allogeneic CD4+ T cell proliferation and to change Th2 polarization. The ITP-MSC-DCs had a deficient capacity to induce anergic and regulatory T cells. Jagged-1 expression and Notch2 signalling pathway activation were down-regulated in ITP-MSCs. The application of exogenous Jagged-1 and anti-Jagged-1 Ab revealed an involvement of Jagged-1 in the development of regDCs. And NICD2 overexpression of ITP-MSCs displayed elevated Jagged-1 expression and an enhanced ability to induce regDCs, which was suppressed after treatment with anti-Jagged-1 Ab. The attenuated function of control-MSCs following Notch2 knockdown was reversed by exogenous Jagged-1. Thus, the Notch2 signalling pathway may modulate regDC differentiation in a Jagged-1-dependent manner. Following pre-treatment with aspirin, both ITP-MSCs and control-MSCs exhibited an up-regulation of the Notch2/Jagged-1 signalling pathway and an enhanced ability to induce regDCs.
Summary/Conclusions: ITP-MSCs show defects in the induction of regDC development, which may play a role in the pathogenesis of ITP. The Notch2/Jagged-1 signalling pathway is involved in the impaired function of ITP-MSCs, and agents targeting this pathway may have great potential for the treatment of ITP.

S825

NOVEL RARE VARIANTS IN EXON 4 OF GP6 HAVE LARGE EFFECTS ON GPVI EXPRESSION AND PLATELET FUNCTION

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Background: One of the initial steps in primary haemostasis is the binding of collagen by the GPVI signalling receptor on platelets. We have an interest in genetic variation in GP6 with an effect on expression and function. Platelets from GPVI-deficient patients are not activated by collagen, but do not show any severe bleeding phenotype. Therefore the GPVI receptor is a promising target for chronic anti-thrombotic treatment. Common variants in the *GP6* gene are known to influence GPVI expression, but they only account for 16% of the observed variation seen in large cohort studies.
Aims: We hypothesized that a large proportion of the variation would be explained by rare variants in GP6 with a greater effect on expression and function.
Methods: The study was performed in the Cambridge Platelet Function cohort of 1500 health consented volunteers. GPVI function and expression was determined by flow cytometry and levels confirmed by Western blotting and ELISA. We planned on Sanger sequencing *GP6* and *FCER1G* in the extreme cases with combined function and expression defects of GPVI. Where possible pedigrees of index cases with rare and functionally relevant GP6 variants, were recruited. Site-directed mutagenesis was performed to introduce individual identified variants and final plasmids verified by Sanger sequencing to test the functionality of the variants. The effect of the variants on thrombus formation was determined by the van Kruchten methodology.
Results: We identified two outlier individuals with approximately 50% reduction in GPVI expression levels and reduced functional responses. Sequencing in one of the individuals, identified a rare G/A variant at position 584 in the *GP6* coding sequence, causing a serine to asparagine substitution at residue 195 (S195N). This variant had a frequency of 0.011% in 72.590 Caucasian individuals of the ExAC database. Sequencing of the second individual and their 4 pedigree members revealed a pattern consistent with the inheritance of a novel rare C/A variant at position 580 in the *GP6* coding sequence, encoding a proline to threonine substitution at residue 194 (P194T). This variant was unobserved in the ExAC database. Both the S195N and P194T variants occur in a key

structural motif of the second immunoglobulin-like domain of the receptor. In cell lines the S195N variant resulted in no expression of GPVI, whereas P194T did have expression. Both variants did effect thrombus formation in flowing whole blood over collagen-coated surfaces.
Summary/Conclusions: We have used a combination of platelet GPVI expression and function phenotypes, combined with sequencing of outliers, to identify rare variants with large effect on the native configuration of the cellular receptor and thereby its function and expression.

S826

LONG-TERM RISK OF CARDIOVASCULAR EVENTS FOLLOWING SPLENECTOMY-A DANISH POPULATION-BASED COHORT STUDY
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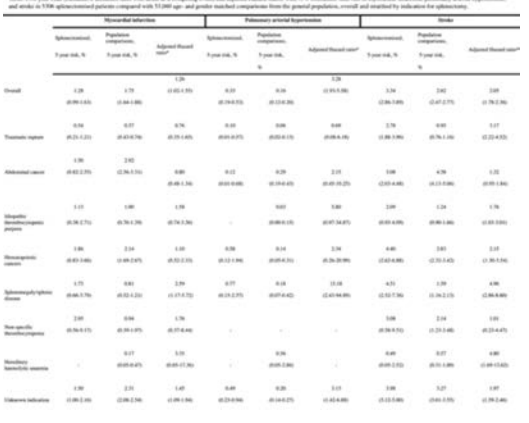
Background: The most frequent medical indications for splenectomy are hematological disorders and although, splenectomy has been used for decades to treat various medical and surgical conditions, it is known to be associated with various short and long term complications. An increased risk of venous thromboembolism following splenectomy is well documented while data on long-term risk of cardiovascular events following splenectomy are scarce.
Aims: This study aimed to assess risk of acute myocardial infarction (MI), pulmonary arterial hypertension (PAH), and stroke following splenectomy among patients splenectomised for a variety of indications compared with the risks in the general population and to determine whether these events were related to the procedure or to the underlying pathology by comparing the risk among patients who underwent splenectomy with that among non-splenectomised patients with similar medical conditions.
Methods: We used population-based medical databases to identify patients splenectomised in Denmark during 1996-2012, and constructed an age- and sex-matched general population comparison cohort and a disease-matched comparison cohort. We classified splenectomised patients into 8 subgroups based on the underlying indication for splenectomy: (1) traumatic rupture of the spleen; (2) idiopathic thrombocytopenic purpura (ITP); (3) unspecified thrombocytopenia; (4) hematopoietic cancer; (5) hereditary haemolytic anaemia; (6) abdominal cancer; (7) splenomegaly/other splenic diseases; (8) no indication recorded. We computed 5-year cumulative incidence rates (treating death as a competing event) and adjusted hazard ratios (aHRs) with corresponding 95% confidence intervals (CIs) of MI, PAH, and stroke for the 3 cohorts. We controlled for age, sex, and pre-existing chronic obstructive pulmonary disease, obesity, pulmonary embolism and heart failure.
Results: We identified 5,306 splenectomised, 53,060 population comparisons and 11,651 disease-matched comparisons. The most frequent indications for splenectomy were traumatic rupture (19.5%), abdominal cancers (16.6%), hematopoietic cancers (7.9%), and ITP (7.1%). Within 5 years of follow-up (table), 1.28% of splenectomised patients had MI compared with 1.75% in the general population, yet corresponding aHR was 1.26 (95%CI 1.02-1.55). The 5-year cumulative incidence of PAH was 0.33% among splenectomised and 0.16% [aHR 3.28(95%CI 1.93-5.58)] in the general population, and for stroke 3.34% *versus* 2.62% [aHR 2.05(95%CI 1.78-2.36)]. When comparing the splenectomised cohort with the disease-matched cohort, only stroke-risk was elevated, occurring in 2.99% of the splenectomised *versus* 2.32% in disease-matched [aHR 1.49(95% CI 1.23-1.81)].

Table 1.



Summary/Conclusions: The higher risk of stroke in splenectomised compared with the disease-matched and the general population indicates that splenectomy increases the risk of stroke. Risk of MI and PAH was not higher in splenectomised patients than in disease-matched comparisons, indicating that the higher risk in splenectomised patients compared with the general population can largely be explained by the underlying indication.

S827

QUANTIFICATION OF IGG ANTI-ADAMTS13 ANTIBODY LEVEL CAN PREDICT THE LIKELIHOOD OF MORTALITY IN ACUTE THROMBOTIC THROMBOCYTOPENIC PURPURA
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Background: Acute Idiopathic Thrombotic Thrombocytopenic Purpura (TTP) is a life threatening disorder caused by inhibition of the Von Willebrand factor cleaving protein ADAMTS13 by acquired antibodies, usually of the IgG subclass. Its prevalence has been estimated at four to six cases per million per year and is characterized by hemolytic anemia, thrombocytopenia, fever, neurological symptoms and renal dysfunction. Untreated, mortality has been documented at 90% but even with treatment, it remains around 15-20%. Plasma Exchange (PEX) is the mainstay of treatment but immunosuppressive/immunomodulatory therapy is often also required. Since January 2009, the United Kingdom TTP registry has been collecting information on all acute presentations of TTP across the country. In addition to testing for ADAMTS13 activity, confirmation of the presence of Anti-ADAMTS 13 IgG antibodies forms part of the initial work up.
Aims: A retrospective review to evaluate whether the presenting IgG ADAMTS13 antibody level can predict the likelihood of mortality in acute presentations of TTP.
Methods: 360 acute events were identified from the registry involving 314 patients treated in 63 hospitals. Only acquired TTP was considered with all cases of congenital TTP excluded. Acute TTP was defined as ADAMTS13 protease activity below 10% (FRETS vWF-73 assay, NR: 60-120%) or between 10-20% with a detectable IgG ADAMTS13 antibody present. Acute events were then stratified into groups depending on the presenting IgG ADAMTS13 antibody level (0-29%, 30-59%, 60-89%, >90%).
Results: Of the events identified, 68% of those involved were female with a median age of 46 (range 5-90 years) and a median ADAMTS13 at diagnosis of<5% (range<5%-57%). There was no statistically significant difference when considering these factors between the subgroups. 40 deaths were noted in total, accounting for 11.1% of all events. There was a statistically significant increase in mortality in patients with a presenting IgG ADAMTS13 antibody level above 30% compared to those with a level below 30% (p=0.0023). Subgroup analysis of those presenting with an IgG ADAMTS13 antibody level above 30% found no significant difference in mortality if the level was 30-59%, 60-89% or above 90% (p=0.83).
Summary/Conclusions: There is an increased risk of mortality seen in patients with acute TTP and a presenting IgG ADAMTS13 antibody level above 30%. Such patients should be considered for more aggressive treatment from initial diagnosis. Mortality does not appear to increase with further increases in the antibody level.

Non-malignant hematopoietic disorders

S828

MASITINIB FOR THE TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT AND SMOLDERING SYSTEMIC MASTOCYTOSIS: A RANDOMIZED, PLACEBO-CONTROLLED, INTERNATIONAL, PHASE 3 STUDY

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Background: Indolent systemic mastocytosis (ISM) and smoldering systemic mastocytosis (SSM) are life-long conditions associated with a significant reduction in quality-of-life. Masitinib (AB1010) is a selective oral tyrosine kinase inhibitor targeting wild-type KIT (WT-KIT), LYN and FYN. Masitinib does not inhibit KIT-D816V mutated mast cells (MC) but does target normal MCs. This is in part via dual inhibition of LYN and FYN modulating MC degranulation in a KIT-independent manner, and secondly by greatly reducing the normal MC burden through inhibition of WT-KIT.
Aims: To assess the efficacy and safety of masitinib (6 mg/kg/day over 24-weeks with a possible extension period) against placebo in severely symptomatic ISM/ISM patients (pt) who were unresponsive to optimized symptomatic treatments.
Methods: Severely symptomatic ISM/SSM pts were defined as having at least one baseline symptom among: pruritus score ≥9, number of flushes/week ≥8, Hamilton rating scale for depression score ≥19, or Fatigue Impact Scale (FIS) total score ≥75. Treatment-effect was tested using repeated measures methodology for rare diseases via the generalized estimating equation (GEE) model in a modified intention-to-treat (mITT) population. Response was defined as a ≥75% improvement from baseline for any one of the aforementioned severe symptoms. Primary endpoint (4R75%) was cumulative response (timeframe W8-W24) in at least one severe baseline symptom. Long-term analysis was performed over the timeframe of W8-W96. Secondary endpoints included outcomes related to safety, patient-reported symptomatic endpoints and objective endpoints representative of MC activity or burden.
Results: A total of 135 pts were enrolled. Masitinib showed a significant improvement over placebo in its primary endpoint, 18.7% *vs* 7.4%, respectively, odds ratio of 3.6 (95%CI 1.2-10.8, P=0.008). Masitinib sustained a significant 4R75% response over the long-term with odds ratio of 3.5 (95%CI 1.3-9.7, P=0.016). This result was corroborated by statistically significant outcomes in various sensitivity and secondary analyses. For example, at W24 the mean change of tryptase level relative to baseline in pts with baseline tryptase level >20µg/L was a decrease of 18.0% in the masitinib arm *vs* an increase of 2.2% in the placebo arm, *i.e.* an absolute difference of 20.2% (P<0.001). The response of urticaria pigmentosa to masitinib was also statistically significant when compared with placebo (P=0.02), an observation supported by abolition of Darier's sign (P=0.02). Toxicities were predominantly gastrointestinal or skin events, consistent with masitinib's known safety adverse events (AE) profile and typically manageable via dose reduction. Severe AE reported with a >4% difference between treatment-arms were diarrhea (9.8%), rash (5.7%), and asthenia (4.1%). The most frequent serious AEs in the masitinib arm were diarrhea (4.3%) and urticaria (2.9%). There were no deaths or life-threatening AEs in the masitinib arm. Long-term safety assessment revealed comparable incidence of AEs between treatment-arms.
Summary/Conclusions: Masitinib generated a significant therapeutic benefit across a diverse range of symptoms in pts with severely symptomatic ISM/SSM who were unresponsive to optimized symptomatic treatments. Moreover, the response criterion of ≥75% improvement in at least one severe baseline symptom constitutes a clinically meaningful effect. Data from the study's extension period showed that masitinib was capable of maintaining remission of symptoms for over 2 years. This is an important observation given that ISM and SSM are a life-long conditions requiring chronic management. Masitinib was associated with increased frequency of AEs during the first 6 months of treatment, although no toxicities were life-threatening, and over the long-term the incidence of AEs was similar between masitinib and placebo. In summary, masitinib has shown a positive benefit/risk ratio and may be an important future treatment option for severely symptomatic ISM/SSM pts.

S829

STAT3 GAIN-OF-FUNCTION MUTATIONS CAUSE AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME LIKE DISEASE BY DYSREGULATING EXPRESSION OF FAS AND BCL2 PROTEINS AND CAN BE THERAPEUTICALLY TARGETED BY BH3 MIMETICS

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Background: Autoimmune Lymphoproliferative Syndrome (ALPS) like disease is typically characterized by lymphadenopathy, hepatosplenomegaly, autoimmune cytopenias and an elevated number of double negative T cells (DNT cells, $CD3^+$, $TCR\alpha/\beta^+$, $CD4^-$, $CD8^-$). 70% of patients suffering from classical ALPS harbor germline or somatic mutations in genes involved in the apoptotic FAS death receptor signaling pathway (*FAS*, *FASLG* or *CASP10*). For about 30% of ALPS or ALPS-like patients the genetic cause is unknown.
Aims: The objective of this study was to identify novel gene candidates underlying ALPS like disease of unknown genetic cause and to test potential targeted therapeutic approaches.
Methods: 30 patients with clinical ALPS like symptoms, but without classical mutations were analyzed by whole-exome sequencing. Candidate genes were identified using an in-house developed bioinformatic analysis pipeline for gene prioritization. Candidate mutations were validated by Sanger sequencing. Their impact on Fas signaling and apoptosis was studied and potential therapeutic approaches tested.
Results: We identified two patients with *de novo* germline mutations (p.R278H, p.M394T) of the *Signal Transducer And Activator Of Transcription 3 (STAT3).* Elevated levels of phosphorylated STAT3 (pSTAT3-Tyr705) indicated constitutive activation of STAT3. Patient 1 presented with Coombs positive hemolytic anemia, thrombocytopenia, generalized progressive, non-infectious, non-malignant lymphadenopathy and splenomegaly at the age of nine. Immunophenotyping revealed increased numbers of DNT cells (20% in peripheral blood) and over time the patient developed panhypogammaglobulinemia. Patient 2 presented with early-onset insulin dependent diabetes mellitus and non-infectious, non-malignant lymphadenopathy, splenomegaly, thrombocytopenia, neutropenia and mild anemia. DNT cells and immunoglobulin levels were normal. We could demonstrate that constitutive activation of STAT3 led to decreased *FAS* expression on primary patient T cells and to reduced FAS ligand induced apoptosis similar to the effect of *FAS* mutations in classical ALPS. Therapeutic targeting of STAT3 is challenging and so far none of the developed STAT3 inhibitors has been approved for clinical use. To determine alternative targets we analyzed STAT3 signaling in primary and transformed lymphocytes of the patients. *STAT3* gain-of-function mutations led to increased expression of STAT3 target genes, including *SOCS3* and key anti-apoptotic factors of the BCL2 family of proteins (*BCL-2, BCL-XL*), whereas expression of pro-apoptotic factors (*BAX, BAK*) was decreased. Consistently, patient cells were resistant to cell death induced by stimuli (*e.g.* IL-21, staurosporine) mediating cell death via the BCL2 protein family. Treatment with a STAT3-specific inhibitor (S3I-201) normalized the expression of these factors and rescued the apoptotic response. Cells harboring *STAT3* mutations were significantly more sensitive than normal cells to death induced by the BH3 mimetic inhibitor ABT-737 that targets BCL2,

BCL-XL and BCL-W providing proof-of-concept evidence and indicating a novel therapeutic option.
Summary/Conclusions: We report here on dominant *STAT3* gain-of-function mutations that caused clinical phenotypes similar to ALPS like disease. We demonstrated that constitutive active STAT3 caused decreased *FAS* expression and a skewed balance of pro- and antiapoptotic BCL-2 factors resulting in apoptosis evasion that may be therapeutically targeted by BH3 mimetics.

S830

PRECLINICAL PHARMACOKINETICS AND PHARMACODYNAMICS OF AG-519, AN ALLOSTERIC PYRUVATE KINASE-R ACTIVATOR
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Background: Pyruvate kinase (PK) deficiency is a rare genetic disease causing chronic hemolytic anemia. Symptoms vary in severity and include splenomegaly, iron overload and jaundice, and current treatments are supportive only. PK deficiency is caused by mutations in the red blood cell (RBC) isoform of PK (PK-R), a key enzyme in RBC glycolysis. Functional PK-R deficiency leads to increases in the upstream metabolite 2,3-diphosphoglycerate (2,3-DPG) and decreases in the product adenosine triphosphate (ATP) in blood. Small molecule activation of mutant PK-R could restore the glycolytic pathway, and decrease hemolysis, leading to patient benefit. AG-519 is an orally available allosteric activator of both mutant and wild type PK-R currently in clinical development.
Aims: To explore the pharmacokinetic/pharmacodynamic (PK/PD) relationships of AG-519 with PK-R activity, ATP and 2,3-DPG in wild type PK-R mice, and to use data from animal studies to project the pharmacokinetic profile and efficacious dose of AG-519 in humans.
Methods: *In vitro* intrinsic clearance (CL_{int}) was calculated in microsomes and hepatocytes from all species tested. *In vivo* PK/PD was analyzed in PK-R wild type C57/BL6 female mice. AG-519 or vehicle was given orally as a single dose, or for 5 and 13 twice-daily (BID) doses, at 1, 10, 50 and 150mg/kg. Blood or plasma concentrations of AG-519, ATP and 2,3-DPG were measured at 0, 3, 6, 12, 24, 36, 48 and 72 h following the last dose by liquid chromatography with tandem mass spectrometry, with PK-R activity measured by colorimetric assessment of the reaction rate in blood cell lysates. For *in vivo* pharmacokinetic profiling of AG-519 in Sprague Dawley rats, Beagle dogs and cynomolgus monkeys, clearance (CL), volume of distribution at a steady state (Vss), terminal half-life (t½), and oral bioavailability (F) were calculated. The human profile was predicted by allometric scaling with correction factors.
Results: AG-519 showed moderate CL (1.13-2.51L/h/kg), moderate Vss (2.08-6.44L/kg), moderate to long t½ (6.3-9.8h), rapid absorption (T_{max} ≤1.2h) and moderate oral bioavailability (6.9-19.5% with suspension formulation) in mouse, rat, dog and monkey. There was good *in vitro* to *in vivo* correlation in the CL estimates across species. In mouse PK/PD studies, dose-dependent increases in blood ATP levels were observed after 5 and 13 BID doses, but not after a single dose. In addition, dose-dependent decreases in 2,3-DPG were observed following both single and multiple doses of AG-519. There were dose-dependent increases in blood PK-R activity after single and multiple AG-519 doses. The AG-519 exposure to biomarker response (2,3-DPG, ATP and PK-R activity) relationship was described by an E_{max} model. With the projections of an effective t½ (4.1-7.1h), CL (0.414L/h/kg), Vss (2.95L/kg), and F (22.5%) in humans, twice-daily dosing of 111mg (range 62-134mg) of AG-519 in humans is anticipated to achieve the $EAUC90_{(0\text{-}12h)}$ (the exposure required to achieve 90% of maximal effect on all three biomarkers) of 421 h•ng/mL.
Summary/Conclusions: AG-519 showed a favorable pharmacokinetic profile in several animal species, allowing prediction of its profile in humans. This, along with the clear PK/PD relationship established in the mouse model demonstrating activation of PK-R, allowed prediction of the AG-519 efficacious dose in humans. These data supported the decision to bring AG-519 into a phase 1 healthy volunteer study (NCT02630927).

S831

HAPLO-IDENTICAL TRANSPLANTATION FOR ACQUIRED SEVERE APLASTIC ANEMIA IN A MULTI-CENTER PROSPECTIVE STUDY
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Background: Severe aplastic anemia (SAA) is a life-threatening disorder for which allogeneic hematopoietic stem cell transplantation (HSCT) is the available curative approach. Haplo-identical donor (HID) as an alternative source, should be considered in the absence of matched related or unrelated donor after failing to respond to immunosuppressive therapy (IST). Obvious superiority arises from the immediate availability of a suitable haplo-identical donor for most patients within appropriate time-frame. However, initial outcomes of HID for SAA were far from satisfactory owing to poor engraftment, refractory graft-*versus*-host disease (GVHD) and delayed immune reconstitution.
Several small-sample studies using various approaches of haplo-identical transplantation revealed the mixed success, with short-term survival rates reaching 67%>100% and graft failure rates ranging from 0% to 25%. However, the recent reports of haplo-identical transplantation for SAA are rather heterogeneous and almost all series are limited by small numbers of patients.
Aims: To further prove the therapeutic effect of haplo-identical SCT in the treatment of SAA, we conducted a prospective multi-center study using uniform protocol, and compared outcomes of haploidentical SCT with those of all contemporaneous transplantation from MRD.
Methods: This was a disease-specific, multicenter, prospective study. All eligible patients treated at 11 participating institutions were included. Patients were administered with BU/CY+ATG conditioning regimen, G-BM combined with peripheral blood stem cells without in vitro T-cell depletion and a combination of CsA, MTX and MMF as graft-*versus*-host disease (GVHD) prophylaxis. The primary endpoint was engraftment. Various outcomes of HID were compared with those of contemporaneous transplantation from matched related donors (MRD).
Results: For patients receiving HID-SCT, all cases surviving for more than 28 days achieved donor myeloid engraftment. The median time for myeloid engraftment was 12 (range, 9-25) and for platelet was 15 (range, 7-101) days with a cumulative platelet engraftment incidence of 94.1±0.1%. With a median follow up of 18.3 (3.0-43.6) months and when compared with MRD, HID recipients had more cumulative incidence of grade II-IV acute GVHD (aGVHD; 33.7% *vs* 4.2%, *P*<0.001), more chronic GVHD (cGVHD; 22.4% *vs* 6.6%, *P*=0.014) at 1 year, but similar grade III-IV aGVHD (7.9% *vs* 2.1%, *P*=0.157), 3-year estimated overall survival (OS, 89.0% *vs* 91.0%, *P*=0.555) and 3-year estimated failure free survival (FFS, 86.8% *vs* 80.3%, *P*=0.659). Multivariate analysis showed no significant difference in primary engraftment and survival outcomes between two cohorts.
Summary/Conclusions: Haplo-identical transplantation using our protocol was proved to be an effective and safe choice in SAA patients who failed previous IST. It could be recommended for those who lack a MRD as higher priority.

S832

SUCCESSFUL HEMATOPOIETIC STEM CELL TRANSPLANTATION IN TEN PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA USING AN INTENSIVE IMMUNOMYELOSUPPRESSIVE CONDITIONING REGIMEN: THE RESULTS OF A SINGLE INSTITUTE
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Background: Severe congenital neutropenia (SCN) is a rare heterogeneous genetic disorder categorized as a bone marrow failure syndrome. The main clinical feature of patients with SCN is recurrent bacterial infections from early infancy due to severe chronic neutropenia. Majority of SCN patients have benefitted by the treatment with granulocyte colony-stimulating factor (G-CSF). However, patients on long-term G-CSF therapy have a relative risk of developing myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). The only curable treatment for SCN patients is hematopoietic stem cell transplantation (HSCT). Recently, HSCT with reduced intensity conditioning (RIC) regimens have been applied to the treatment for SCN patients prior to malignant transformation. However, the optimal conditions of HSCT for SCN patients have not been established.
Aims: In this study, we conducted bone marrow cell transplantations (BMT) in ten patients with SCN using an immunomyelosuppressive conditioning regimen to minimize early and late transplant-related morbidity in Hiroshima University Hospital.
Methods: A total of eleven HSCT procedures were performed in ten patients with SCN from 2009 to 2015. Four of ten patients had experienced the engraftment failure of initial HSCT and three of them were referred to our hospital for re-transplantation. Nine of ten patients (90%) had a heterozygous mutation in the *ELANE* gene. Bone marrow cells (BM) were obtained from five HLA-matched related (MRD), three HLA-matched unrelated (MUD), and three HLA-mismatched unrelated (7/8) donors (MMUD), respectively. Conditioning regimen consisted of fludarabine (125 mg/m^2), cyclophosphamide (140 mg/m^2), melpharan (90 mg/m^2), and total body irradiation (3.6 Gy). Antithymocyte globulin (ATG, 10–12 mg/kg) was also administered, but two patients from MRD did not receive ATG, and one patient from MUD received low-dose ATG (i.e., 2.5 mg/kg instead of 10–12 mg/kg). Short-term methotrexate and tacrolimus were administered for the prophylaxis of graft-*versus*-host disease (GVHD).

Results: Engraftment of neutrophils was observed within post-transplant 24 days in all but 1 case who developed graft failure. This case, who had undergone initial HSCT with MUD-derived bone marrow using our conditioning regimen with low-dose ATG (2.5mg/kg), was rescued by second HSCT with BM from another MUD receiving the same conditioning regimen with a 12mg/kg of ATG. Two patients received HSCT from HLA-matched related donor developed stable mixed chimerism without neutropenia in peripheral blood. Although one patient who received donor lymphocyte infusion due to mixed chimerism developed acute GVHD grade II and limited chronic GVHD, the others did not develop severe GVHD (acute GVHD gradeII-III 9%, chronic GVHD 9% respectively). All patients are alive for 6 months to 8 years after HSCT with no signs of severe infections or transplantation-related morbidity.
Summary/Conclusions: Our results demonstrate that BMT together with a sufficient immunosuppressive conditioning regimen may be a feasible and effective treatment for SCN patients, irrespective of initial engraftment failure. The excellent results in our cohort suggest that indications for proceeding to HSCT could be extended to patients without malignant transformation. The further analyses of accumulated cases are necessary to assess the efficacy, safety, and less late adverse effects related to HSCT including fertility.

Transfusion medicine

S833

RED CELL ALLOIMMUNISATION RISK IN PATIENTS WITH DIFFERENT TYPES OF INFECTIONS

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Background: Red cell alloantigen exposure can lead to antibody associated morbidity. A valid alloimmunisation prediction score will support identification of high-risk patients, consequently followed by offering them extended matched red cell products. Murine models have suggested inflammation as an important modulator of the humoral response towards red cell antigens.
Aims: We set out to quantify the alloimmunisation risk of various types of infections in humans.
Methods: We performed a multicenter case-control study in a source population of patients who received their first and subsequent red cell transfusions during an eight year follow-up period. Cases, defined as patients developing a first transfusion-induced red cell alloantibody, were each compared with two randomly sampled controls, defined as non-alloimmunised patients receiving at least the same number of red cell units. Logistic regression analysis, stratifying for potential confounders, was used to evaluate the association between red cell alloimmunisation and the presence of various types of infections during a 5-week 'alloimmunisation risk period'. Tissue-invasive bacterial infections were defined as 'severe' or 'mild' according to the expected induced systemic inflammatory response accompanying the type of infection. Bacteremiae were subclassified into Gram-positive or Gram-negative bacteremiae according to the causative microorganism. Viraemia and disseminated viral zoster infections were defined as 'disseminated viral infections', hereby contrasting local viral infections.
Results: Within a cohort of 24,063 newly transfused patients, 505 cases and 1,010 matched controls received a median of eight (interquartile range 4-16) red cell transfusions. During the risk period, 159 cases (31.5%) and 314 controls (31.1%) were diagnosed with at least one infectious episode. Alloimmunisation incidences increased upon red cell antigen exposure during severe, but not mild, bacterial infections (adjusted relative risk (RR) 1.34 with 95% confidence interval (CI) 0.97-1.85; figure). RRs were further pronounced when these bacterial infections were accompanied with long-lasting fever (RR 3.06, CI 1.57-5.96). Disseminated viral disorders demonstrated a RR of 2.41 (CI 0.89-6.53, figure). Surprisingly, bacteraemia with Gram-negative, but not with Gram-positive, microorganisms, coincided with a 2-fold reduction in alloimmunisation incidences (RR 0.58, CI 0.13-1.14, figure 1). Fungal infections, as well as elevated CRP values, and (level of) leucocytosis were not associated with red cell alloimmunisation.

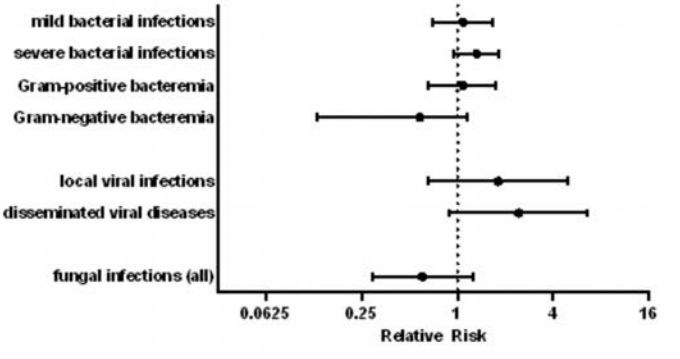

Figure 1.

Summary/Conclusions: Our findings corroborate animal studies suggesting that red cell transfusions in a setting of severe bacterial or viral infections increase the risk of alloimmunisation, contrasting Gram-negative bacteraemia which substantially reduce alloantibody formation. Data on recent or existing infections should thus be incorporated into an alloimmunisation prediction score, enabling selecting high-risk patients who will benefit most from extended matched red cell transfusions.

S834

THE USE OF PEGYLATED CARBOXYHEMOGLOBIN BOVINE IN PATIENTS FOR WHOM BLOOD IS NOT AN OPTION

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Background: Severe hypoxia due to low hemoglobin (Hb) levels causes significant morbidity and mortality. This is of great concern for patients who cannot receive blood transfusions due to personal (i.e., Jehovah's Witness) or medical (i.e., hyperhemolysis) reasons, or simply due to the lack of availability. PEGylated-carboxyhemoglobin (PEG-HbCO; SANGUINATE™) is a novel therapeutic agent designed to release carbon monoxide to reduce vascular inflammation and then transfer oxygen to hypoxic tissue and cells. PEG-HbCO has been used in 24 patients under the USA emergency Investigational New Drug (eIND) program. A retrospective analysis of these patients was performed.
Aims: Perform post-hoc analysis of clinical findings; their relationship to individual patient diagnosis/associated comorbidities and PEG-HbCO intervention.
Methods: 23 patients with hemoglobin levels under 5 g/dL and 1 sickle cell disease patient with Hb of 8.7 were determined to be near death or at serious risk of severe morbidity and were treated with PEG-HbCO. Applications included AML, sickle cell disease (SCD) comorbidities, trauma and hemorrhagic shock. Patients ranged in age from 19 to 61. A unit of PEG-HbCO consists of 500 mL (40 mg/mL). Total doses ranged from 1 unit up to 8 units given over 9 days.
Results: No serious adverse events associated with PEG-HbCO were reported. All patients provided with timely emergency treatment with PEG-HbCO were reported by the investigator to show clear signs of improved mental function that was temporally associated with infusion of PEG-HbCO despite continuing low Hb levels. Improvements in cerebrovascular oximetry, cerebral blood flow, pulmonary infiltrates, renal function and serum biochemistry (bilirubin, liver function, LDH) were reported in various patients. A SCD patient with acute chest syndrome showed clearing of pulmonary infiltrate within 24 hours of PEG-HbCO infusion. Nine of eleven (81%) severely anemic patients showed improvement upon administration of PEG-HbCO, defined by an initial improvement in mental alertness and culminating in hospital discharge (excluding trauma patients with ISS score ≥25).
Summary/Conclusions: The varied results seen in these critical care patients represent preliminary evidence of substantial improvement in clinically significant endpoints used by the clinicians to assess response to treatment in the urgent care setting. Most important is the evidence of response despite no immediate improvement in the hemoglobin level which, if left untreated, may have lead many of these patients to significant morbidity and potential mortality. While severe anemia persisted for most of these patients, it appears that the life-threatening hypoxia did not. There is a significant unmet medical need to treat hypoxia in patients for whom blood is not an option. PEG-HbCO is under development for the treatment of the life-threatening effects of acute severe anemia (Hb ≤5 g/dL) in patients who cannot receive red blood cell transfusions. PEG-HbCO is also in clinical development for the treatment of SCD comorbidities, delayed graft function following renal transplantation and reduction of delayed cerebral ischemia following subarachnoid hemorrhage.

S835

INTRODUCTION OF A PREOPERATIVE ANAEMIA CLINIC IMPROVES PERI-OPERATIVE TRANSFUSION USE

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Background: Preoperative anaemia is a potentially modifiable risk factor for perioperative morbidity and mortality. Strategies for anaemia correction have been outlined by the UK Joint Blood and Transplant Professional Advisory Committee, the 2015 NICE (National Institute of Clinical Excellence) guidelines for blood transfusion and a guideline by the British Committee for Standards in Haematology. Here we present the results of a pilot study, where we introduced a preoperative clinic aimed at correcting treatable anaemia without delaying cardiac surgery at Papworth hospital, UK.
Aims: The aims of this study were to evaluate whether introduction of a preoperative anaemic clinic improves patient blood management or clinical outcome following major cardiac surgery.
Methods: All patients listed for major cardiac surgery between May to December 2015, Papworth Hospital, UK were screened for anaemia at surgical pre assessment clinic. 107 patients with a haemoglobin <130g/l (males) and <120g/l (females) were recruited and underwent further testing for ferritin, vitamin B12, plasma folate, creatinine and TSH. Patients were then reviewed at clinic by a consultant haematologist, who decided upon appropriate anaemia management. 25 patients with a ferritin level<100g/L received 1g of Ferinject® (Fe-Carboximaltose) as a single dose infusion up to 6 weeks preoperatively. For another 23 patients the above ferritin criterion was met, but for a variety of reasons preoperative iron could not be administered. 4 patients received oral iron. The remaining patients were advised to commence B12, folate or combinations thereof or proceeded to surgery in the absence of a treatable cause of anaemia. We collected information on blood products administered, total hospital stay, intensive care stay and mortality. Data were compared to a previously published historic control group of anaemia patients undergoing cardiac surgery (n=165;Pap60) and compared by t-test.
Results: Patients assessed in the preoperative anaemia clinic received significantly less red cell transfusion than patients proceeding directly to surgery in the Pap60 study (2.2±3.2 units *vs* 3.9±4.0 units; mean±S.D., p=0.027) and had significantly shorter hospital stay (12.9±10.6 days *vs* 16.0±9.9 days; mean±S.D., p<0.01). However there was no difference in transfusion use or hospital stay between iron treated and non-iron treated, low ferritin patient subgroups, nor were these significantly different in comparison to the whole anaemia clinic cohort.

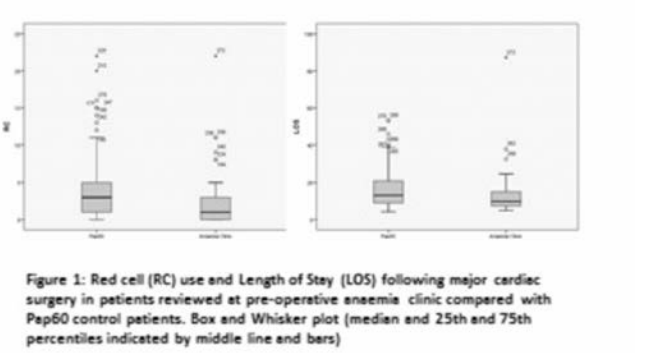

Figure 1: Red cell (RC) use and Length of Stay (LOS) following major cardiac surgery in patients reviewed at pre-operative anaemia clinic compared with Pap60 control patients. Box and Whisker plot (median and 25th and 75th percentiles indicated by middle line and bars)

Figure 1.

Summary/Conclusions: In conclusion, we have found that our preoperative anaemia clinic improves overall patient blood management in the peri-operative period with a reduction in blood transfusion usage. However intravenous iron when given in the context of major cardiac surgery seems to have no additional benefit in this pilot study.

S836

LUSPATERCEPT DECREASES TRANSFUSION BURDEN AND LIVER IRON CONCENTRATION IN REGULARLY TRANSFUSED ADULTS WITH BETA-THALASSEMIA

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Background: Luspatercept (ACE-536), a fusion protein containing a modified activin receptor type IIB, is being developed for the treatment of β-thalassemia. Luspatercept binds to GDF11 and other ligands in the TGF-β superfamily to promote late-stage erythroid differentiation. Luspatercept corrected the effects of ineffective erythropoiesis in a thalassemia mouse model (Suragani R, Blood 2014) and was well tolerated and increased hemoglobin in a phase 1 clinical study (Attie K, Am J Hematol 2014).
Aims: This is an ongoing, phase 2, multicenter, open-label, dose-finding study followed by a long-term extension study to evaluate the effects of luspatercept in adults with β-thalassemia, including a subgroup on regular transfusions (≥4 units RBC/8 weeks). Endpoints included RBC transfusion burden (units/12 weeks), liver iron concentration (LIC) by MRI, safety, and quality of life questionnaires.
Methods: Inclusion criteria included age ≥18 yr and genetic confirmation of β-thalassemia. Luspatercept was administered SC every 3 wks for up to 5 doses in the dose-ranging study (completed) with a 2-month follow-up unless enrolled directly into a 2-year extension study (ongoing). Six sequential cohorts (n=35 total) were treated at escalating doses from 0.2 to 1.25 mg/kg. An additional expansion cohort (n=29) was treated with a starting dose of 0.8 mg/kg with escalation up to 1.25 mg/kg. Of the 64 patients (pts) treated in the dose-ranging study (including 30 pts regularly transfused), 51 enrolled in the extension study. RBC transfusions during the study were administered based on each patient's usual pre-transfusion hemoglobin threshold.
Results: Data (as of 25 Sept 2015) were evaluable for 28 of 30 pts on regular RBC transfusions. Median age was 37.5 yr, ranging from 21 to 54 yr, and 57.1% had prior splenectomy. At baseline, median transfusion burden was 8 units/12 weeks (range 4-18 units). Mean (±SD) LIC was 4.5±4.6 mg/g dw. 27 pts were on iron chelation therapy (ICT) at baseline. 21/28 (75%) pts achieved ≥33% decrease and 16/28 (57%) achieved ≥50% decrease in transfusion burden over any 12-week period compared with the 12 weeks prior to treatment (mean decrease was 56% in 23 pts with at least 12 weeks on study). 4/8 (50%) pts with baseline LIC ≥5 mg/g dw had a decrease in LIC ≥2 mg/g dw (mean decrease 3.1 mg/g dw, 35.1%) during the 16-week base study; 14/14 (100%)

pts with baseline LIC<5 mg/g dw maintained LIC<5 mg/g dw. Luspatercept was generally well tolerated, with no related serious adverse events reported to date. Adverse events in this regularly transfused subgroup were mostly mild-moderate and the most frequent related adverse events (≥4 pts) were bone pain, myalgia, arthralgia, headache, asthenia, and musculoskeletal pain.
Summary/Conclusions: Luspatercept treatment was well-tolerated and led to decreased RBC transfusion requirements in regularly transfused patients with β-thalassemia. The decrease in RBC transfusions correlated with decreases in liver iron concentration. These changes represent a significant reduction in disease burden for regularly transfused patients with β-thalassemia. A Phase 3 study of luspatercept in adults who require regular RBC transfusions due to β-thalassemia is ongoing (BELIEVE study; clinicaltrials.gov NCT02604433).

S837

BENEFIT OF RITUXIMAB AS SECOND LINE TREATMENT CHOICE FOR AUTOIMMUNE HEMOLYTIC ANEMIA IN CHILDREN: A PROSPECTIVE FRENCH COHORT

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Background: Childhood autoimmune hemolytic anemia (AIHA) requires in 30-50% of cases immunosuppressive therapy for steroid resistance or prolonged dependence. Although there are no pediatric guidelines for the second line strategy, in small retrospective studies or rare controlled studies, rituximab appears to be indicated.
Aims: This prospective observational national study reports the practice, efficiency and tolerance of rituximab in non-selected children with isolated AIHA or AIHA in context of Evans syndrome (AIHA/ES).
Methods: For this study, all children under 18 years old, living in France, who had been treated, by rituximab for an AIHA, whatever the context was, between January 1, 2000 and December 31, 2012, registered in the OBS'CEREVANCE cohort, were included. The end point for analysis was December 31, 2013, ensuring more than 1 year's follow-up for each patient. Response criteria were defined, relapse free survival (RFS) curves were provided, and tolerance was assessed from patient's medical records.
Results: Sixty one children with AIHA were given rituximab, 375 mg/m²/dose for a median of 4 weekly doses. All patients had previously received steroids, 26 had received one to four supplementary line of treatment. The median age at rituximab initiation was 8.5 [0.3; 17.6] years. The median delay from diagnosis to rituximab was 9.9 [0.2; 153.1] months. With a median follow-up of 4 years (0.3 - 11) from rituximab initiation, 46 patients responded (75%), 40 complete response (CR) and 6 partial response (PR). 20 patients (43%) relapsed in a median delay of 10.8 (1.7-57.6) months after treatment by rituximab The proportion of relapses among responder patients was significantly lower in patient who were treated early after diagnosis (p=0.03). In all children rituximab allowed decrease of steroid therapy. Compared to AIHA/ES, in isolated AIHA, the proportion of CR was significantly higher (74% *vs* 54%, p=0.02), and RFS was significantly higher (p=0.04). Ten patients were aged less than one year at rituximab initiation. In this subgroup, the median time between diagnosis and rituximab initiation was 1.6 months, shorter than 12 months in the rest of the cohort (p=0.006). Seven patients responded (70%), 6 CR and RFS was 71%. Two children presented allergic reactions. No case of progressive multifocal leuco-encephalopathy was observed. A total of 40 children received IVIg replacement, whose duration was temporary in 31 and prolonged in 9. Five patients died and among them one severe sepsis associated with agranulocytosis could be reliable to rituximab therapy.
Summary/Conclusions: In our hands, in this large series of childhood AIHA, rituximab was a valuable second line treatment, allowing steroid withdrawal in 60-70% of cases, with 30% long term responders, even in infants, mainly in isolated AIHA and when it was precociously used. Two serious concerns on safety are abrupt severe neutropenia, and prolonged hypogammaglobulinemia: adequate screening of underlying primitive immune deficiency before treatment is mandatory.

Late Breaking Oral Session

LB2233

OOVERALL SURVIVAL IN RELAPSED/REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS RECEIVING INOTUZUMAB OZOGAMICIN *VS* STANDARD CARE IN THE PHASE 3 INO-VATE STUDY

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Background: CD22 is expressed in most (>90%) cases of B-cell acute lymphoblastic leukemia (ALL) and is an attractive target for the treatment of B-cell malignancies. Inotuzumab ozogamicin (InO), a humanized anti-CD22 antibody conjugated to calicheamicin, has demonstrated significantly superior response *vs* standard care (SC) in the first 218 randomized patients with relapsed/refractory (R/R) ALL in the phase 3 INO-VATE trial (data presented previously, DeAngelo, European Hematology Association [EHA] 2015 meeting [LB2073]).
Aims: To assess overall survival (OS) and progression free survival (PFS) of adults with R/R ALL receiving InO *vs* SC
Methods: In this ongoing global, 2-arm, randomized phase 3 trial (NCT01564784), patients with R/R ALL (including ~15% of patients in each arm with Philadelphia-positive ALL) due to receive salvage (S) 1 or 2 therapy were randomized to InO (starting dose 1.8 mg/m^2/cycle [0.8 mg/m^2 on day 1; 0.5 mg/m^2 on days 8 and 15 of a 21-28 day cycle (≤6 cycles)]) or SC (either fludarabine/cytarabine [ara-C]/granulocyte colony-stimulating factor [FLAG], ara-C plus mitoxantrone, or high-dose ara-C). Study was designed with two primary endpoints: 1) OS and 2) complete remission (CR)/CR with incomplete hematologic recovery (CRi) assessed in first 218 patients randomized (results previously presented). Overall study-wise type-I error was controlled by splitting 1-sided α to 0.0125 for each endpoint. Safety was assessed in all patients who received ≥1 dose of study drug. Per protocol, the final OS analysis was to occur upon observing ~248 events; 252 events (122 with InO and 130 with SC) were observed on March 8, 2016; data as of this date are presented.
Results: The ITT analysis population included 326 patients with both arms being well balanced for baseline stratification factors. The OS hazard ratio (HR) between InO and SC was 0.77 (97.5% CI, 0.58-1.03) with 1-sided P=0.0203 and median OS 7.7 [95% CI, 6.0-9.2] *vs* 6.7 [95% CI, 4.9-8.3] months. The second primary objective of demonstrating a statistically significant improvement in final OS with InO *vs* SC was not met at prespecified significance level of 0.0104. The 2-year OS rate for InO *vs* SC was 23% (95% CI, 16-30%) *vs* 10% (95% CI, 5-16%). It was noted, however, that the OS departed from the proportional hazards assumption. Given this, a restricted mean survival time (RMST) analysis was applied and showed: mean OS was 13.9 months for InO *vs* 9.9 months for SC, a difference that met statistical significance. PFS was significantly longer with InO *vs* SC (HR, 0.45 [97.5% CI, 0.34-0.61]; 1-sided P
Summary/Conclusions: Compared with SC, InO provided evidence of longer OS and significantly prolonged PFS in adult patients with R/R ALL.

LB2234

PLATELET TRANSFUSION IN CEREBRAL HAEMORRHAGE (PATCH): A RANDOMISED CONTROLLED TRIAL

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Background: Antiplatelet therapy use at time of intracerebral haemorrhage (ICH) is associated with higher mortality. Reduction of haematoma growth using platelet transfusion might improve outcome.
Aims: To investigate whether platelet transfusion improves outcome compared to standard care at three months in people with spontaneous ICH taking antiplatelet therapy.
Methods: This was a multicentre prospective, randomised, open, blinded-endpoint (PROBE) parallel group trial, conducted at 60 hospitals in The Netherlands, Scotland and France. We enrolled patients with spontaneous supraten-

torial ICH aged ≥18 years using antiplatelet therapy for ≥7 days preceding ICH, if Glasgow Coma Scale was ≥8. Participants were randomised (1:1, with a secure web-based system using permuted blocks, stratified by study centre and type of antiplatelet therapy) to receive either platelet transfusion (intervention) within six hours of start of symptoms and 90 minutes of diagnostic brain imaging, or standard care without platelet transfusion (comparator). The primary outcome was modified Rankin Scale (mRS) score assessed blinded to treatment allocation at three months after ICH. PATCH was registered with the Netherlands trial register (NTR1303).
Results: Between February 2009 and October 2015, 97 participants were randomised to platelet transfusion and 93 to standard care. Mean age with standard deviation was 74.0, 10.2 years in the interventional and 73.5, 11.2 years in the comparator group. The odds of death or dependence at three months were higher after platelet transfusion compared to standard care (adjusted common OR 2·05, 95% CI 1·18 to 3·56), with no evidence of treatment effect modification in sub-groups of interest. Platelet transfusion did not reduce ICH growth at 24 hours.
Summary/Conclusions: Platelet transfusion seems inferior to standard care for people using antiplatelet therapy before ICH.

LB2235

HIV-1-CONTAINING PLATELETS ARE PRESENT IN HIV-1 INFECTED PATIENTS *IN VIVO* AND ARE INTERNALIZED BY MACROPHAGES *IN VITRO*

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Background: Mainly regarded as actors of primary hemostasis, human platelets are also capable of directly interact and internalize, by endocytosis, the human immunodeficiency virus (HIV)-1 *in vitro*. Although patients with acquired immunodeficiency syndrome can present thrombocytopenia and, as we showed earlier, when highly viremic, present HIV-containing platelets, the *in vivo* relevance of virus endocytosis by platelets and its role in HIV pathogenesis remain to be elucidated. We speculate that HIV enclosed within platelets can be sheltered from host immune system response and transported, by circulating platelets, to macrophages from different tissues, where the virus can establish viral reservoirs.
Aims: We therefore investigated whether HIV-1-containing platelets could be internalized by macrophages, triggering productive infection and possibly establishing a viral reservoir vectored by platelets.
Methods: To approach the question, we obtained platelet-enriched plasma (PRP) from seropositive patients (n=40) treated or not with highly active antiretroviral therapy (HAART) and established a flow cytometry/confocal microscopy method to: i) quantify the amount of HIV-1-containing platelets present in HIV-1 seropositive patient PRP ; and ii) observe the interaction of HIV-1-containing platelets from patient PRP with human peripheral blood monocyte-derived macrophages (M0) *in vitro*, and determine qualitatively and quantitatively the distribution of the virus.

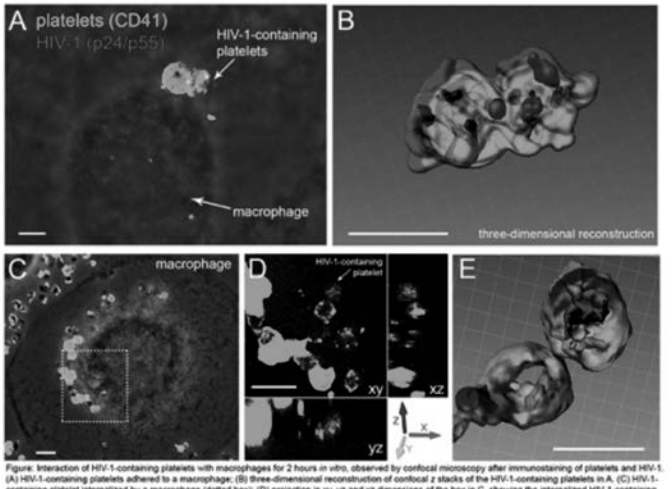

Figure: Interaction of HIV-1-containing platelets with macrophages for 2 hours *in vitro*, observed by confocal microscopy after immunostaining of platelets and HIV-1. (A) HIV-1-containing platelets adhered to a macrophage; (B) three-dimensional reconstruction of confocal *z* stacks of the HIV-1-containing platelets in A. (C) HIV-1-containing platelet internalized by a macrophage (dotted box); (D) projection in xy, yz and xz dimensions of the box in C, showing the internalized HIV-1-containing platelets in detail; (E) three-dimensional reconstruction of confocal *z* stacks of the HIV-1-containing platelets indicated in D.

Figure 1.

Results: Here, we show that HIV is consistently retrieved within platelets of HIV-infected patients. These patients with detectable HIV-1-containing platelets had lower numbers of $CD4^+$ (< 300/ul) and total T lymphocytes in the blood when compared with patients lacking detectable HIV-1 in platelets. In contrast, detection of HIV-1-containing platelets was not correlated to patient viral load, treatment or thrombocytopenia. This suggests that HIV-1 can also circulate, sheltered within platelets, in the blood of patients with undetected cell-free virus and unaltered platelet count. When incubated for 2 hours with M0 *in vitro*, platelets derived from patient PRP interacted rapidly with M0, either being internalized by M0 or remaining attached to M0 surface (figure). Viral p24/p55 capsid antigens were detected within M0 after the interaction period. The higher the number of HIV-1-containing platelets detected in PRP (detected by flow cytometry), the higher the number of HIV-1-containing platelets interacting with M0 (detected morphologically). The amount of HIV-1-containing platelets associated to macrophages correlated negatively with patients $CD4^+$ and total T lymphocytes numbers, highlighting that association of HIV-1-containing platelets with M0 could occur *in vivo* in patients with bad prognosis.
Summary/Conclusions: Although the outcome of HIV-1 retention by platelets and a platelet mediated-immunity during viral infection are still under investigation, this study demonstrates that HIV-1 can circulate in the bloodstream of seropositive, HAART-treated patients with undetected blood viral load using platelets as carriers, and that macrophages internalize HIV-1-containing platelets derived from these patients. The results highlight an alternative pathway, via platelets, for viral dissemination to tissue macrophages. Considering that inhibitors of platelet function have been employed for the treatment of different diseases, the study contributes to reveal a novel and promising therapeutical target for blocking HIV-1 spread and establishment of viral reservoirs.

LB2236

PHASE 3 RANDOMISED CONTROLLED STUDY OF DARATUMUMAB, BORTEZOMIB AND DEXAMETHASONE *VERSUS* BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: CASTOR

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Background: Daratumumab (D), a human anti-CD38 IgGκ monoclonal antibody, induces deep and durable responses with a favorable safety profile in patients with relapsed or refractory multiple myeloma (RRMM). We report a pre-specified interim analysis of the first randomised controlled study of daratumumab (CASTOR; NCT02136134).
Aims: To compare the efficacy and safety of daratumumab plus bortezomib (V) and dexamethasone (d) *versus* Vd alone in patients with RRMM in a phase 3 study.
Methods: Patients who received ≥1 prior line of therapy were randomised (1:1) to 8 cycles (q3w) of Vd (bortezomib: 1.3 mg/m^2 subcutaneously on Days 1, 4, 8, 11; dexamethasone: 20 mg orally on Days 1, 2, 4, 5, 8, 9, 11, 12) with or without daratumumab (16 mg/kg intravenously qw in Cycles 1-3, Day 1 of Cycles 4-8, then q4w until progression). The primary endpoint was progression-free survival (PFS).
Results: 498 patients (DVd, 251; Vd, 247) were randomised. Baseline demographics and disease characteristics were well balanced. Patients received a median of 2 prior lines of therapy (range 1-10). 66% received prior bortezomib; 76% received prior immunomodulatory drug (IMiD); 48% received prior proteasome inhibitor (PI) and IMiD; 33% were IMiD-refractory; and 32% were refractory to last line of prior therapy. With a median follow-up of 7.4 months, daratumumab significantly improved median PFS (61% reduction in the risk of progression/death) for DVd *versus* Vd (Figure 1). Addition of daratumumab to Vd also significantly delayed median time to disease progression (TTP) *versus* Vd (not reached [NR] *vs* 7.3 mo; hazard ratio, 0.30; 95% confidence interval, 0.21-0.43; P <0.0001). Daratumumab significantly increased overall response rate (ORR; 83% *vs* 63%, P <0.0001), in addition to doubling the rates of very good partial responses (VGPR) or better (59% *vs* 29%, P <0.0001) and complete responses (CR) or better (19% *vs* 9%, P = 0.0012) for DVd *versus* Vd, respectively. The median duration of response was NR for DVd *versus* 7.9 months for Vd. All planned sensitivity analyses demonstrated that DVd was better than Vd, which was consistent with the results from the primary analysis. In addition, pre-specified subgroup analyses on PFS demonstrated that the

treatment effect of DVd over Vd was consistent across all selected subgroups. Most common (>25%) treatment-emergent adverse events (TEAEs; DVd/Vd) were thrombocytopenia (59%/44%), peripheral sensory neuropathy (47%/38%), diarrhoea (32%/22%) and anaemia (26%/31%). Most common grade 3/4 TEAEs (>10%) were thrombocytopenia (45%/33%), anaemia (14%/16%), neutropenia (13%/4%). 7%/9% (DVd/Vd) of patients discontinued due to a TEAE. Daratumumab-associated infusion-related reactions (IRR; 45% of patients) mostly occurred during the first infusion (98% of patients with IRR); most were grade 1/2 (grade 3/4, 9%/0%).

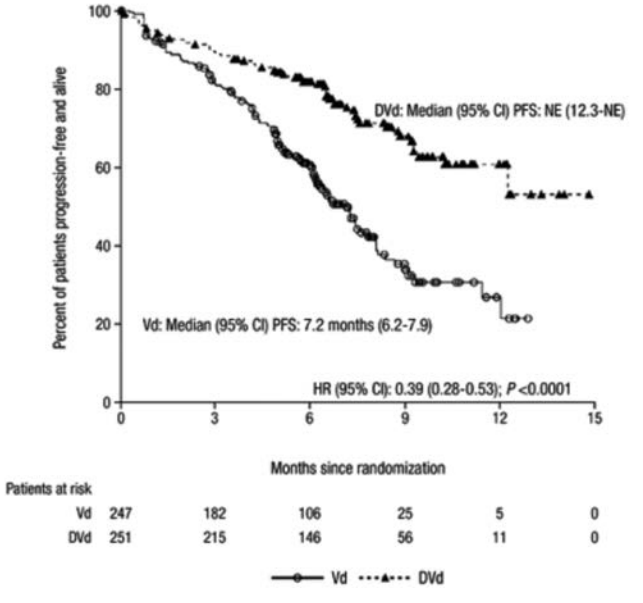

Figure 1.

Summary/Conclusions: Daratumumab significantly improved PFS, TTP, and ORR in combination with Vd *versus* Vd alone. DVd doubled rates of both VGPR or better and stringent CR/CR *versus* Vd alone. Safety of DVd is consistent with the known safety profile of daratumumab and Vd. The addition of daratumumab to Vd should be considered a new standard of care for patients with RRMM currently receiving Vd alone.

LB2237

THE ANTI C1S COMPLEMENT ANTIBODY TNT009 INDUCES RAPID COMPLETE REMISSIONS OF ANAEMIA IN PATIENTS WITH PRIMARY COLD AGGLUTININ DISEASE

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Background: Cold agglutinin disease (CAD) is a difficult to treat autoimmune haemolytic anemia in which IgM antibodies bind to erythrocytes at low temperature and fix complement. This complement deposition opsonizes erythrocytes which undergo extravascular haemolysis in the liver.
Aims: Based on preclinical studies (Shi *et al.*, 2014 Blood), we hypothesized that TNT009, an antibody directed against complement component C1s, could stop haemolysis in CAD patients.
Methods: This represents an interim analysis of a first-in-human trial, where we have included four female CAD patients. Patients received a test dose of 10 mg/kg TNT009, followed by a full dose of 60mg/kg 1-4 days later, and three additional weekly doses of 60 mg/kg.
Results: All infusions were well tolerated without premedication and without relevant adverse effects. Out of four patients, three had been transfusion-dependent either previously (one patient within the last year) or recently (2 patients within 14 days of their enrolment into the trial). These three patients unequivocally responded immediately to TNT009 infusion, and are described below. TNT009 blocked the classical pathway of complement as demonstrated by an immediate drop in CH50 and rise in C4. TNT009 rapidly abrogated extravascular haemolysis, normalizing bilirubin levels in all three patients from a median of 1.7mg/dL (range: 1.6-2.6) to 0.7mg/dL (0.6-0.7) within 24 hours. Infusion of TNT009 immediately decreased the destruction of reticulocytes which rose from 131 (103-140) to 185 (175-286) x10E9/L within 24h. Haptoglobin levels normalized within one week and confirmed the complete halt of haemolysis. Haemoglobin levels rose from a median haemoglobin of 7.5g/dL (range: 6.8-8.3) before the test dose by 1.6 g/dL (1.1-1.6) within the first week, and completely normalised (>12 g/dL Hb by end of study in two patients; one patient still under observation) within 5-6 weeks. Normalisation of these parameters corresponded to complete remission of CAD-induced haemolysis. The time course of all these changes was highly significant (Friedman ANOVA $p<0.001$). Haemolysis recurred when effective drug levels were completely cleared from the circulation about 4 weeks after the last dose of TNT009. Re-exposure to TNT009 in a named patient programme recapitulated the immediate onset of effect, and the rapid and complete stop of haemolysis in these patients. The only patient who did not respond to TNT009 was suffering from secondary CAD and had a 70% bone marrow infiltration with chronic lymphocytic leukaemia accompanied by CLL-driven severe hypocomplementaemia.
Figure 1: Early changes within one week after TNT009 infusion (60mg/kg) in the three responders. Individual patients are depicted by the same symbols throughout. Haptoglobin is slightly offset for better visibility of similar values.

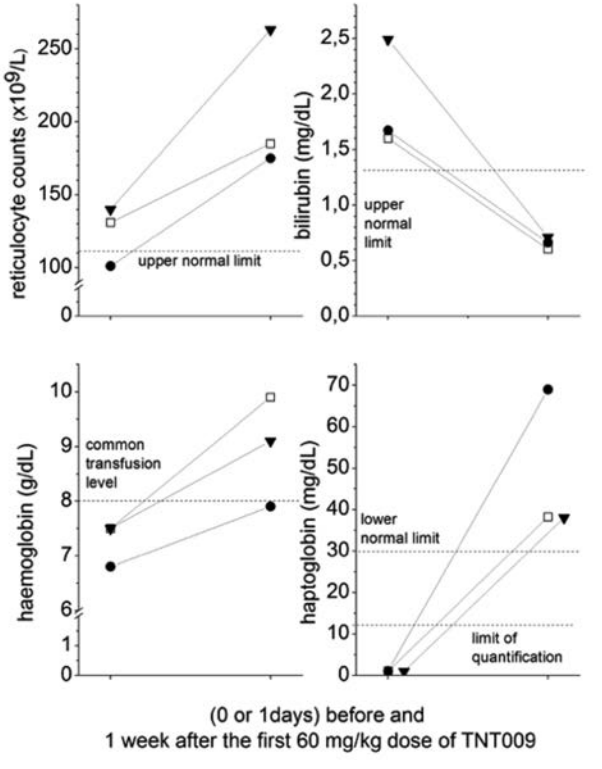

Figure 1.

Summary/Conclusions: TNT009 has an immediate onset of action, and showed remarkable and unprecedented efficacy in CAD patients in need of treatment. The three responding patients became transfusion independent while on TNT009. The results are encouraging and instrumental in planning for upcoming pivotal studies.

LB2238 *This abstract is part of the Presidential Symposium*

AN OPEN-LABEL, RANDOMISED, PHASE 3 STUDY OF DARATUMUMAB, LENALIDOMIDE, AND DEXAMETHASONE (DRD) *VERSUS* LENALIDOMIDE AND DEXAMETHASONE (RD) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): POLLUX

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Background: Daratumumab (D), a human anti-CD38 IgGκ monoclonal antibody, was associated with rapid, deep, and durable responses and a favorable safety profile when combined with lenalidomide and dexamethasone (Rd) in a phase 1/2 study of relapsed or relapsed and refractory multiple myeloma pts (Plesner T, *et al.* ASH 2015, Abs. 507).
Aims: To compare the efficacy and safety of D in combination with Rd *vs* Rd alone in pts with RRMM in a randomised, open-label, multicenter, phase 3 study (POLLUX; NCT02076009).
Methods: Pts who received at least 1 prior line of therapy for myeloma were randomised (1:1) to R 25 mg orally on Days 1-21 of each 28-day cycle and d 40mg weekly, with or without D (16mg/kg qw for 8 weeks, q2w for 16 weeks, then q4w until progression). The primary endpoint is progression-free survival (PFS). Secondary endpoints are time to progression (TTP), overall response rate (ORR), rate of very good partial response (VGPR) or better, minimal residual disease-negative rate, overall survival (OS), duration of response, time to response, and safety.

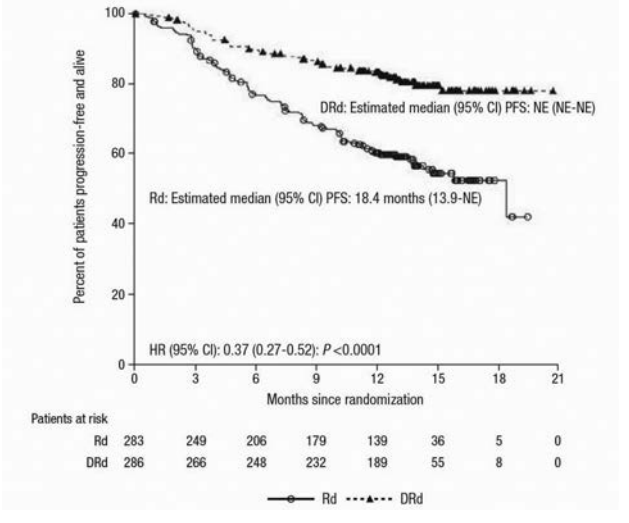

Figure 1.

Results: 569 pts were randomised. Median age of pts was 65 years old. Pts received a median of 1 prior line of therapy (range 1-11), with 19% of pts with ≥3 prior lines of therapy. Eighty-six percent received a prior PI, and 55% received prior IMiD, including 18% prior R, with 44% having received both a PI and an IMiD; 27% were refractory to the last line of prior therapy, 18% were PI refractory, and none were R refractory. After a median follow-up of 13.5 months, D significantly improved median PFS (63% reduction in the risk of progression/death) for DRd *vs* Rd (Figure 1). Addition of D to Rd significantly delayed TTP *vs* Rd (not reached [NR] vs estimated median of 18.4 mo; hazard ratio, 0.34; 95% CI, 0.23-0.48; P<0.0001). D significantly increased ORR (93% *vs* 76%, P<0.0001) and rates of VGPR or better (76% vs 44%, P<0.0001) and complete response (CR) or better (43% *vs* 19%, P<0.0001) for DRd *vs* Rd, respectively. The median duration of response was NR for DRd *vs* 17.4 months for Rd. All preplanned sensitivity analyses were consistent with results from the primary analysis. Pre-specified subgroup analyses of PFS demonstrated that the treatment effect of DRd over Rd was consistent across all pre-specified subgroups. The most common (<25%) TEAEs (DRd/Rd) were neutropenia (59%/43%), diarrhea (43%/25%), fatigue (35%28%), upper respiratory tract infection (32%/21%), anemia (31%/35%), constipation (29%/25%), cough (29%/13%), thrombocytopenia (27%/27%), and muscle spasms (26%/19%). Most common grade 3/4 TEAEs (>10%) were neutropenia (52%/37%), thrombocytopenia (13%/14%), and anemia (12%/20%). The rate of Grade 3/4 infections/infestations was 28% in the DRd group and 23% in the Rd group and the most common Grade 3/4 infections/infestations TEAE (≥5%) was pneumonia (8%/8%). Similar rates of treatment discontinuation due to TEAEs were observed (7%/8%). D-associated infusion-related reactions (IRR; 48% of pts) mostly were grade 1/2 (grade 3/4, 5%/0%); most (92% of IRRs) occurred during the first infusion.
Summary/Conclusion: DRd was superior to Rd alone. A significant reduction in the risk of disease progression/death was demonstrated with DRd *vs* Rd. DRd induced deep and durable responses, including doubling stringent CR/CR rates and significantly increasing the rate of VGPR or better *vs* Rd alone. DRd was associated with a manageable safety profile consistent with the known safety profile of D and Rd. The combination of D and Rd potentially represents a new standard of care for pts with >1 prior treatment.

E-POSTERS

Acute lymphoblastic leukemia - Biology

E838

IMPROVING NELARABINE EFFICACY IN REFRACTORY/RELAPSED T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA BY TARGETING ABERRANT PI3K/MTOR SIGNALING

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Background: The introduction of novel chemotherapy protocols has improved the outcome of T-cell acute lymphoblastic leukemia (T-ALL) patients, but refractory and/or relapsing disease remains a major concern. In this context, a major contribution was provided by the introduction of the nucleoside analogs and in particular nelarabine, approved for salvage treatment of refractory/relapsed T-ALL patients.
Aims: A serious nelarabine drawback is that it induces a dose-dependent neurotoxicity. To improve nelarabine efficacy, it is essential to study its molecular targets, testing selective inhibitors of such targets, to be administered in combination with nelarabine, allowing for a lower dosage of the chemotherapeutic.
Methods: Human T-ALL cell lines (HBP-ALL, DND41, Jurkat, MOLT-4, MOLT16, CEM, CEM-R drug-resistant, ALL-SIL, Loucy, HSB2, Peer, PF382, P12-ICHIKAWA) and primary T-ALL refractory/relapsed lymphoblasts from patients were incubated with increasing concentrations of nelarabine alone or combinated with PI3K/mTOR inhibitors for cell viability assays and absolute cell counting by flow cytometry. Flow cytometry was also performed to analyze apoptosis and for phenotyping analyses. Protein expression was evaluated by Western Blot. ENT1/ENT2 gene expression was measured by quantitative real time PCR in T-ALL settings.
Results: We examined cell viability of cell lines and primary T-ALL refractory/relapsed cells. Cell viability assays indicated the presence of T-ALL cell lines sensitive to nelarabine (IC_{50}<5μM) and others which displayed higher IC_{50} (>15μM). The most resistant cell line (IC_{50}>300μM) was Loucy, which is representative of ETP-ALL, a T-ALL subtype with a very poor prognosis. Nelarabine sensitive cells showed a significant increase of apoptotic cells after 48h treatment with 2-5 μM nelarabine, as demonstrated by flow cytometric analyses of stained with AnnexinV FITC/PI cells and western blot analyses of caspases 8, 9, 3, and PARP. In contrast, resistant T-ALL cells were not perturbated. It has been reported that the levels of expression of ENT 1/2 nucleoside transporters were related to in vitro nelarabine sensitivity of T-ALL cell lines and primary samples. The expression of ENT1/2 transporters could be also dependent on interactions between leukemic cells and tumor microenvironment. No significant differences in ENT1/2 mRNA levels between samples sensitive or resistant to nelarabine were seen. Modulation of ENT1/2 gene expression was not related to nelarabine treatment, even if in some cases the co-culture with human stromal cells HS-5, which mimic the bone marrow microenvironment, partially supported cell survival. Upregulated phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signaling is a common feature of T-ALL, where it portends a poorer prognosis by influencing leukemic cell proliferation, survival, and drug-resistance. We analyzed the effects of nelarabine on the phosphorylation status of key components of the PI3K/mTOR pathway in T-ALL settings. Sensitive T-ALL cells showed a strong decrease in the phosphorylation of Ser473 p-Akt and Ser235/236 p-S6 ribosomal protein (S6RP). In contrast, resistant cells treated with nelarabine alone, showed a hyperactivation of PI3K/mTOR and MEK/ERK signaling pathways. The combination of nelarabine with the pan PI3K inhibitors ZSTK474 or BKM120 was synergistic in reducing cell viability and in inducing strong apoptosis in all the resistant cell lines and in relapsed T-ALL patient samples with upregulated PI3K/mTOR signaling.
Summary/Conclusions: Nelarabine combined with PI3K inhibitors efficiently reduced cell viability and induced apoptosis in T-ALL settings, allowing for a lower dosage of nelarabine and therefore, synergizing with conventional therapies in relapsed/refractory T-ALL patients with upregulation of PI3K signaling.

E839

ROLE OF G9A AND EPIGENETIC CHANGES IN LEUKEMIA CELL MIGRATION AND THEIR REGULATION BY VLA4 INTEGRIN

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Background: T-Acute Lymphoblastic Leukemia (T-ALL) originates from neoplastic T cell precursors in the thymus. Usually, T-ALL cells have to migrate from the thymus into other tissues, where residual cells might persist after treatments. The nuclear deformability of these tumor cells, and the signals from the cell receptors called integrins are critical to allow cancer cells to squeeze and cross physical barriers. Epigenetics, a novel cancer hallmark that comprises histone modification, histone variants and DNA modifications, leads a new drug generation to prime cancer cells to death. But some epigenetic changes, such as H3K9me3, have been linked with cancer invasion, and might have additional functions leading cancer progression.
Aims: The objective of this study was to explore how epigenetic changes induced by the integrin VLA4/α4β1 control, in a transcriptional independent manner, the nuclear deformability and infiltration of ALL cells.
Methods: Human T (Jurkat) leukemia cell lines, and normal primary T cells were cultured in suspension or on integrin ligands for different times and the levels of H3K9me3 were determined. Nuclear deformability was evaluated using several biophysical techniques, such as Atomic Force Microscopy. To identify the molecular mechanism induced by α4β1 integrin, and how this would be relevant for T-ALL cell migration, different biochemical and cell biology methods were performed.
Results: We showed that acute lymphoblastic leukemia (ALL) cells cultured on VCAM1 or Fibronectin (ligands of α4β1 integrin) presented higher levels of H3K9me3 than cells in suspension. We confirmed that ALL cells were more sensitive to this effect than normal T-cells. We determined that G9a, a histone methyltransferase, localized at the nuclear envelope and was responsible for H3K9 methylation when T-ALL cells were cultured on VCAM1. We used stable Jurkat cells depleted for G9a to demonstrate that H3K9 methylation contributed to the nuclear stiffness and the ability of ALL cells to migrate in response to chemotaxis and in 2D and 3D environments.
Summary/Conclusions: Our results reveal novel functions for G9a in modulating ALL migration, which might contribute to tumor cell dissemination and leukaemia progression.

E840

DYNAMICS OF EARLY RESPONSE TO TREATMENT OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IS ASSOCIATED WITH GENE DEFECTS

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Background: Genetic abnormalities such as *BCR-ABL1* translocations, *MLL* and *CRLF2* rearrangements and *IKZF1* deletions influence blast clearance during induction phase of childhood B-cell precursor ALL (BCP-ALL). Minimal residual disease (MRD) at different time-points of the induction protocol reflects chemosensitivity and may provide information about resistance of leukemic cells to cytotoxic drugs.
Aims: We investigated early response to treatment at three time-points of induction protocol (days 8, day 15 and 33) in patients with childhood ALL with respect to genetic abnormalities.
Methods: 481 children (median age 4.33 yrs), with newly diagnosed BCP-ALL treated between 02-2009 and 08-2015 with the ALL-IC BFM09 protocol in centers of the Polish Pediatric Leukemia/Lymphoma Study Group were enrolled into analysis. Targeted copy number screening was performed on available DNA samples (n=457) using the P335-B2 SALSA MLPA kit (MRC-Holland, Netherlands). *IKZF1* deletions were confirmed with breakpoint-specific multiplex-PCR. CRLF2 protein expression on leukemic cells was determined by flow cytometry (FCM). Steroid resistance was defined as the presence of ≥1000 leukemic cells/μl of peripheral blood after 7 days of steroid therapy. MRD was measured at day 15 (MRD15) and day 33 (MRD33) of induction therapy using 8-color flow cytometry. Chemosensitivity of leukemic cells to L-asparaginase, antracyclines and steroids was tested *in vitro* using an MTT assay (n=27).
Results: The frequency of cytogenetic abnormalities and microdeletions in selected genes were as follow: *BCR-ABL1* fusion n=11 (2.4%), *MLL* rearrangements n=24 (5.2%), *IKZF1*del n=96 (20%), *PAX5* del n=100 (22%), *PAR1* del n= 42 (10%), *CDKN2A* del n=128 (28%), *CDKN2B* del (26%), *BTG1* del n=39 (8.5%), *ETV6* del n=101 (22%), *EBF1* del n=21 (4.5%), *RB1* del n=30 (6.5%). TLSPR expression was observed in 6% of patients (22/358). Poor steroid

response was present in 42 cases (42/452=9.3%) and was not associated (all p>0.15) with any of the analyzed genetic factors. Median MRD15 and MRD33 were 5.67% and 0.01% respectively. In univariate analysis median MRD level at day 15 was significantly higher among patients with *BCR-ABL1* fusion [12.69(2.5-37.78)% *vs* 0.31(0.03–3.28)% p=6x10^{-4}], *IKZF1*del [1.005(0.072-12.54)% *vs* 0.3(0.03-2.7)%, p=4x10^{-4}] and *ETV6*del [0.6(0.075-6.615)% *vs* 0.32(0.029-3.94)%, p=0.02]. Also median MRD level at day 33 was elevated in patients with *BCR-ABL1* fusion [0.03(0.0001-0.08)% *vs* 1x10^{-4}(0.0001-0.005)%, p=0.02] and in patients with *IKZF1* deletion [1x10^{-4}(0.0001-0.05)% *vs* 1x10^{-4}(0.0001-0.003)%, p=0.02]. Multivariate analysis of MRD15, MRD33 and ΔMRD was done for variables with univariate significance of p<0.15 (log_{10}WBC, age at diagnosis, sex, poor steroid response, hypodiploidy, *MLL* rearrangements, *BCR-ABL1* fusion, *IKZF1* del, *ETV6* del, *EBF1* del, *PAR1* del). A mixed effects model was used to evaluate intraindividual changes of MRD15 and MRD33 and factored in genetic covariates. The following variables were significant for MRD15 and MRD33 timepoints: log_{10}WBC (β=0.28, p=1x10^{-7}) and (β=0.09, p=0.05), age at diagnosis (β=0.22, p=1x10^{-7}) and (β=0.23, p=1x10^{-6}), *IKZF1* del (β=0.14, p=1x10^{-3}) and (β=0.08, p=0.08), respectively. Additionally the following variables were significantly associated with the absolute difference of MRD between MRD33 and MRD15: logWBC (β=-0.15, p=4x10^{-4}), steroid resistance (β=-0.38, p=1x10^{-6}), IKZF1 del (β=-0.19, p=2x10^{-2}), and PAR1 del (β=-0.13, p=3x10^{-3}). MTT confirmed increased resistance to asparaginase of IKZF1 del positive blasts (p=0.01).
Summary/Conclusions: Poorer blast clearance during induction protocol in ALL patients harboring *IKZF1* deletions may result partly from chemoresistance to L-ASP, which was noted for the first time in both clinical and in vitro settings.

E841

THE WEE1 INHIBITION DEEPLY SENSITIZES ACUTE LYMPHOBLASTIC LEUKEMIA CELL LINES AND PRIMARY CELLS TO THE CYTOTOXIC EFFECT OF DIFFERENT ANTINEOPLASTIC COMPOUNDS
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Background: Although the efficacy of Checkpoint kinase inhibitors have been established in different kind of tumors, only few studies have been performed in order to evaluate their effectiveness on hematological disorders. The Wee1, ATR/Chk1 and ATM/Chk2 pathways are involved in cell cycle regulation, DNA damages response and DNA repair. Wee1 is a checkpoint kinase, involved mainly in the regulation of G2/M transition through the phosphorylation of both Cyclin-dependent kinase 1 (CDK1) and 2 (CDK2).
Aims: In this study we evaluated the in vitro/ex vivo efficacy of a Wee1 inhibitor, MK-1775, in single agent and in combination on Acute Lymphoblastic Leukemia (ALL) cell lines and primary samples.
Methods: A panel B-/T-ALL cell lines and different primary cells isolated from adult ALL patients were treated in single agent or in combination with different compound (nucleoside analogue, Clofarabine; tyrosine kinase inhibitor, Bosutinib; Chk1/Chk2 inhibitor, PF-00477736) and then was evaluated the reduction of the cell viability (MTS reagent), the reduction of the proliferation(Trypan blue exclusion dye assay), cell cycle modification (Propidium iodide staining), induction of apoptosis (Annexin V/Pi staining), protein modification (Western blot) and protein expression modification (Prime PCR plate).
Results: MK-1775 deeply reduced the cell viability in a dose and time-dependent manner in all the treated cell lines. The reduction of both cell viability and proliferation were associated with the increment of apoptosis and the activation of different DNA damage markers (gH2AX and Parp-1), confirming the cytotoxicity of MK-1775 in single agent. We hypothesized that targeting Chk1, a kinase upstream, of Wee1, would be more effective in reducing cell proliferation. Indeed, the concomitant inhibition of Chk1 and Wee1 kinases, using the PF-0477736 in combination with MK-1775, synergized in the reduction of the cell viability, inhibition of the proliferation index and induction of apoptosis. We undertook further studies to understand the chemo-sensitizer activity of the compound, thus MK-1775 was combined with different drugs (Clofarabine, Bosutinib Authentic, Bos, and a particular isomer of Bosutinib, Bos-I).The combination between MK-1775 and clofarabine showed an additive effect in terms of reduction of the cell viability and induction of apoptosis. Finally the Wee1 inhibitor was combined with the tyrosine kinase inhibitors Bos and Bos-I. Both isomers in combination with MK-1775 showed an additive effect in term of reduction of the cell viability and induction of apoptosis. The cytotoxic effect of Bos-I was stronger on the Philadelphia-negative cell lines in comparison to the positive counterpart. Western blot analysis highlighted that this compound, but not the Bos, interfered with the Chk1/Chk2 and Wee1 pathway. The results found on the different cell lines were confirmed also on primary leukemic cells.
Summary/Conclusions: In our opinion the results of this study identify the Wee1 kinase as a promising target for the treatment of ALL. As monotherapy the inhibition of Wee1 increases the genetic instability of leukemic cells, promoting cell death caused by progressive addition of DNA damages. As chemosensitazer agent, the MK-1775 inhibits the DNA damage response pathway, increasing the cytotoxicity of different compounds. Supported by ELN, AIL, AIRC, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project. Martinelli G. & YenT.J. equal contribution

E842

CHARACTERISATION OF JAK3 KINASE DOMAIN MUTANTS
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Background: Janus kinase 1 (JAK1) and JAK3 are cytosolic tyrosine kinases that are required for cytokine receptor signaling. Recent studies have identified both JAK1 and JAK3 mutations in ALL, AML and lymphoma. JAK3 is mutated in 16% of T-ALL cases. The maojority of these mutations are found within the pseudokinase and kinase domains of JAK3.
Aims: To determine the transforming mechanism of newly identified JAK3 kinase domain mutants L875H, P906S and E958K and to characterize their sensitivity to various JAK inhibitors.
Methods: We studied six JAK3 mutations, two pseudokinase (M511I and R657Q) and four kinase domain (L857Q, L875H, P906S and E958K) mutants. We expressed these proteins in Ba/F3 (proB-cell) and ex vivo cultured primary mouse T-cells. CRISPR/Cas9 genome editing was used to generate common gamma chain (Il2rg) and Jak1 knock out cells.
Results: All six JAK3 mutants were able to transform Ba/F3 cells to growth factor independent growth. The JAK3 L857Q and L875H mutants were able to transform the Ba/F3 cells in the absence of Jak1 or Il2rg, indicating that these two kinase domain mutants do not require binding to a cytokine receptor and do not require Jak1 as a signaling partner. In contrast, the JAK3 M511I, R657Q, P906S and E958K mutants required the presence of Jak1 and Il2rg. Moreover, the growth of these four JAK3 mutants was decreased by overexpression of wild type JAK3 showing that the JAK3 mutants compete with wild type Jak3 for binding to the receptor. Using a proT-cell model, the two JAK3 kinase domain mutants L857Q and L875H showed increased transformation to cytokine independent growth compared to the remaining JAK3 mutants. We next determined the sensitivity of the cells transformed by the JAK3 mutants to either JAK3 selective inhibitors (tofacitinib and decernotinib) or JAK1 selective inhibitors (ruxolitinib and baricitinib). Decernotinib was able to inhibit all JAK3 mutants. While tofacitinib was able to inhibit most JAK3 mutants, the L875H mutant was resistant to this compound. Whilst the JAK1 selective inhibitors were able to inhibit the JAK3 M511I, R657Q, P906S and E958K, they were not able to inhibit the JAK3 L857Q and L875H mutants that were shown to transform cells independently of JAK1.
Summary/Conclusions: The majority of JAK3 mutant proteins require binding to a cytokine receptor complex for full activation and transformation of cells to cytokine independent growth and are sensitive to JAK1 selective inhibition. All data indicated that the transforming mechanism of the two kinase domain mutants L857Q and L875H differ from the other JAK3 mutants. They can activate downstream signaling independent of the receptor complex and are not sensitive to JAK1 selective inhibition. In addition, the L857H mutants show a high tolerance for the JAK inhibitor tofacitinib.

E843

IDELALISIB SENSITIVITY AND MECHANISMS OF DISEASE PROGRESSION IN RELAPSED TCF3-PBX1 ACUTE LYMPHOBLASTIC LEUKEMIA
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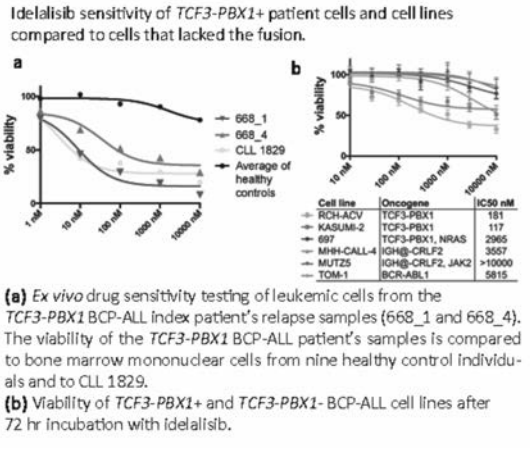
Background: *TCF3-PBX1* (*E2A-PBX1*) is a recurrent gene fusion in B cell precursor lymphoblastic leukemia (BCP-ALL) resulting from translocation t(1;19)(q23;p13). The majority of human *TCF3-PBX1* BCP-ALLs are pre-B-cell receptor positive. *TCF3-PBX1* BCP-ALL patients typically respond to chemotherapy; however, many relapse and subsequently develop resistant disease with few effective treatment options. Mechanisms driving disease progression and therapy resistance have not been studied previously in *TCF3-PBX1* BCP-ALL.
Aims: Here, we aimed to identify novel options for treating *TCF3-PBX1* BCP-ALL by profiling leukemic cells from a 25-year-old male who relapsed after chemotherapy and allogeneic bone marrow transplant. In addition, we sought to identify molecular mechanisms underlying disease pathogenesis and progression.

Methods: Bone marrow (BM) aspirates and a skin biopsy were collected from the index patient at diagnosis and relapse. The sensitivity of BM mononuclear cells was assessed against a library of 302 investigational and approved anti-neoplastic drugs. To understand molecular mechanisms underlying the pathogenesis and progression of the disease, we performed in-depth molecular profiling of the patient samples. Exome sequencing was used to identify somatic mutations and copy number aberrations at diagnosis and relapse, while RNA sequencing was performed to identify aberrant gene expression. Drug classes effective at inhibiting the viability of the patient cells were further investigated using *TCF3-PBX1*+ BCP-ALL and control cell lines that lacked the fusion.
Results: Drug sensitivity testing showed that leukemic blasts from relapse samples of the index patient were sensitive to several classes of targeted drugs. Among the most effective was idelalisib, inhibitor of phosphatidylinositide 3-kinase delta (p110δ) and approved as a second line treatment for CLL and follicular lymphoma. Testing of two relapse samples from the index patient (668_1 and 668_4), positive control CLL and healthy controls showed that the index BCP-ALL and CLL cells were similarly sensitive to idelalisib (Figure 1a). To determine whether idelalisib sensitivity is common to all BCP-ALLs, we tested the sensitivity of *TCF3-PBX1* positive (n=3) and negative (n=3) BCP-ALL cell lines. Two *TCF3-PBX1*+ cells lines were sensitive while all negative cell lines were resistant (Figure 1b). The *TCF3-PBX1*+ 697 cell line had a mutation to *NRAS*, likely resulting in RAS pathway activation and altering sensitivity to idelalisib. Idelalisib sensitivity of *TCF3-PBX1*+ BCP-ALL cells was further supported by evidence showing TCF3-PBX1 directly regulates expression of *PIK3CD*, the gene encoding p110δ. RNA sequencing of the relapse samples showed high *CXCR4* expression, which was not observed in a cohort of diagnostic phase *TCF3-PBX1* BCP-ALLs (N=15). CXCR4 mediates interactions with CXL12 expressing BM stromal cells and has been implicated in contact mediated drug resistance. Idelalisib inhibits CXCR4 signaling providing a rationale for using this drug to counter drug resistance. The index patient's leukemia acquired mutations to both *TP53* alleles at relapse. In addition, the patient's leukemic cells had an *MTOR* mutation, which was associated with high sensitivity to mTOR inhibitors, which has not been observed before in *TCF3-PBX1* BCP-ALL.



(a) *Ex vivo* drug sensitivity testing of leukemic cells from the *TCF3-PBX1* BCP-ALL index patient's relapse samples (668_1 and 668_4). The viability of the *TCF3-PBX1* BCP-ALL patient's samples is compared to bone marrow mononuclear cells from nine healthy control individuals and to CLL 1829.
(b) Viability of *TCF3-PBX1*+ and *TCF3-PBX1*- BCP-ALL cell lines after 72 hr incubation with idelalisib.

Figure 1.

Summary/Conclusions: Our results suggest idelalisib is a promising treatment option for patients with *TCF3-PBX1* BCP-ALL, while other drugs could be useful depending on the genetic context of individual patients.

E844

INFANT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA SHOWS CHARACTERISTIC GENETIC FEATURES COMPARED TO CHILDHOOD CASES

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Background: Survival rates of infants (<1 year of age) diagnosed with acute lymphoblastic leukemia (ALL) remain poor with a 4-year event free survival of 47% (Interfant-99). Beside their often highly aggressive initial clinical presentation, these patients usually do not respond well to current therapies. For reasons not yet understood, nearly all of those patients present with B cell-ALL with T cell-ALL remaining an absolute rarity in infants.
Aims: Given its rarity, the biology of infant T-ALL is not understood. Also, it is not clear whether it presents a distinct disease compared to T-ALL diagnosed in later childhood. The aim of our present study therefore was to identify the landscape of genetic alterations of infant T-ALL by whole-exome sequencing (WES), transcriptome and microRNA sequencing.
Methods: We analyzed a total of three infant T-ALL patients and six childhood T-ALL patients as comparison. All sequencing was performed on a HiSeq 2500 (Illumina). Obtained sequence reads were aligned to the human reference genome. Resulting variation calls were annotated by Variant Effect Predictor. MuTect and VarScan2 were employed for identification of somatic nucleotide variations (SNVs). Correlation analyses based on public databases for miRNA-mRNA targeting were performed to reveal miRNA-mRNA pairs expressed in a negative correlation fashion.
Results: In total, 4504 genes had SNVs in any of the three infant patients, 1305 were recurrent in at least two and 557 in all three infant patients. We did not detect any SNVs in Notch1, however, we found SNVs in Notch2 and Notch3. On transcriptome level, 676 genes were differentially expressed ($|\log FC| \geq 1$, FDR<0.05) between the patient groups. Out of these genes, 194 were downregulated in infants and 482 were upregulated when compared to childhood cases. The hierarchical clustering of these significantly differentially expressed genes clearly separated the two patient groups. To analyze the differences between infant and childhood T-ALL samples on an epigenetic level, we performed miRNA sequencing and found 55 miRNAs which were differentially expressed between infant and childhood cases. Correlation analysis for differentially expressed miRNA-mRNA target pairs revealed 34 miRNA-mRNA pairs (Spearman's Rho ≤ -0.6, FDR ≤0.05). Pathway analyses (Ingenuity Pathway Analysis/IPA, Qiagen) showed 51 pathways which were differentially regulated between infant and childhood patients (p-value<0.05). Most perturbed pathways fell into categories with functions in the immune system or were cancer-related, including differentiation, proliferation, apoptosis or cell survival signaling. Examples include ERK5 and TLR signaling. Both toll-like-receptors 2 (TLR2) and 4 (TLR4) were predicted to be inhibited by IPA leading to an aberrant expression of their downstream targets. TLR2 was not significantly downregulated in T-ALL infant samples with a logFC of -1.21 (FDR=0.5), because of variant expressions within the groups. However, one infant case showing a slightly higher expression of TLR2 was carrying an SNV in its coding sequence leading to a missense variant. Several TLR2 targets including SELP, ITGA2B, IL1B, CXCL8 and CD86 were significantly downregulated in infant compared to childhood T-ALL samples.
Summary/Conclusions: Our analyses on exome, transcriptome and miRnome level suggests that infant T-ALL has distinct disease features compared to childhood T-ALL. Differences were observed on both transcriptomic and epigenetic level. Moreover, we describe several differentially expressed pathways including ERK5 and TLR signaling pathways.

E845

IDENTIFICATION OF THE GENOMIC AND TRANSCRIPTOMIC CHANGES CORRELATED WITH THE EX VIVO RESISTANCE TO DAUNORUBICIN AND DOXORUBICIN IN PEDIATRIC ACUTE LEUKEMIAS

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Background: Drug resistances in leukemias correlates with numerous genomic and transcriptomic changes, but the significant majority is still unidentified. Daunorubicin (DNR) and doxorubicin (DOX) are chemotherapeutics of the anthracycline family frequently used in acute leukemias (AL) treatment.
Aims: The objective of the experiment was to identify changes on the genome and transcriptome level and compare them with *ex vivo* resistance to DNR and DOX in pediatric patients with acute lymphoblastic (ALL) or myeloblastic (AML) leukemia.
Methods: In order to determine the *ex vivo* drug resistance profiles to DOX and DNR, MTT cytotoxicity assay was performed on mononuclear cells from 155 patients with ALL or AML. Gene expression profiles (51 patients with ALL, 16 with AML) were prepared on the basis of cRNA hybridization to oligonucleotide arrays of the human genome (Affymetrix). CGH array profiles, (34 patients with ALL, 12 with AML) were prepared on the basis of gDNA hybridization to oligonucleotide arrays of the human genome (Agilent). Data were analyzed using bioinformatics tools (Partek GS, GeneSpring, Feature Extraction, CytoGenomics) and data bases (UCSC Genome Browser, GeneCards, PantherDB). Validation of array results was performed by RT-qPCR with the use of UPL probes for 20 genes comparing to 4 reference genes. The relative expression was calculated by Pfaffl method of quantification in REST-2009 (Qiagen).
Results: Ontological analysis of selected genes in transcriptomic profiles revealed, that hydrolases were mostly overexpressed in lymphoblasts resistant to DNR and DOX, but contrarily hydrolases showed differential expression level in AML cells resistant to DNR. Moreover, in both leukemic blasts resistant to DNR, chemokines were overexpressed. Additionally, in myeloblasts resistant to DNR, genes involved in transcription from RNA polymerase II promoter were downregulated, in ALL cells resistant to DOX-overexpressed, and in ALL blasts resistant to DNR genes showed differential expression. On the basis of achieved aCGH results, we singled out a couple of recurrent genomic changes identified among children with AL. Amp8p12-p11.21 is statistically significant

rearrangement in resistant DNR profile, additionally del9p21.3 and amp22q11.22 are in resistant DOX profile. In blasts sensitive to DNR, we also identified del5q32-35.3, amp14q32.33, del15q11.1-11.2 and del21.11.2-p11.1. What is more, the results of relative expression analysis of candidate and reference genes are presented in Table 1.

Table 1. Microarray and qPCR expression level analysis for all valifated genes.

Gene	Microarray					Realtime PCR			
	DNR		DOX			DNR		DOX	
	Ratio	P value	Ratio	P value	Efficiency	Ratio	P value	Ratio	P value
CANDIDATE GENES									
ABCG1	3.631	0.006	1.147	0.620	1.87	0.828	0.328	1.622	0.042
ANXA1	3.278	0.003	3.481	0.001	1.91	3.753	0.001	3.353	0.001
ARAP1	1.652	0.511	1.298	0.008	1.97	1.442	0.026	1.675	0.001
CDKN1A/P21	1.598	0.393	1.181	0.101	1.86	1.127	0.671	1.375	0.329
DUSP2	11.080	0.051	1.691	0.113	1.95	1.926	0.013	1.926	0.015
FGR	2.581	0.009	2.154	0.001	1.95	3.205	0.001	3.162	0.001
HK3	3.231	0.434	1.596	0.019	1.95	3.748	0.000	5.425	0.000
IFIT3	4.419	0.345	0.696	0.246	1.89	1.756	0.265	1.695	0.202
ITGAM	2.556	0.002	0.951	0.406	1.90	2.436	0.001	2.728	0.001
PCDH9	0.426	0.009	0.369	0.058	1.98	0.258	0.007	0.351	0.256
RETN	2.395	0.000	2.201	0.000	1.91	2.201	0.021	2.734	0.008
RUNX1	0.597	0.055	0.973	0.899	1.91	1.079	0.713	1.000	0.998
SERPINA3	2.237	0.001	1.760	0.002	1.93	4.009	0.000	3.387	0.001
TCF7	2.003	0.022	1.637	0.110	2.10	1.046	0.890	0.881	0.766
WDR26	1.733	0.135	0.950	0.659	1.88	1.221	0.361	1.156	0.360
WNK1	2.716	0.046	1.472	0.036	1.92	1.007	0.563	0.996	0.988
CASP1	2.107	0.367	0.820	0.481	1.86	2.121	0.220	1.495	0.003
ITGB2	2.417	0.069	2.130	0.022	1.85	3.209	0.019	6.537	0.001
TBL1XR1	0.517	0.029	0.981	0.922	1.96	0.897	0.772	0.858	0.695
TTC28	0.486	0.018	0.762	0.478	1.95	1.188	0.732	2.467	0.102
REFERENCE GENES									
TUBA1B	4.257	0.060	1.168	0.158	1.91	1	Nd	1	Nd
SDHA	0.188	0.013	0.995	0.437	1.93	1	Nd	1	Nd
GAPDH	1.048	0.743	1.089	0.235	1.91	1	Nd	1	Nd
ACTB	1.696	0.081	1.172	0.118	1.92	1	Nd	1	Nd

Summary/Conclusions: The multidimensional analyses revealed, that resistance to DNR and DOX are the result of recurrent genomic and transcriptomic changes in leukemic blasts. We presume, that chromosome aberrations del9p21.3 and amp22q11.22 may associate with resistance to DOX, and amp8p12-p11.21 with lack of sensitivity to DNR and DOX. Additionally, basing on the Real-Time PCR results, we selected presumable target genes, such as *ANXA1, ARAP1, FGR, HK3, SERP1, ITGAM, DUSP2, RETN, ITGB2* in DOX and IDA profiles. All of them were overexpressed. Interestingly, *CASP1* was unique target gene in DOX resistant profile and *PCDH9* in DNR resistant profile. Overexpression of *CASP1* may influence on immune response in leukemia. Furthermore, the decreased expression level of cadherins (such as *PCDH9*) may have impact on the interactions of hematopoietic progenitors and bone marrow stromal cells, resulting breakdown of adhesive mechanisms and enhancement of cell proliferation. This study was supported by Grant from the National Science Centre No. DEC-2011/03/D/NZ5/05749.

E846

SLOWER EARLY RESPONSE TO TREATMENT AND DISTINCT EXPRESSION PROFILE OF CHILDHOOD HIGH HYPERDIPLOID ACUTE LYMPHOBLASTIC LEUKAEMIA WITH DNA INDEX<1.16

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Background: Acute lymphoblastic leukaemias (ALL) with 51-67 chromosomes in leukaemic cells are defined as high-hyperdiploid (HHD). Besides the number of chromosomes, the hyperdiploid ALL can be determined also by DNA index (DNAi; established by flow cytometry), representing ratio of DNA content in leukaemic *vs* normal diploid cells. In childhood, HHD leukaemias comprise 25-30% of all cases, typically arise from B lymphocyte precursors and are generally associated with good prognosis. However, several studies show heterogeneity in HHD-ALL and suggest that the favourable prognosis is associated rather with cases presenting with higher ploidy defined by DNAi >=1.16 or with a presence of specific single or combined trisomies. Thus, cases with DNA index >=1.16 and<1.6 are often considered as "typical" high hyperdiploid ALLs while leukemias with >50 chromosomes and DNA index<1.16 are only rarely studied separately.
Aims: In this study we analysed childhood HHD-ALL patients divided into groups with lower (<1.16) and higher (>=1.16) DNAi to reveal biological and clinical differences between the two subgroups.
Methods: Eighty-nine childhood HHD-ALL patients were analysed by single nucleotide polymorphism array to determine extra chromosomes and to correlate the data with DNA content established by flow cytometry. Moreover, we analysed treatment response, presence of secondary aberrations, mutations in Ras pathway genes (NRAS, KRAS, FLT3, PTPN11) and also gene expression profile (GEP) to reveal possible differences between the two subgroups.
Results: Our results show that vast majority of cases with 51 to 54 chromosomes have DNAi between 1.1 and 1.16 and cases with 55 and more chromosomes have usually DNAi >=1.16. The groups with lower and higher DNAi have distinct response to early treatment - the better response of the group with higher DNAi is probably associated with specific chromosomal gains (trisomy of chromosome 10 or combined with trisomies 4 and/or 17) highly enriched in the group with higher DNAi. Moreover, patients with lower and higher DNAi show distinct GEP in unsupervised clustering analysis; importantly, analysis of the differentially expressed genes and number of its genomic copies suggests, that gene dosage effect probably does not play a driving role in the different behaviour of the two HHD-ALL subgroups.
Summary/Conclusions: The distinct GEP suggests that cytogenetically defined HHD-ALL is in fact composed of two biologically distinguishable subgroups. These differences should be taken into account when defining ALL with excellent prognosis; if treatment deintensification is considered for childhood HHD ALL, only cases with DNAi >=1.16 and gain of chromosome 10 plus chromosomes 4 and/or 17 should be taken into account. Support: The study was supported by grants from Ministry of Health, Czech Republic: IGA NT/14350-3 and project 00064203 (UH Motol).

E847

DIFFERENT PROGNOSTIC ROLE OF RECURRENT COPY NUMBER ABERRATIONS IN B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA WITHOUT RECURRENT FUSION GENES IN 3 CONSECUTIVE AGE COHORTS

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Background: In B-lineage acute lymphoblastic leukemia negative (B-NEG ALL) for recurrent fusion genes (*BCR-ABL1*, *MLL*-rearrangements, *TCF3-PBX1, ETV6-RUNX1*), the most common copy number aberrations (CNAs) - *i.e. IKZF1*, *PAX5*, *EBF1*, *BTG1*, *CDKN2A*, *RB1*, *CRLF2* deletions - increase with age progression (Messina et al, 2016). While the prognostic role of *IKZF1* deletion (Δ*IKZF1*) has been extensively studied, novel data on the potential role of other CNAs are currently emerging. With the number of novel prognostic markers increasing exponentially, ALL risk assessment is becoming complex; thus, it is crucial to provide a simplified, integrated predictor, possibly adjusted for age-cohort.
Aims: 1) To correlate the most common CNAs with the clinico-biological features of B-NEG ALL patients; 2) to assess their prognostic role; 3) to evaluate whether their impact on outcome varies across age-cohorts.
Methods: CNAs of the above mentioned genes were studied by Multiplex Ligation-dependent Probe Amplification (Salsa MLPA P335 ALL-IKZF1 kit, MRC-Holland) in 157 B-NEG ALL, including 56 adults, 56 adolescents/young adults (AYA) and 45 children. CNAs were correlated with age, gender, Hb levels, white blood cell (WBC) and platelets counts, and with RAS and JAK/STAT pathway mutations. The impact of CNAs on disease free survival (DFS) was analyzed.
Results: Overall, 46.6% of children, 82.4% of AYA and 87.5% of adults, carried at least 1 CNA. In particular, deletions of *IKZF1* were detected in 41.4%, of *CDKN2A* in 36.9%, of *PAX5* in 25.5%, of *ETV6* in 17.8%, of *EBF1* in 12.1% and of *BTG1* in 10.2% of patients, with *IKZF1*, *CDKN2A*, *PAX5* and *BTG1* deletions being significantly more frequent in adults and AYA than in children, as already mentioned. *EBF1* and *CDKN2A*-deleted patients had a significantly higher WBC count. *BTG1* deletions occurred exclusively in males (p=0.002). Furthermore, JAK/STAT mutations were significantly associated with *EBF1* (p=0.033) and *CDKN2A* deletions (p=0.026). Survival analyses on the whole cohort with clinical data available (N=133) showed that *CDKN2A*-deleted patients had a significantly worse DFS than *CDKN2A*-WT cases at 5 years (39.7% *vs* 64.1%, p=0.005); *EBF1*-deleted patients also had a significantly poorer DFS than *EBF1*-WT cases (33.3% *vs* 60.1%, p=0.003). A trend towards a worse DFS was observed for *BTG1*-deleted cases, with its negative impact being more evident when considering only AYA (30% *vs* 61.4%, p=0.095). *CDKN2A* and *EBF1* deletions retained their negative impact on DFS also when considering the adult cohort only. Indeed, *CDKN2A*-deleted had a shorter DFS than *CDKN2A*-WT cases (12.8% *vs* 54%, p=0.03); similarly, DFS of *EBF1*-deleted was inferior than *EBF1*-WT cases (0% *vs* 38.3, p=0.002); a trend was observed combining adults with AYA, while no impact was found in children. At variance, a trend towards a better DFS for *ETV6*-deleted *vs ETV6*-WT cases was observed (100% *vs* 65%, p=0.065) in children, recalling the favorable prognostic impact of *ETV6-RUNX1*. In the present cohort, the role of Δ*IKZF1* remains controversial: nevertheless, by combining adults and AYA, patients harboring Δ*IKZF1*-only or no CNAs had a trend towards a longer DFS than the remaining cases (62.6% *vs* 39.1%, p=0.068).
Summary/Conclusions: Besides the heterogeneous distribution, we also found a different impact on prognosis of CNAs across age cohorts, with *CDKN2A* and *EBF1* deletions influencing adult patients' outcome and *BTG1* negatively impacting on AYA survival; notably, these lesions did not impact on the outcome of childhood ALL. These findings indicate that, apart from the mutational status, risk assessment of ALL should also include the evaluation of selected CNAs.

E848

CALCINEURIN COMPLEX ISOLATED FROM T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) CELLS IDENTIFIES NEW SIGNALING PATHWAYS WHOSE INHIBITION SYNERGIZE WITH CALCINEURIN INHIBITION TO PROMOTE T-ALL CELL DEATH

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Background: Calcineurin (Cn) is a calcium activated protein phosphatase, composed of a catalytic subunit (PPP3CA) and a regulatory subunit (PPP3CB). It is critically involved in calcium signaling in diverse cells and is involved in many aspects of normal T cell physiology, however the role of Cn and/or its downstream targets in leukemogenesis are still ill-defined.
Aims: Available evidence shows that Nuclear Factor of Activated T cells (NFAT) transcription factors are mediators of Cn action in different cancers. However, it is possible that NFAT factors are not the only targets of Cn in leukemogenesis, as Cn can dephosphorylate other factors possibly relevant to its oncogenic properties. Aim of this study was to identify putative downstream targets/effectors involved in the pro-oncogenic activity of Cn in T-cell acute lymphoblastic leukemia (T-ALL), evaluate if the interactors are enriched in cellular signaling pathways relevant for T-ALL pathogenesis and if novel drug combinations can be proposed to enhance the efficacy of chemotherapeutic drugs and/or reduce the side effects of clinically relevant immunosuppressive drugs targeting Cn.
Methods: In order to identify putative downstream targets/effectors involved in the pro-oncogenic activity of Cn in T-ALL we used tandem affinity chromatography, followed by mass spectrometry (MS) to purify novel Cn-interacting partners. Novel interactions were validated by immunoprecipitation following transient overexpression in 293T cells or as native interactions in T-ALL cells. Drug synergisms using relevant drug combinations were evaluated through the execution of cell viability assays (bioluminescent method and annexin V-PI staining). Effects of Cn activity modulation on cellular signaling pathways found to be enriched in our MS list were evaluated by western blotting and flow cytometry.
Results: We identified numerous PPP3CA interactors, including proteins implicated in leukemia pathobiology such as NPM1, BCL11B, GSK3b and KDM1. The identified Cn-interacting proteins were found to be part of diverse cellular signaling pathways including eIF2 signaling, cell cycle control of chromosomal replication, mammalian target of Rapamycin (mTOR) signaling and 14-3-3 mediated signaling. Interestingly, modulation of Cn activity (by inhibitors and activators) lead to alterations in the identified signaling pathways including PI3K-AKT-mTOR signaling and eIF2 signaling. In addition, jointly targeting pathways enriched in our Cn complex (such as PI3K-mTOR signaling, cell cycle control of chromosomal replication mediated by topoisomerase II) together with Cn unveiled novel synergistic pro-apoptotic drug combinations.
Summary/Conclusions: The signaling pathways reported here will contribute to identify novel drug combinations which could enhance the efficacy of chemotherapeutic drugs and/or reduce the side effects of clinically relevant immunosuppressive drugs targeting Cn.

E849

OVEREXPRESSION OF PTP4A3 IN ETV6-RUNX1 ACUTE LEUKEMIA

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Background: Acute leukemia is the most common cancer in childhood. The ETV6-RUNX1 (E/R) fusion gene is present in 25% of childhood precursor B cell acute lymphoblastic leukemia (pB-ALL). E/R fusion is acquired *in utero* by a translocation between chromosomes 12 and 21 (t[12,21][p13;q22]). Translocation creates an aberrant fusion transcription factor which induces genome-wide repression of regulatory sequences and gene transcripts. The family of protein tyrosine phosphatase 4A (PTP4A) proteins are phosphatases with dual specificity. The family consists of three closely related members (PTP4A1-3) and they participate in a wide range of cellular activities, including cell proliferation, migration and invasion. Kinases and phosphatases have an intricate relationship in balancing intracellular signalling activity, and this balance is often deregulated in malignancies.
Aims: There are indications that members of the PTP4A family are involved in pathogenesis of various hematological malignancies. This motivated us to investigate the role of PTP4A3 in acute lymphoblastic leukemias by using gene expression data on patient samples, bioinformatic analyses and experimental manipulation of cell lines.
Methods: We investigated expression of members of PTP4A family across acute and chronic leukemias, cell lines and healthy cells of hematopoietic origin in a large collection of patient samples assayed by Affymetrix HU2.0 microarray and retrieved from GEO database. After normalization and manual curation, a two-dimensional "leukemia-map" was generated by t-SNE to visualize the data. We used experimental manipulation of cell lines to validate our findings from the "leukemia-map".
Results: PTP4A3 showed markedly increased expression among pre-B-ALL and decreased expression in acute myeloid leukemias, chronic leukemias and healthy cells. In further scrutiny, strong expression of PTP4A3 coincided with the E/R subtype of pB-ALL, showing approximately four-fold higher expression as compared to other pB-ALL leukemias (p=5.449805e-50). To validate the finding, two more patient data sets were retrieved to confirm the higher expression of PTP4A3 in the E/R subtype. In agreement, induction of E/R fusion in Nalm-6 cells increased the level of PTP4A3 mRNA. At primary transcript level, as assayed by global nuclear run-on sequencing (GRO-seq), the decrease of PTP4A3 expression after E/R knockdown was evident. Further, we identified a number of potential E/R binding sites upstream of PTP4A3 gene. Inhibition of PTP4A3 phosphatase activity in E/R positive REH cells was better tolerated as compared to other tested ALL cells.
Summary/Conclusions: We show that expression of PTP4A3 is markedly upregulated in the E/R subtype of acute pB-ALL. Our data hint that PTP4A3 expression is regulated by the E/R fusion itself, suggesting further studies on the surrounding regulatory elements. The potential role of PTP4A3 inhibitors will be further evaluated in combination with the known anti-leukemic drugs.

E850

DROPLET DIGITAL PCR ANALYSIS FOR DIAGNOSIS AND MINIMAL RESIDUAL DISEASE MONITORING IN ADULT PH+ ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: *BCR-ABL1* tyrosine kinase inhibitors (TKIs) are considered an important component of treatment for adult patients affected by Philadelphia-positive (Ph+) acute lymphoblastic leukemia (ALL). In fact, recent studies reported that treating Ph+ ALL with the combination of imatinib and multi-agent chemotherapy improved the overall outcome. Currently, no data are available on the impact of TKIs on minimal residual disease (MRD) in Ph+ ALL. In fact, although the real-time quantitative PCR (RQ-PCR) method, usually employed for monitoring the *BCR-ABL1* residual transcript, is sensitive and easy to perform, it lacks a full standardization and international quality validation.
Aims: Here, we describe a highly sensitive and reproducible droplet digital PCR (ddPCR) test to monitor *BCR-ABL1* transcript level in Ph+ ALL. The aim of the study is to demonstrate that ddPCR represents a sensitive and rapid method for both diagnosis and MRD monitoring of Ph+ ALL patients.
Methods: *BCR-ABL1* expression analysis by ddPCR was performed in twenty-two newly diagnosed adult Ph+ ALL patients. The diagnosis was confirmed by qualitative RT-PCR specific for the *BCR-ABL1* p190 fusion gene detection. ddPCR experiments were successfully performed in all twenty-two patients at the onset; several follow-up points were evaluated in thirteen patients. ddPCR experiments were performed using primers and probes specific for *BCR-ABL1* p190. *GUSB* was used as control gene. Fifty ng and 750 ng of cDNA templates were used for the onset and for the post-treatment samples, respectively. To increase the limit of detection (LOD), three replicates were run for the post-treatment samples. ddPCR experiments were performed by Bio-Rad's QX200 system and ddPCR data were analyzed with QuantaSoft analysis software (version 1.7.4). Target concentration was expressed as *BCR-ABL1* copies/mg.
Results: First, we defined the LOD of the *BCR-ABL1* p190 ddPCR system, a 10-fold dilution series (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of a pool of p190 positive patients using a diluent-pool of healthy volunteers. This analysis showed remarkable linearity, trueness, and precision down to 10^{-5}. After converting to log-log scale, linear regression showed no concentration-dependent bias, and R^2 equaled 0.996. Because the negative samples showed no background, even the detection of a single droplet per well was considered a positive result. The median concentration of the *BCR-ABL1* transcript at the onset was 233.8 (min 3.24-max 1744) x 10^3 *BCR-ABL1* copies/mg. Concerning the analysis of follow-up samples, among the thirty-four points that were negative to qualitative nested RT-PCR, twenty-three (68%) resulted to be positive by ddPCR analysis, with a median concentration of 44.95 (min 0.27-max 573.3) *BCR-ABL1* copies/mg. Follow-up points that were negative in ddPCR remained negative even when the experiments were repeated increasing the depth of the analysis, evaluating a total quantity of 4.5 micrograms of RNA.
Summary/Conclusions: This study indicates that, as compared to RQ-PCR, ddPCR increases the depth of the quantitative analysis of *BCR-ABL1* p190 fusion transcript by allowing the evaluation of larger amounts of RNA. Moreover, our preliminary data revealed that the amount of the *BCR-ABL1* fusion transcript at diagnosis is heterogeneous and that the ddPCR is much more sensitive than nested qualitative RT-PCR analysis, as the 68% of samples negative to nested PCR during the follow-up resulted to be positive by ddPCR. Therefore, we suggest that ddPCR represents a precise, sensitive and rapid method for both diagnosis and MRD monitoring of Ph+ ALL patients.

E851

ABNORMAL EXPRESSION OF THE CYSTEINE AND GLYCINE-RICH PROTEIN 2 GENE IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Cysteine and glycine-rich protein 2(CSRP2) is a member of the CSRP family of genes, encoding a group of LIM domain proteins, which may be involved in regulatory processes important for development and cellular differentiation. It has been reported that determination of CSRP2 protein level had a significant meaning for cardiovascular and respiratory diseases. In view of the important function of CSRP2 in cell growth and differention, CSRP2 may also have a role in carcinogenesis. However, the expression of CSRP2 has not been explored in leukemia, especially in B-cell acute lymphoblastic leukemia (ALL).
Aims: The purpose of this study was to investigate the expression of human CSRP2 messenger RNA in B-cell acute lymphoblastic leukemia by real-time fluorescent quantitative reverse transcription-polymerase chain reaction assay.
Methods: A real-time quantitative RT-PCR based on TaqMan fluorescence methodology was used to quantify the CSRP2 mRNA copy number in the bone marrow cells from patients with leukemia and in 11 human leukemic cell lines. Normal marrow samples from the allogeneic stem cell transplantation donors were served as control. Informed consent was obtained for every marrow sample.
Results: Expression levels of the CSRP2 gene in leukemic cell lines, leukemia patients and normal donor marrow are shown in Figure 1. These results showed that the relative levels of CSRP2 gene expression in marrow from the 212 newly diagnosed B-cell ALL was significantly higher than those of marrow from the 43 healthy donors(p<0.01). No statistical significant difference was observed in 18 newly diagnosed T-cell ALL, 73 AML and 43 healthy donors (p's >0.05), but it was higher in SupB15, BV-173 cells from B-cell ALL cell lines than in other cells from AML or T-cell ALL cell lines. Focusing on B-cell ALL patients, the median level of CSRP2 in 212 newly diagnosed patients was 42.13%(range 0-740.55%), while the median level in 290 treated patients who achieved CR decreased to 0.55%(range 0-21.21%). However, in 17 refractory patients and 22 relapsed patients, the median level was 60.83%(range 2.89-548.47%) and 43.17%(range 0.08-346.81%), respectively. In newly diagnosed B-cell ALL, 8 patients with MLL-AF4 translocation showed higher CSRP2 expression levels compared with 124 patients without this translocation(p<0.01). Figure1.Expression levels of CSRP2 in leukemic cell lines, leukemia patients and normal donor marrow. HD denotes healthy donors; De nove denotes newly diagnosed; CR denotes complete remission; Horizontal lines represent median CSRP2 expression for each group.* indicates p<0.05 compared with HD; & indicates p<0.05 compared with De novo B-cell ALL; # indicates p<0.05 compared with CR B-cell ALL.

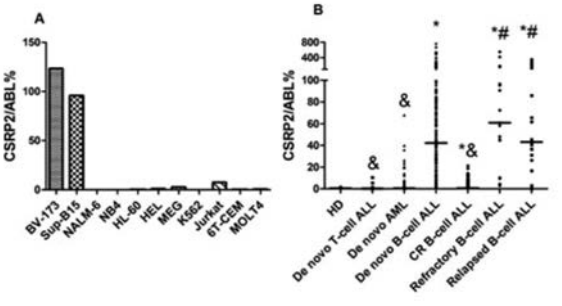

Figure 1.

Summary/Conclusions: These findings suggest that abnormal expression of CSRP2 in leukemia may be involved in the pathomechanism of B-cell ALL.

E852

IL7R OVEREXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): PROGNOSTIC IMPLICATIONS IN B-LINEAGE ALL WITHOUT RECURRENT FUSION TRANSCRIPTS

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Background: The IL7 receptor alpha chain (IL7R) heterodimerizes either with the IL2RG to form the IL7 receptor or with CRLF2 to form the receptor for the thymic stromal lymphopoietin (TSLP) both in T and B-cells. Signaling from these receptors activates the JAK/STAT pathway that is frequently altered both in T- and B-lineage acute lymphoblastic leukemia (ALL), due to mutations of its members. Among them, *IL7R* mutations are relatively common in adult B-ALL without recurrent transcripts (B-NEG) and in T-ALL. In a cohort of adult ALL previously studied by gene expression profiling (GEP) we observed that *IL7R* expression levels were variable, with a considerable number of B-NEG and T-ALL cases overexpressing it, thus suggesting that its deregulation may rely on different mechanisms.
Aims: To evaluate the clinical relevance of *IL7R* expression levels in adult B-NEG and T-ALL, we correlated the levels of *IL7R* expression with patients' outcome and analyzed the transcription profile of *IL7R*-high cases.
Methods: *IL7R* expression levels were inferred from previously performed GEP studies (Affymetrix) of adult ALL, including 78 B-NEG and 68 T-ALL. Martingale residual analysis was used to select the optimal *IL7R* cut-off, which was computed separately for these two groups. Differences in survival curves of *IL7R*-high and *IL7R*-low were calculated by the log-rank test. GEP of *IL7R*-high was analyzed and correlated with the molecular and immunophenotypic features. Screening of the JAK/STAT genes recurrently mutated was performed by Sanger in 66/78 B-NEG (*IL7R*, *CRLF2*, *JAK2)* and 38/68 T-ALL (*IL7R, JAK1/3, STAT5B*).
Results: *IL7R* expression levels were extremely heterogeneous both in B-NEG (mean 656.9, range 43.8-3000) and in T-ALL (mean 1047 range 58.9-5874). Martingale residual analysis provided a cut-off of 500 for B-NEG and 1000 for T-ALL. By using these cut-points, 47% of B-NEG and 41% of T-ALL cases were defined as *IL7R*-high. In B-NEG, correlation between *IL7R* expression levels and outcome showed that *IL7R*-high cases had a significantly shorter OS at 4 years than *IL7R*-low (31% *vs* 62%, p=0.04). Similarly, in T-ALL a trend towards a poorer OS was observed in *IL7R*-high patients than *IL7R*-low (27% *vs* 40%, p=0.2). The comparison of *IL7R*-high and *IL7R*-low by supervised GEP analysis identified a set of differentially expressed genes both in B-NEG (157 genes) and T-ALL (208 genes); only 11 transcripts overlapped. Indeed, functional annotation analysis of upregulated genes in *IL7R*-high highlighted an enrichment of JAK/STAT, Wnt and ErbB signaling members in B-NEG cases and of JAK/STAT and Notch pathway members in T-ALL, suggesting the involvement of different downstream cascades. As per the associations with other biological features, *IL7R* levels were significantly lower in *TAL1* cluster than in other subgroups (p=0.005). Notably, we observed a significant association between *IL7R* high expression and JAK/STAT pathway mutations both in B-NEG and T-ALL (p=0.001 and 0.03, respectively), thus indicating that *IL7R*-high might be a marker of an underlying JAK/STAT mutation.
Summary/Conclusions: Overall, our findings indicate that *IL7R* altered expression may represent a novel prognostic marker in B-NEG cases and, to a lesser extent, in T-ALL. Given the different GEP observed between *IL7R*-high B-NEG and *IL7R*-high T-ALL, it is intriguing to speculate that *IL7R* deregulation may activate different pathways. Furthermore, *IL7R* overexpression should prompt the investigation of JAK/STAT mutations both in B-NEG and T-ALL. However, given the overall incidence of JAK/STAT mutations it is likely that *IL7R* overexpression might be also sustained by other mechanisms that are currently under investigation.

Acute lymphoblastic leukemia - Clinical

E853

NEGATIVE PRE TRANSPLANT MINIMAL RESIDUAL DISEASE, ASSESSED BY FLOW CYTOMETRY, IMPROVES OUTCOME IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA, ESPECIALLY IF CLEARENCE IS ACHIEVED EARLY DURING INDUCTION

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Background: With the introduction of pediatric-inspired induction regimen, prognosis of adult acute lymphoblastic leukemia (ALL) has substantially improved, leading to an increased assessment of minimal residual disease (MRD) during all induction course and a progressive use of MRD-driven therapeutic approach. In most modern pediatric protocols, persistence of MRD during the first courses of induction therapy is an adverse prognostic factor and constitutes a strong indication for early allogeneic bone marrow transplantation (BMT). However, it remains unclear if an effort towards MRD eradication should be attempted before BMT, as relatively few data are available on outcome of MRD positive adult ALL patients.
Aims: The aim of this study was to analyze the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk and to evaluate the impact of day 33 MRD status in pre-BMT MRD negative patients.
Methods: We retrospectively analyzed the outcome of 53 consecutive ALL patients receiving allo-BMT, with available pre-BMT multicolour flow cytometry (MFC) MRD assessment. Median age at transplant was 30 years. Disease phase was CR1 in 20 (38%), CR2 in 17 (32%), and CR3 in 14 patients (30%). Thirty-five patients (66%) had B lineage ALL, whereas 18 (34%) had T-ALL. Relapse-free survival (RFS) was calculated from the time of transplantation until last follow-up or documented leukemic relapse. Overall Survival (OS) was calculated from the time of transplantation until death by any cause or last follow-up. Median follow up was 62 months.
A positive MFC MRD was defined by the presence of no less than 25 clustered leukemic cells/10^5 total events (threshold of$2.5x10^{-4}$ residual leukemic cells) at four-color flow-cytometry.
Results: Relapse occurred in 30 patients (57%). Three-year RFS was 42.2% (median 10 months). The probability of relapse was significantly affected by disease status at BMT (CR1 *vs* CR2 or p<0.001), karyotype (p<0.03) and MFC MRD (both day 33 and pre-BMT, p<0.03). Pre-BMT MFC-MRD was a strong predictor of longer relapse free survival: 5 relapses occurred in the 17 MFC-MRD negative patients and 3-years RFS was 69.4% (median not reached), compared to 25 relapses observed in 36 MFC MRD positive patients, with a 3-year RFS of 29.4% (median 5 months). Multivariate analysis revealed that MFC MRD and disease status at transplantation were significant predictors of RFS duration (p<0.001 and p<0.05, respectively). Eleven patients with pre-BMT MRD negative had available day +33 assessment. Two relapses were observed among the 3 day 33 MRD positive patients (66%), compared to three relapses among 8 day 33 MFC MRD negative patients (37%). MRD evaluation was a strong predictor of long term survival, as 3- years OS was 65% for MFC MRD negative compared to 49.8% for MFC MRD positive patients (p<0.05).
Summary/Conclusions: Pre transplant MFC MRD evaluation is a feasible and reliable predictor of relapse risk in adults ALL patients. Patients with positive MFC MRD have a very high relapse risk and a poor outcome after BMT. Although small numbers prevented us to drive conclusion, persistence of MRD at day 33 seems to confer a worse prognosis in pre-BMT MRD patients. In the era of targeted therapy, at least for B-ALL, persistence of MRD after induction could be an indication for innovative approach, such as attempts of MRD eradication using recently developed immunoconjugated monoclonal anti-CD-22 antibodies or anti-CD3/anti-CD-19 bispecific antibodies.

E854

MOLECULAR DETECTION OF ALL IN THE PERIPHERAL BLOOD IS MORE SENSITIVE THAN FLOW CYTOMETRIC ANALYSIS OF THE BONE MARROW IN PATIENTS WITH TREATMENT-RELATED HYPOCELLULARITY

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Background: Minimal residual disease detection (MRD) is an important tool for early detection and monitoring relapse. Traditionally, bone marrow is collected and analyzed by flow cytometry (FC) to evaluate MRD status in acute lymphoblastic leukemia patients (ALL). More recently, highly sensitive molecular methods such as high throughput sequencing (HTS) allow for more sensitive detection of MRD below 1 in 10^{-5}. Although the bone marrow is considered to be more representative of leukemic burden than sampling the blood, bone marrow collection is more invasive, traumatic, and costly than peripheral blood draws and therefore is performed only at specified and limited intervals. Paired bone marrow and blood samples from ALL patients can be assessed and compared for MRD at different time points in therapy.
Aims: In this study, we analyzed paired blood and bone marrow samples from the Adaptive Biotechnologies assay of IgH and TCRG in order to determine whether HTS performed on a peripheral blood sample is as informative as HTS and FC on a bone marrow sample in the assessment of MRD.
Methods: ALL patients enrolled in the Seattle Children's Pediatric Leukemia Adoptive Therapy (PLAT-02) trial undergo MRD assessment pre and post CD19 CAR T cell infusion by FC of the bone marrow. We have used high throughput sequencing (HTS) in paired blood and bone marrow samples from these patients. Samples taken prior to therapy evaluate the complete B cell repertoire and detect any dominant sequences which may be used for subsequent tracking. Samples taken at intervals during therapy assess the levels of MRD up to 63 days post-infusion.
Results: We have obtained 38 paired bone marrow and peripheral blood samples to date. We have observed a significant correlation in both the frequency of total reads and the number of malignant cells per million in paired peripheral blood and bone marrow samples by HTS. Of these, 26/38 (68%) of bone marrow samples were determined either aparticulate or hypocellular. Importantly, 8/14 (57%) of patients who were MRD negative in the bone marrow by FC were MRD positive in the blood by HTS. While it may only be true of patients receiving this particular form of treatment, there were some samples assayed by immunosequencing in which peripheral blood was a more sensitive measure of tumor burden than simultaneously acquired bone marrow. These data suggest, that, in cases where the bone marrow is hypocellular or aparticulate, peripheral blood samples evaluated by HTS are more sensitive than traditional flow cytometric analysis in the bone marrow for detection of MRD.
Summary/Conclusions: The impact of B cell aplasia may be important in increasing the sensitivity of HTS in the detection of MRD. The ability to detect an ALL B cell clone in the blood through HTS has many clinical implications; blood can be sampled more often, is less of a burden to patients, and can provide earlier prediction of relapse.

E855

DE-ESCALATION OF INTENSITY DID NOT AFFECT THE LONG-TERM OUTCOME NEITHER IN T-ALL NOR IN BCP-ALL IF NON-INTERRUPTIVE TREATMENT IS APPLIED: RESULTS OF THE RUSSIAN ALL STUDY GROUP

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Background: More aggressive pediatric-like protocols and high portion of allogeneic HSCT are now a standard approach to treatment in adult acute lymphoblastic leukemia (ALL). Here we report the results of the adult (15-55 yy) Ph-negative ALL protocol based on the different approach: de-escalated but non-interruptive treatment with low numbers of allo-HSCT. The study is registered on the ClinicalTrials.gov public site; NCT01193933.
Aims: To assess non-intensive and non-interruptive approach to ALL treatment.
Methods: The ALL-2009 is based on: (1) the replacement of prednisolone (Pdn) 60mg/m^2 with dexamethazone (Dexa) 10mg/m^2 if blast cells are ≥25% in b/m after prephase (7d); (2) de-intensified but non-interruptive 5 months induction/consolidation treatment (5 wks Pdn/Dexa with 3 instead of 4 Dauno/Vncr pulses, 4 weeks of 6MP with 5 L-asp, 2 instead of 4 ARA-C blocks, 1 instead of 2 Cph injections during induction; induction-like 3 consolidations for 3wks, 2wks, 4wks –continuously without intervals), followed by (3) 2 late (at 6 mo) intensifications– with 1 day HD MTX (1,5 g/m^2)and with 1 d HD ARA-C (2 g/m^2), both with L-asp and 3 d dexa and (4) 2-yrs continuous 6MP/MTX maintenance with doses modification according to myelosuppression with monthly 3-days dexa/vncr/L-asp pulses (∑ L-asp=590.000IU/m^2). The protocol was identical for all risk groups. For T-ALL patients auto-HSCT after non-myeloablative BEAM conditioning followed by the protocol maintenance was an option as late consolidation. Allo-HSCT was indicated only for extremely high-risk BCP-ALL (t(4;11), L>100). No central MRD monitoring was performed. Since Apr 2009 till Feb 2016, 298 Ph-negative ALL pts with a median age 28 years (15-55), f/m 137/161 were recruited. In 6 pts phenotype was unknown, B-lineage ALL was diagnosed in - 62,4% (n=182), T-lineage ALL-36,6% (n=107), biphenotypic -1% (n=3). In BCP-ALL 31 patients (n=17%) were not qualified by the risk in the data-base; 72,8% (n=110) were in the high risk (HR) group (WBC ≥30; EGIL BI, LDH >2N; late CR; t(4;11)-positive). In T-ALL 7 patients (n=8%) were not qualified by the risk in the data-base; 76% (n=76) were in the high risk (HR) group (WBC ≥100; EGIL TI/II,TIV, LDH >2N; late CR). The analysis was performed in february 2016.
Results: CR rate in 284 available for analysis pts was 86,7% (n=246), induction death occurred in 9,1% (n=26), resistance was registered in 4,2% (n=12). Late

responders constituted 13,1% (n=20) in BCP-ALL and 28,9% (n=24) in T-ALL. In 89 CR T-ALL pts 35 auto-HSCT were performed at a median of 5,8 mo of CR. Allogeneic BMT was performed only in 18 of 256 patients who survived induction (7,0%). Totally 49 pts (19,9%) had relapsed. At 60 mo OS for the whole cohort constituted-58,9% (BCP-ALL -53,3%, T-ALL-67,5%), DFS-64.9% (BCP-ALL-56,2%, T-ALL-79,5%), relapse probability (RP)-25% (BCP-ALL - 30,6%, T-ALL-15,8%). In a univariate analysis among various risk factors (age< ≥30y, initial risk group, WBC, LDH, immunophenotype, late response >35d) age (≥30 y) for BCP-ALL became statistically significant for OS and RP, WBC for DFS and RP, risk group for DFS, LDH for RP, t(4;11) for OS, DFS and RP. In T-ALL in a univariate analysis only LDH was significant for OS. In a multivariate analysis no common risk factors were significant non in T-ALL, nor in BCP-ALL.
Summary/Conclusions: Our data demonstrate that the de-escalation of intensity but constant non-interruptive treatment is rather effective in adult ALL producing more than 50% OS in BCP-ALL and 65% in T-ALL. We believe that this approach, without intensive highly myelosuppressive consolidation courses and high portion of allogeneic HSCT may become an alternative and reproducible approach in adult Ph-negative ALL, though we have to stress that the compliance of the pts to the protocol should be very strict.

E856

SERIAL MONITORING OF BCR-ABL BY REAL-TIME POLYMERASE CHAIN REACTION IN NEWLY DIAGNOSED PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH IMATINIB

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Background: The positive impact of imatinib on treatment outcome in patients with Philadelphia Chromosome-Positive (Ph+) acute lymphoblastic leukemia (ALL) is well known, and the kinetics of the BCR-ABL transcript correlated with the patient's clinical course.
Aims: The aim of this study was to evaluate the relationship between imatinib dose intensity and molecular response of BCR-ABL.
Methods: Imatinib (600 mg/day orally) was administered continuously with combination chemotherapy, starting from eighth day of remission induction treatment, then through 5 courses of consolidation or until allogeneic hematopoietic cell transplantation (HCT). Patients who were not transplanted were maintained on imatinib for 2 years. Molecular response monitoring was performed at the central lab with quantitative RT-PCR assays for peripheral blood or bone marrow BCR-ABL RNA in serial; at the time of diagnosis, at hematologic complete remission (HCR), and every 3 months thereafter. The molecular response was defined as complete (MCR) if the *BCR-ABL/G6PDH* ratio was less than $1x10^{-5}$.

Table 1.

Risk Factor	Molecular relapse-free survival		
	P	HR	95% CI
Using G-CSF during induction treatment			
No	0.034	0.376	0.152-0.944
Yes		1	
Dose intensity of imatinib			
Initial dose intensity within 7 weeks			
≥90%	0.017	0.405	0.192-0.853
<90%		1	
Total dose intensity during entire study period			
≥80%	0.902	1.070	0.361-3.173
<80%		1	
Early complete molecular response within 3 months after remission induction			
Yes	0.001	0.251	0.111-0.570
No		1	
Underwent allogeneic stem cell transplantation			
Yes	0.002	0.284	0.130-0.621
No		1	

Results: Between October 2005 and February 2009, total 87 patients, aged 16-71 years, with newly diagnosed Ph+ALL were enrolled. With median follow-up of 5 years among survivors (range: 2.6-8.9 years). At the time of diagnosis, the BCR-ABL transcript amount showed no correlation with WBC count and peripheral blast percent. Eighty-two patients (94%) achieved HCR at a median 25 days (range, 14-69 days) and 45 patients (57.7%) of 78 evaluable patients achieved MCR at the same time. Within three months after induction treatment, 59 patients (67.8%) achieved molecular complete remission. Total MCR rate was 88.5% during the entire study period and the median time from treatment to MCR was 54 days (range, 13-384 days). Among these 77 MCR patients, 32 experienced molecular recurrence which was defined as BCR-ABL transcript positive conversion from $1x10^5$. On subsequent follow-up, regardless of allogeneic HCT, 24 additional patients achieved MCR at 3 to 9 months. Of 54 patients who underwent allogeneic HCT while in first CR, with quantitative PCR being performed at a median 2.1 months (range, 0.1-4.0 months) prior to allogeneic HCT, 43 (80%) showed BCR-ABL transcript amount below $1x10^5$. After allogeneic HCT, 49 of 50 evaluated patients (98%) achieved MCR. Median time of MCR duration was 13 months (range, 0.9-60.3 months) and median molecular relapse-free survival was 28 months. Patients with loss of MCR at any time had a higher cumulative incidence of leukemia relapse and a lower relapse-free survival (RFS). Thirty two patients who lost of MCR had significantly inferior RFS (P<0.0001) and OS (P=0.001) then 41 who maintained MCR. MCR achievement within 3 months after remission induction was significant predictor of RFS (P=0.004) and OS (P=0.003). In univariate analyses, the duration of MCR was affected by using G-CSF during remission induction (P=0.0046), initial dose intensity of imatinib within 7 weeks (P=0.001), ≥90% *vs*<90%, total imatinib dose intensity during whole study period, and allogeneic HCT (P=0.002). Multivariate analyses after adjusting the Cox model was described in Table 1.
Summary/Conclusions: Prospective assessment of the extent of molecular response and imatinib dose intensity during imatinib-based treatment and post-allogenic HCT is likely to be useful in identifying subgroups of Ph+ALL patients at a high risk of relapse and we will be able to apply the risk-adapted or minimal residual disease based therapeutic approaches for Ph+ALL patients.

E857

KPT-8602 IS A SECOND-GENERATION XPO1 INHIBITOR WITH IMPROVED IN VIVO TOLERABILITY AND POTENT IN VIVO ACTIVITY AGAINST ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Human exportin-1 (XPO1) is a transport protein that exports a wide variety of different cargo proteins from the nucleus to the cytoplasm. Selective Inhibitor of Nuclear Export (SINE^{TM}) compounds block the formation of the XPO1-cargo complex leading to accumulation of tumor suppressors in the nucleus and show potent anti-cancer activity. Selinexor (KPT-330) is currently in Phase-II/IIb clinical trials for treatment of both hematologic and solid tumors, while KPT-8602 is in Phase 1/2 clinical trials for treatment of relapsed refractory multiple myeloma.
Aims: We investigated the *in vitro* and *in vivo* activity of KPT-8602, a second-generation clinical stage XPO1 inhibitor with improved tolerability, against acute lymphoblastic leukemia (ALL).
Methods: We performed co-localization experiments and a phenotypic reporter assay to evaluate the anti-XPO1 activity of KPT-8602. We cultured 5 T-ALL and 3 B-ALL cell lines with different concentrations of the drug and assessed cell viability after 72h exposure. CRISPR/Cas genome editing was used to mutate XPO1 in ALL cell lines. For the *in vivo* experiments, NSG mice were injected with a T-ALL patient derived xenograft (PDX) sample, and animals were treated with KPT-8602 10mg/kg or placebo once a day by oral gavage for 3 weeks. Mice were bled weekly to measure the total white blood cell count and number of human leukemic cells.
Results: KPT-8602 strongly inhibited XPO1-cargo interaction as well as XPO1-dependent nuclear export at nanomolar concentrations and induced potent cytotoxicity on T-ALL and B-ALL cell lines *in vitro* (EC_{50} values ranging from 25 to 140 nM). To determine the drug-target specificity, we used CRISPR/Cas mediated genome editing to introduce XPO1 mutations that prevented KPT-8602 binding and resulted in loss of inhibitory activity, indicating that the inhibitory effects of KPT-8602 are completely dependent on XPO1 inhibition. Daily oral treatment with KPT-8602 could block leukemic expansion in mice engrafted with a JAK3 mutant T-ALL at an early stage of disease development. Also when KPT-8602 treatment was initiated at day 28 after injection (when more than 5% of human leukemic cells were detected in the blood), we observed a significant reduction of leukemia cell numbers, without affecting normal erythropoiesis. KPT-8602 treatment during 3 weeks led to prolonged survival of the animals, compared to placebo treated animals (p=0.0041).
Summary/Conclusions: KPT-8602 is a highly specific second-generation XPO1 inhibitor with potent anti-ALL activity both *in vitro* and *in vivo*. It displays better tolerability compared to selinexor, which allows for daily dosing and warrants further evaluation of this new drug in patients.

E858

DETAILED CHARACTERIZATION OF LAIP PROFILE AND HETEROGENEITY AT TIME OF DIAGNOSIS PREDICTS MINIMAL RESIDUAL DISEASE IN B-CELL PRECURSOR ALL

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Background: Early and accurate risk stratification in B-cell precursor acute

lymphoblastic leukemia (BCP-ALL) is essential for individualized treatment strategies in order to improve prognosis and lower treatment associated toxicity. Leukemia-associated immunophenotype (LAIP) heterogeneity is common at time of diagnosis (Øbro et al, Leukemia, 2011) yet current immunophenotyping methods do not include heterogeneity. Furthermore, studies of the LAIP prognostic value have not shown consistent results.

Aims: To characterize in detail the LAIP and the frequency of immunophenotypically distinct subpopulations by flow cytometry at time of diagnosis and to evaluate their prognostic value for end-of-induction minimal residual disease (MRD) in BCP-ALL.

Methods: Flow cytometry data from time of diagnosis was re-evaluated retrospectively in 125 patients with BCP-ALL consecutively diagnosed between October 2011 and June 2015 and treated according to the NOPHO ALL 2008 protocol. The expression of intracellular and surface B-lineage markers (CD45, CD19, CD34, CD38, CD10, CD20, CD22, nTDT, cyCD79a, and cyCD22) and cross-lineage expressed markers (CD123, CD66c, CD133, CD13, CD33, and CD15) was scored as negative, positive dim, positive (normal) or positive bright, and broad or bimodal expression was registered. Corresponding normal populations in unaffected bone marrow samples were used as reference (methodological details will be presented). Groups were compared by the Mann-Whitney U test. The prognostic potential of LAIP expression was assessed in a multiple regression model with end-of-induction flow-MRD as outcome adjusting for age, WBC at diagnosis, cytogenetic group (high risk comprised hypodiploid, MLL, and iAMP21, non-high risk comprised t(12;21), hyperdiploid, t(1;19), dic(9;20), and no cytogenetic aberration) and NOPHO diagnostic treatment regime (high/standard risk).

Results: Distinct immunophenotypic subpopulations defined by bimodal expression of one or more markers were observed in 66 patients (53%), with nine patients (7%) having three or more subpopulations. The most frequently bimodal marker was CD34, displaying bimodality in 37 patients (30%). In patients with a homogeneous expression of CD34 we found higher end-of-induction flow-MRD in patients with CD34 positive blasts compared with CD34 negative (p=0.0008, figure 1). Accordingly, in patients with bimodal expression of CD34 we assessed the expression level of the dominant blast subpopulation and found that patients with a CD34 positive dominant blast subpopulation displayed higher end-of-induction MRD (p=0.0095, figure 1). When including all patients (CD34 homogeneous negative/dim/normal/bright and heterogeneous predominantly negative/positive) in an adjusted multiple regression model, we confirmed the prognostic value of CD34 score on MRD (p=0.0003). Since risk stratification of patients without identified cytogenetic aberrations is more difficult, we analyzed this subgroup separately, and found that CD34 expression is an independent predictor of high MRD (p=0.0074, multiple regression, n=37) in this group as well.

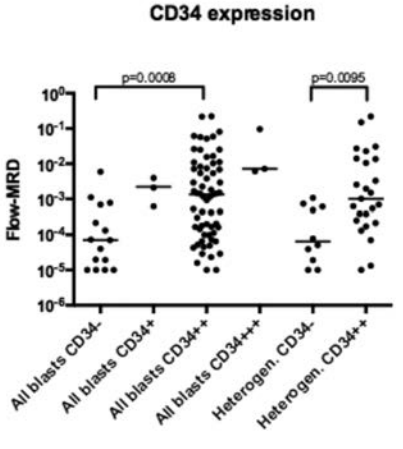

Figure 1. Flow-MRD in patients according to CD34 expression. Lines represent median values. -: negative, +: positive dim, ++: positive (normal), +++: positive bright.

Summary/Conclusions: Detailed characterization of the CD34 expression level of blasts and immunophenotypically distinct subpopulations seems to bring valuable information on BCP-ALL prognosis regardless of age, WBC and cytogenetic findings. Detailed and standardized LAIP characterization is a readily accessible prognostic tool and could potentially be taken into consideration in future risk evaluations of BCP-ALL patients.

E859

PHASE I STUDY OF MOXETUMOMAB PASUDOTOX IN ADULT PATIENTS WITH RELAPSED OR REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Moxetumomab pasudotox (MP), previously known as HA22 or CAT-8015, is a recombinant immunotoxin composed of the Fv fragment of a humanized IgG4 anti-CD22 monoclonal antibody fused to a 38-kDa truncated fragment of Pseudomonas exotoxin A. CD22 is expressed on the surface of blasts in over 90% of patients with precursor B-acute lymphoblastic leukemia (ALL) as well as in other lymphoid neoplasms and is an attractive target for monoclonal antibody-based therapy. MP and its predecessor compound, BL22 have been investigated for the treatment of patients with relapsed hairy cell leukemia (HCL) as well pediatric patients with relapsed B-ALL and have demonstrated promising activity.

Aims: This single center, phase I study was conducted to determine the maximum tolerated dose and potential efficacy of MP in adult patients with relapsed and/or refractory B-ALL.

Methods: Patients were eligible if they were 18 years or older, with previously treated B-ALL (either relapsed after having achieved prior response or refractory to prior therapy) and had adequate performance status and organ function with bilirubin ≤1.5mg/dL, liver enzymes ≤2 x upper limit of normal (ULN) and creatinine ≤2mg/dL. Patients with cardiac ejection fraction less than 40%, active hepatitis or HIV infection, history of microangiopathic hemolysis, symptomatic CNS involvement, recent prior therapy (including systemic chemotherapy in the past 14 days, monoclonal antibodies in the past month, or allogeneic stem cell transplant in the past 100 days) were excluded. Dose escalation in the phase I portion of the study was based on a standard 3+3 design starting at a dose of MP 30μg/kg every other day (qod) for 6 doses with subsequent dose levels at 40 and 50μg/kg qod for 6 doses and 50μg/kg qod for 10 doses. All patients received appropriate premedication with hydroxyzine, ranitidine, corticosteroids and acetaminophen and were monitored for toxicity including previously identified risks of capillary leak syndrome and hemolytic uremic syndrome (HUS). All other supportive managements was based on standard institutional practice.

Results: Between December 2013 and September 2015, 16 patients with relapsed or refractory B-ALL were treated on this trial. Median age of the patients was 30 years (range, 18-67 years). Median number of prior therapies was 3 (range, 1-6). 4 patients had a prior allogeneic stem cell transplant and 3 had prior therapy with inotuzumab ozogamycin. 10 patients were refractory to their last prior therapy and 6 had relapsed from prior therapy (duration of prior CR less than 6 months in 2 and greater than 6 months in 4). Performance status was 0 in 2, 1 in 10 and 2 in 4 patients. Four patients had normal karyotype and 10 complex cytogenetic abnormalities which included t(4;11) in 1 patient. Another patient had t(1;19) and one patient had miscellaneous abnormality. CD22 expression was detected in all patients including 50-69% expression in 4, 70-89% expression in 4, and ≥90% expression in 8. The median number of courses of MP administered was 1 (range, 1-4) with 6 patients treated at 30 μg/kg, 4 patients at 40 μg/kg, and 6 patients at 50 μg/kg x 6 doses. The final dose regimen of 100 μg/kg x 10 doses was not studied due to early termination of the study. Only one DLT (grade 3 HUS) occurred at 50 μg/kg x 6 dose level; however, the study was terminated because the 10-dose schedule was not tolerated in a parallel pediatric trial. One patient with 5 prior regimens achieved a complete response (CR) which lasted 2.5 months. No other responses by standard criteria were reported. Non-hematological toxicity of grade 3/4 included reversible capillary leak syndrome (CLS) in 1 patient, weight gain in 2, ascites in 1, tumor lysis syndrome (TLS) in 1, elevation of liver enzymes in 3, and HUS in 1 patient. Grade 2 adverse events including CLS, edema and elevation of liver enzymes were noted in another 5 patients. No deaths directly attributable to therapy were reported.

Summary/Conclusions: MP at the doses studied in this trial is feasible and can produce response in patients with multiply relapsed B-ALL. The recommended dose for phase II trials is 50μg/kg qod x 6 doses.

E860

INTENSIVE VS. SEMI-INTENSIVE CHEMOTHERAPY IN OLDER ADULTS (55-65 YR) WITH PHILADELPHIA CHROMOSOME-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Treatment of older adults (55-65 yr.) with ALL is difficult to standardize. The election between intensive *vs* semi-intensive therapy is frequently based on physician and/or patient preferences. Specific analyses of outcomes according to the intensity of the therapy in this age group are scarce.
Aims: To analyze and compare the baseline characteristics, the results of treatment and the outcomes of older adults (55-65yr.) with Ph-negative ALL included in intensive high-risk (HR) protocols (ALL03 and ALL11, J Clin Oncol 2014; 32:1595-604, Blood 2015; 126: 1333) *vs* semi-intensive protocols (ALLOLD07, Leuk Res 2016; 41: 12-20) from the Spanish PETHEMA Group.
Methods: The main clinical and hematologic data, as well as the response to therapy and outcome of older adults (55-65 yr.) with Ph-negative ALL treated intensively or semi-intensively according to the PETHEMA protocols were analyzed and compared. Intensive protocols (ALL03 and ALL11) included intensive induction and consolidation therapy followed by allogeneic HSCT according to MRD clearance. Semi-intensive protocol (ALLOLD07) includes non-intensive induction chemotherapy with non-genotoxic drugs, followed by semi-intensive consolidation and standard maintenance therapy (EWALL backbone); allogeneic HSCT with RIC regimen was allowed in fit patients in CR1 according to physician's criteria.
Results: From 2003 to 2015, 46 patients were treated intensively *vs* 32 treated semi-intensively. Except for age (intensive: 58[55-65] yr. *vs* semi-intenive: 61 [56-65], p=0.01), the main clinical and hematological characteristics at baseline were comparable in the two groups. Death in induction and relapse rate were higher in the semi-intensive group, with a trend for a lower CR duration in this group. However, the OS probability was not significantly different in the two groups (Table 1). The rate of HSCT realization in CR1 was low in both groups (8% *vs* 5%).

Table 1.

	LAL AR03/11 (n=46)	LAL OLD07 (n=32)	P value
Death in induction	0	4/31* (13%)	0.023
Failure	6/46 (13%)	7/31* (23%)	0.210
CR	40/46 (87%)	20/31* (64%)	
Treatment-related mortality in CR	9/40 (23%)	2/20 (10%)	0.307
Relapse	17/40 (43%)	15/20 (75%)	0.017
Median CR duration, (95% CI)	24.6 (19.6; 29.7)	9.6 (4.6; 14.6)	0.085
Median overall survival (months), (95% CI)	13.2 (0; 26.5)	9.3 (5.5; 13.2)	0.279

Summary/Conclusions: This study confirms the poor prognosis of older adults (55-65 yr.) with Ph-negative ALL, regardless of the intensity of the therapy. The low rate of HSCT in CR1 in this age group and a high rate of death in consolidation in patients treated intensively are of note. The trend for a significantly better CR duration in patients treated intensively did not translate into improved OS. Better therapies are needed in this age group of Ph-negative ALL patients.
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E861

FLOW CYTOMETRY OF CEREBROSPINAL FLUID IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AT DIAGNOSIS AND RELAPSE

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Background: Acute lymphoblastic leukemia (ALL) may involve central nervous system (CNS) in 3-6% of pediatric patients. Cytology (CC) of cerebrospinal fluid (CSF) is currently used to define CNS infiltration, although sensitivity and specificity are low. Flow cytometry (FC) can identify blasts in CSF samples that are negative for cytology with higher sensitivity and specificity. The frequency and the significance of this occult cerebrospinal involvement in children with ALL is still not clear.
Aims: We explored the feasibility of CSF flow cytometric analysis at each lumbar puncture during therapy in primary and relapsed ALL. Moreover, we studied prospectively its clinical significance in comparison with cytology and cell count.
Methods: We included children (aged 1-18 years) with Philadelphia negative ALL and with ALL isolated marrow relapse diagnosed from 12.09.2013 to 31.01.2016 at our Institution, after informed consent acquisition. Treatment schedule and definition of CSF involvement were as per AIEOP-BFM ALL2009 Protocol. At each point of intrathecal therapy, CSF was collected and analyzed within 24 hours by an automated cell counter, by cytology and 8-color flow cytometry (B or T lineage panel). A tiny cluster of events with immunophenotype compatible with blasts at diagnosis was considered positive by FC (FC+).
Results: 784 samples from 76 patients with *de novo* ALL and 92 samples from 13 children with marrow relapse were considered. Median follow up was 14 months (1-28,5 months). At diagnosis 4 T-ALL patients (5%) presented CNS involvement (CNS3): 3 with positive cytology (CC+) and positive FC, 1 with cranial nerve palsy (CC-/FC-). Other 3 patients (pB-ALL) had positive cytology but<5 cell/µl (CNS2): 2 were FC+, 1 FC- (normal T and B cells). 25/76 children (33%) with no CNS leukemia (CNS1) resulted FC+: 3 T-ALL, 22 pB-ALL. 9/25 (36%) CNS1/FC+ patients were high risk (MRD based stratification). FC persisted positive at day 15 in 2 CNS3/FC+ patients, in 1 CNS2/FC+ and in 2 patients that were positive only by FC at diagnosis. Among patients who were FC+ at diagnosis, we found other isolated FC+ samples at subsequent time points: 2 during induction, 4 during consolidation. Moreover, during reinduction a child CNS3/FC+ at diagnosis presented other 3 samples FC+/CC-. Even children who had negative CSF by flow cytometry at diagnosis presented isolated FC+ samples at later time points: 1 during induction, 6 during consolidation. One child (CNS1/FC- at diagnosis) died for infection, one patient (CNS1/FC+ at diagnosis) presented early marrow relapse, the remaining 74 are alive in complete remission (4/74 after hematopoietic stem cell transplantation). Among 13 patients with isolated marrow ALL relapse, 7 (54%) had FC+ CSF at presentation. 4/7 had a second relapse (2 CNS relapse, 2 BM relapse), 3 died. Among 6 FC- patients, 1 had a second marrow relapse, 1 died of transplant related mortality, 1 showed disease progression and 3 are alive and in complete remission.
Summary/Conclusions: Flow Cytometry of CSF is a simple, rapid and cheap method that is able to identify blasts with high sensibility and specificity, allowing the detection of occult CNS infiltration. Our preliminary data suggest that FC CSF positivity may be a risk factor for BM and CNS relapse in children. A longer follow up and a larger group of patients are needed to confirm these findings in ALL at diagnosis and relapse.

E862

USE OF VOXEL-BASED MORPHOMETRY TO DETECT CEREBRAL VOLUMES ABNORMALITIES IN LONG TERM SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Improvements in therapeutic strategies of pediatric Acute Lymphoblastic Leukemia(ALL) over the last decades have highly increased survival rates; consequently, there is a growing population of ALL Long Term Survivors(ALL-LTS). Treatment protocols include Central Nervous System directed Chemotherapy(CT) associated, in selected patients, to Cranial Irradiation(CI). These treatments appear able to determine neurocognitive late effects in ALL-LTS. The anatomical and molecular basis of these alterations are still largely unknown, and neuroimaging studies, such as automated whole brain segmentation of MRI images through Voxel-Based Morphometry(VBM), may be helpful in their understanding. Aim of this study was to better define neuroanatomic regions involved in neurocognitive impairment of a population of ALL-LTS, and to evaluate possible relationships of these abnormalities with type of therapy.
Aims: The primary objective was to detect the neuroanatomical alterations in ALL-LTS who received either CT alone or in combination with CI during childhood. Secondary objective was to assess the neurocognitive performance in the same two groups of LTS.
Methods: In this cross-sectional, controlled study, twentysix ALL-LTS were recruited from the ALL-LTS cohort of patients followed up in the Hemato-Oncology Department of Santobono-Pausilipon Children's Hospital of Naples. Thirteen subjects received CT and CI (group A) while thirteen received CT alone (group B). All signed written informed consent. The two groups were matched for age, sex, ethnicity, education, number of right-handed and left-handed sub-

jects, use of glasses, age at ALL diagnosis, years since therapy-discontinuation and type of therapeutic protocol used. People who underwent HSCT and relapsed subjects were excluded. Imaging were collected using a 3T Siemens Scanner. VBM analyses were conducted on MPRAGE T1 3D images in Statistical Parametric Mapping 8. Analyses of brain volumes were performed as global Gray Matter(GM), White Matter(WM) and Cerebro Spinal Fluid(CSF) volumes and as regional voxel-by-voxel analyses. Neurocognitive performance was assessed through the Wechsler Adult Intelligence Scale-Revised(WAIS-R) for over 16 years and through the Wechsler Intelligence Scale for Children 4th Edition(WISC-4th) for age 9-16 years. d2-R test and Wisconsin Card Sorting Test(WCST) were used to assess respectively attention and concentration abilities and executive function. A two sample t-test was employed for all the between group comparisons.

Results: Absolute volumes of GM, WM and CSF segments of group A *vs* group B did not differ significantly. Group A showed significantly smaller volumes than group B in superior frontal gyrus, posterior medial frontal gyrus, paracentral lobule, inferior parietal lobule, precuneus, temporal medial gyrus, parahippocampal gyrus, medial and posterior cingulate cortex. Significant differences between groups were found for intelligence, performance, attention and memory measures of WAIS-R. No significant differences were observed for WISC-4th, d2-R and WCST, but in both groups, working memory, processing speed, concentration and attention were worse than population norm.

Summary/Conclusions: In our study some neuroanatomic structures showed smaller volumes in ALL-LTS treated with CT in combination with CI than in those treated with CT alone. These findings could be related to specific neurocognitive alterations observed in these subjects, and represent an initial attempt in elucidating their anatomical basis. Further studies on more extensive pediatric series are needed to confirm our observations.

E863

HYPER-CVAD COMPARED TO BFM-LIKE CHEMOTHERAPY FOR THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA. A RETROSPECTIVE SINGLE CENTER ANALYSIS

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Background: Multiple induction regimens have been developed for adult patients with acute lymphoblastic leukemia (ALL). However, there have been no prospective randomized trials that directly compare these regimens.

Aims: In this report, we retrospectively evaluated the outcome of 62 adult ALL patients treated with hyper-CVAD (n=38) and the (*i.e.* Berlin-Frankfurt-Munster, n=24) BFM-like protocols at the American university of Beirut medical center, Lebanon; between November 2000 and december 2015. Feasibility of allogeneic stem cell transplantation (allo-SCT) for those patients, in terms of efficacy and tolerability was also evaluated.

Methods: The median age was 38 years in the hyper-CVAD group and 20 years in the BFM-like group with a male/female ratio of 50/50 and 54/46, respectively. The majority of cases were B cell in origin (89% in the hyper-CVAD group and 79% in the BFM-like group). Thirteen patients (34%) *vs* only 2 patients (8%) were positive for Philadelphia chromosome (Ph+) in both groups respectively, (p= 0.0744). Eight patients received imatinib (7 *vs* 1) and five patients received dasatinib (4 vs1) in the first and second group respectively. Other 2 patients received imatinib than dasatinib in the hyper-CVAD group only.The disease risk score was high in 76% of patients in the hyper-CVAD group *vs* 33% of patients the BFM-like group (p=0.119).

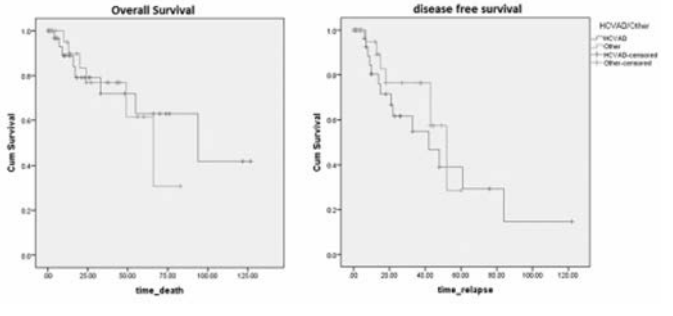

Figure 1.

Results: The median follow-up time was 29 months. Fifteen patients (39%) and ten patients (42%) underwent allo-SCT in the hyper-CVAD and BFM-like group respectively. At the last follow up 28 patients (74%) were in complete remission (CR) in the first group *vs* 18 patients (75%) in the second group. Of those, 20 patients (53%) *vs* 11 patients (46%) were MRD-negative at the last follow up respectively. The 5-year survival rate was similar in hyper-CVAD group compared to the BFM-like group (63 *vs* 62%, p= 0.808) (Figure 1). The 5 years disease free survival (DFS) rate was 29% compared to 28% in the BFM-like group, (p= 0.435) (Figure 2). Both chemotherapies were well tolerated. None of the patients died from drug related toxicity.

Summary/Conclusions: Despite the older age and more patients with high risk category and Ph+ in the hyper-CVAD group, this difference was not translated into a difference in outcome between the 2 groups. The hyper-CVAD regimen seems to be feasible for adult patients with ALL in terms of tolerability and efficacy. There is still a need for large, prospective, randomized studies in order to establish the best chemotherapy protocols for adult ALL patients.

E864

BFM REGIMEN OFFERS SIMILAR EVENT FREE SURVIVAL COMPARED TO HYPERCVAD BUT WITH LESS TOXICITY IN ADOLESCENTS AND YOUNG PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The experience to adapt pediatric protocols to adolescents and young adults (AYA) with acute lymphoblastic leukemia (ALL) is improving. Although there is still concern that toxicity may occur.

Aims: We present two groups of AYA treated with an adult protocol (hypercvad) and BFM developed for children. The study aimed to compare event free survival (EFS) and toxicity in both regimens.

Methods: Patients aged ≥16 and ≤40 between 2008-2015. BFM consisted of 8 blocks. Induction with vcr, pdn, asp, daunorubicine (dau) and intrathecal therapy (IT). Consolidation with ciclophosphamide (cfm), mercaptopurine (mp), cytarabine (cyt), vcr, asp and IT. CNS prophylaxis with methotrexate (mtx), followed by interim maintenance (im) combining vcr, mtx and asp. The other 4 blocks composed by delayed intensification (di) x 2 with vcr, dau, pdn, asp, cfm, mp, cyt, vcr and one im and di (as in induction). Hypercvad was prescribed with the addition of IT to each one of the 8 arms. Maintenance consisted of POMP for 2 years, and in some, combined with etoposide or anthracycline plus IT for 6 months. Rituximab (mab) was allowed at discretion if CD 20+. Ph+ cases received imatinib. Radiotherapy was permitted for mediastinal tumor. Patients changed the initial regimen if non hematological toxicity occurred during induction or by preference and registered in the regimen they continued. Variables analyzed: age (±30), WBC (≥30000 B and ≥100000 T), cytogenetic (t9;22 [ph+] and complex karyotypes), lineage, CD20, and aberrant markers. Relapse and death in CR due to toxicity were censored for the EFS analysis. Toxicity counted as one event in each block/arm (only ≥3 grade) or if admission or transfusion needed. Death during induction was excluded from the toxicity analysis.

Table 1. Toxicities. *Two similar blocks were added together.

	Hypercvad		BFM			
	Odd	Even	Intensification	Consolidation	CNS prophylaxis	Maintenance
Arms/Blocks (%)	53	67	25*	36*	22*	2*
Anemia	14 (26)	41 (61)	5 (20)	15(41)	2(9)	4(15)
Thrombocytopeni	15 (28)	55 (82)	3(12)	8(22)	2(9)	2(7)
Neutropenia	23 (43)	51 (76)	3(12)	12(33)	4(18)	4(15)
Blood support	14 (26)	52 (78)	4(16)	12(33)	3(14)	4(15)
Other toxicities	2 (4)	2 (3)	2(8)	2(6)	2(10)	3(11)
Admission	19 (36)	57 (85)	2(8)	10(28)	3(14)	3(11)
Death	2 (4)	5 (7)	0	0	1(5)	2(7)

Results: Fifty eight cases were treated. Median was 24 years (16-40). WBC was 15400 (0.6-632000). Twelve patients had karyotype and five were high risk (four ph+). Sixteen cases (33%) classified as high risk. Three cases had CNS disease. Fifty one (88%) cases were B and 48% CD 20+ (ten received mab). Seventeen had aberrant markers. Mediastinal tumor was observed in four T cell cases. The EFS of the whole group at 3 years was 30%. Forty five (78%) patients received induction with BFM with 8 deaths (17.7%), and thirteen (22%) with hypercvad with 2 deaths (15.3%). Median follow up was 12 months (1-72) with twenty one individuals in CR. Fourteen (29%) patients changed treatment, thirteen in the BFM group, 4 for pancreatitis and 9 by preference and one due to toxicity in the hypercvad group. Thirty three patients were included in BFM and twenty five in hypercvad. The EFS at three years was 32% for BFM and 28% for hypercvad p=.6. There were seventeen relapses (BFM 8 hypercvad 9) and ten deaths in CR due to toxicity (BFM 3 hypercvad 7). Toxic deaths in BFM were infectious in one and CNS toxicity in two. All toxic deaths in hypercvad were infectious Table 1. None of the variables had statistical difference. More toxic deaths occurred during the even courses and nearly 80% required admission for neutropenia and blood support (compared to a close 30% of the odd courses) in the hypercvad protocol. For the BFM, most admissions happened during consolidation (28%). The other blocks had less toxicity, although neurotoxicity occurred during maintenance. Mucositis presented barely in both groups. Coagulopathy and hepatitis rarely appeared with asp.

Summary/Conclusions: Both regimens had similar EFS but hypercvad had more deaths in CR due to myelotoxicity and more blood support and admission to the hospital. Severe non-myelotoxic events were uncommon.

E865

PATTERN OF CNS RELAPSE IN ACUTE LYMPHOBLASTIC LEUKEMIA BCR-ABL POSITIVE, THE IMPORTANCE OF CHARACTERIZATION OF ABL1 MUTATIONS IN CEREBROSPINAL FLUID

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Background: He incidence of central nervous system (CNS) relapse among patients with BCR/ABL-positive acute lymphoblastic leukemia (ALL) is 8-17%. Although tyrosine-kinase inhibitors (TKI), especially imatinib, are included in the first-line treatment in these patients, their concentration in CNS is too low to effectively prevent CNS relapse, making CNS prophylaxis mandatory.
Aims: To study the frequency, predictors and evolution of BCR-ABL positive ALL patients with CNS relapse in two consecutive clinical trials of the PETHEMA group using imatinib and chemotherapy. As secondary objective we proposed the introduction of a new method for the study of variants of uncertain significance (VUS) in kinase domain of the BCR-ABL from cDNA of cerebrospinal fluid (CSF) blasts, in order to adapt the TKI used in relapse according to the clonal evolution from bone marrow (BM) to CSF cells.
Methods: It has been reviewed data of CNS relapse of patients included in two consecutive clinical PETHEMA trials **(**PETHEMA-LAL-PH-2008 and PETHEMA-LAL-OPH-2007), for treatment of BCR-ABL- *Ph+* ALL patients. In two patients with combined BM and CSF we analyzed the BCR-ABL mutations at diagnosis and at relapse in both sites. Kinase domain of cDNA was amplified in two steps by nested PCR in the samples of BM at diagnosis and BM or CSF at relapse, covering the whole kinase domain from residues Gly227 to Gly514. Further enzymatic fragmentation of the domain yielded ~200 bp of small fragments. Ion Torrent ultra deep-sequencing of the resulting fragments allowed us to evaluate the variants of the chimeric protein.
Results: A total of 138 ALL BCR/ABL positive patients were analyzed and 128 reached complete remission, 30 of them have relapsed (13 [43%] involving CNS [isolated or combined], 16 [53%] BM and 1 in unknown site). The overall survival probability at 2 years was 69% for the "CNS Relapse" group (IC95%: 44-94%; p=0.067) and 44% for the "Not CNS Relapse" group (IC95%: 20-68%; p=0.067, Figure 1a). In a multivariable analysis any clinical variable was associated with CNS relapse probability. In two of the CNS and BM relapsed patients we have performed massive sequencing experiments of ABL1 Kinase domain mutational status. In the first patient ultra-deep NGS of BM samples at diagnosis and at relapse did not show VUS or pathogenic variants, but the CSF study at relapse confirmed the variant c.1159 T>A, p.L387M. The same study in the second patient also found the same pathogenic variant only in CSF blast cells (Figure 1b), whereas, the VUS c.733 A>G, p.K245E was found only in the BM sample at diagnosis.

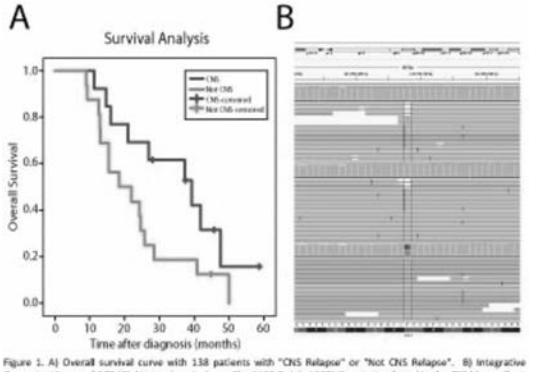

Figure 1. A) Overall survival curve with 138 patients with "CNS Relapse" or "Not CNS Relapse". B) Integrative Genomics Viewer of BCRABL kinase domain (exon 7) c.1159 T>A (p.L387M) mutation found in the CSF blast cells at relapse (bottom). Non-mutated alleles can be seen in bone marrow sample at diagnosis (top), and bone marrow at relapse (middle).

Figure 1.

Summary/Conclusions: In BCR-ABL ALL patients treated with imatinib and chemotherapy CNS relapse was a significant feature, despite CNS prophylaxis, In our series we did not find any clinical variable predicting CNS relapse. We have found the pathogenic variant p.L387M in CSF blasts of two patients with combined CNS and BM recurrence, this variant not being found in BM samples at diagnosis or at relapse. These mutations were sensitive to other TKIs with better penetration to CSF. Based on these results, a mutational study of the kinase domain of the BCR-ABL in blast cells obtained from CSF, should also be integrated in the mutation study of these patients in order to select the TKI according to the clonal evolution from BM to CSF cells.

E866

PROGNOSTIC IMPACT OF COPY NUMBER ALTERATIONS IN A SERIES OF PEDIATRIC PATIENTS WITH DE NOVO B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA IN A SINGLE CENTER

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Background: Gene copy number alterations (CNAs) have been recently proposed as variables for risk stratification in acute lymphoblastic leukemia (ALL). However, their use in clinical practice is controversial, as their prognostic impact could depend on the applied therapeutical protocol and the interaction with other clinical and biological variables.
Aims: To analyze the prognostic impact of CNAs in a series of pediatric patients with B-cell precursor ALL (pre-B ALL) treated in a single center.
Methods: Pediatric patients (0-18 years) with pre-B ALL, diagnosed between 1999 and 2015 and homogeneously treated according to consecutive protocols of the Spanish Hematology and Oncology Pediatric Society (SEHOP) Cooperative Group (SHOP-99, SHOP-2005, SEHOP-PETHEMA 2013). CNAs were studied by MLPA technique with SALSA-P335 kit (MRC-Holland) and Coffalyser software.
Results: We included 55 patients with a median age of 4.7 years (range 1.1-14.9), 56% males. Only one patient had central nervous system infiltration. Median WBC count was $8.4x10^9$/L (range 1.1-296.9). All cytogenetic subtypes were represented. We observed the presence of CNAs in 29 patients (53%), including 17 (31%) cases with one CNA, 8 (15%) patients with 2 CNAs and 4 (7.5%) patients with ≥3 CNAs. The most frequent CNAs were deletions of *CDKN2A/B*, observed in 12 patients (22%), followed by *ETV6* deletions in 11 cases (20%) and *IKZF1* deletions in 9 cases (16%); also, we observed deletions of *PAX5* in 7 cases (13%), of *BTG1* in 4 patients (7%), of *RB1* in 2 cases (4%), *JAK2* deletions in one case and *CRLF2* alterations (PAR region) in one case. *IKZF1* deletions were more frequently observed in *BCR-ABL1* rearranged cases and in patients with B-other subtype (cases with absence of main cytogenetic categories), and *ETV6* deletions predominated in cases with *ETV6-RUNX1* rearrangement. Among those patients with more than one CNA, *IKZF1* was the gene most frequently involved (IKZF1*plus*, 7 cases). The presence of CNAs was significantly correlated to older age (p=0.006), hyperleukocytosis >$20x10^9$/L (p=0.018) and high levels of minimal residual disease (MRD>10%) at day 15 of induction, assessed by flow cytometry (p=0.012). A higher number of CNAs (≥2) was found in boys (p=0.026). Noticeably, the patient with CNS infiltration harbored 3 CNAs. *IKZF1* deletions were associated with high hyperleukocytosis (>$100x10^9$/L, p=0.001), older age (p=0.001), high NCI risk (p=0.008) and high MRD levels at day 15 (p<0.0001). We found the same associations for IKZF1*plus*. *CDKN2A/B* deletions showed a trend to high WBC count (>$100x10^9$/L, p=0.051) and presence of MRD at the end of induction (MRD>0.01%, p=0.082). We found no significant associations for *ETV6* or *PAX5* deletions. After a median follow up 2.6 years (0.3-11.9) all patients are alive and 3 patients relapsed, with an event free survival (EFS) at 5 years of 87±7%. The presence of ≥2 CNAs correlated to worse EFS (64±16% *vs* 100%, p=0.043).
Summary/Conclusions: in our series, the presence of CNAs, mainly *IKZF1* deletions, were associated with poor prognostic factors like older age, high WBC count and high levels of MRD at early timepoints. We are currently expanding our series to confirm our results. GRANTS: PI12/2417, PN I+D+I, ISCIII (SGE, FIS), CIBERER, Fundación AECC, Fundación Sandra Ibarra, Fundación Cris contra el cáncer & "Força Miquel", "Candela" & "Mua" projects.

E867

COMBINED BLINATUMOMAB + DASATINIB/IBRUTINIB THERAPY OF RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS-ANTILEUKEMIC EFFECT ON THE T-HELPER AND T-REGULATORY CELLS REDUCTION BACKGROUND

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Background: Blinatumomab, bispecific anti-CD3/CD19 monoclonal antibody,

is effective as monotherapy in the treatment of relapsed/refractory acute lymphoblastic leukemia (R/R-ALL), but long term results could be improved. T-regulatory (T-reg) and T-helper cells can inhibit effector T-cells used by blinatumomab. Tyrosine-kinase inhibitors (TKI) could be used in the treatment of both Ph-positive (Ph+) and Ph-negative (Ph-) ALL as pre-B-cell receptor signaling inhibitors.
Aims: To study T-cell subpopulations kinetics in R/R-ALL patients during combined blinatumomab+TKI treatment of relapsed ALL patients.
Methods: Two relapsed ALL patients (1 bone marrow Ph+ relapseand 1 extramedullary extraperitoneal with severe abdominal pain Ph- relapse)were treated with blinatumomab 28 mcg/day by continuous infusion 4 weeks cycles with 2 weeks intervals (3 cycles in Ph+ relapse and 2 cycles in Ph- patients). Dasatinib 140 mg/day started In Ph+ patient from the 3rd week of the first blinatumomab cycle and further continuously. Ibrutinib 560 mg/day started in Ph- patient from the 4th week of the first blinatumomab. T-cell subpopulations of the peripheral blood (T-helper, cytotoxic, T-reg, NK) were studied by flow cytometry every week during the whole treatment period. Blinatumomab was provided by Amgen sponsored Expanded Access Program.
Results: The Ph+ ALL patient treated by blinatumomab+dasatinib combination had pleural effusions and nonspecific lung infiltration fully regressed after 2 weeks dasatinib interruption. In that patient the second molecular remission was obtained after the second blinatumomab cycle+dasatinib treatment. In Ph- ALL patient the abdominal pain fully regressed and tumor size slightly decreased after the first blinatumomab cycle+ibrutinib. T-cell subpopulations study revealed only T-helper (lowered from 0,437 to 0,111x10*9/L in Ph+ ALL pt and was decreased to 0,112-0,111x10*9/L in Ph- ALL pt) and T-reg cell (was decreased to 0,006-0,002x10*9/L in Ph+ pt and lowered from 0,009 to 0,006x10*9/L in Ph- ALL pt) pools reduction. Absolute counts of cytotoxic T cells and NK cells remained within the normal reference values in both patients.
Summary/Conclusions: The correlation of the efficacy of blinatumomab treatment that uses effector cells in antileukemic effect against relapsed ALL with T-helper and T-reg pools reduction possibly indicates an overcoming the negative influence of those T-cell subpopulations. TKIs may act in synergy with blinatumomab during their concomitant use.

E868

LOW EXPRESSION OF LYMPHOID ENHANCER-BINDING FACTOR-1 (LEF1) ASSOCIATES WITH POOR PROGNOSTIC FACTORS IN PEDIATRIC ACUTE LEUKEMIA

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Background: *LEF1* expression has been described in different types of leukemia, but its prognostic impact remains controversial. In adult patients with acute lymphoblastic leukemia (ALL), *LEF1* overexpression correlates to high-risk factors (hyperleukocytosis, *BCR-ABL1* rearrangement and complex karyotype). In contrast, overexpression of *LEF1* is a favorable prognostic factor in childhood ALL and in acute myeloblastic leukemia (AML), both in adults and children.
Aims: to analyze the prognostic impact of *LEF1* expression in a series of pediatric patients diagnosed with acute leukemia in a single center.
Methods: we included pediatric patients (0-18 years) with de novo acute leukemia, treated according to consecutive protocols of the Spanish Hematology and Oncology Pediatric Society from 2003 to 2015. mRNA expression levels of *LEF1* were assayed by quantitative PCR and analyzed with the 2^{-DDCt} method, with non-neoplastic samples used as controls for the relative quantification. Patients were grouped into quartiles (Q1-Q4) according to *LEF1* expression level: *LEF1*^{high} (Q4) and *LEF1*^{low} (Q1-Q3). Correlations with clinical and biological variables and survival analysis were performed with non-parametric, chi-square and Kaplan-Meier tests and log-rank comparison for subgroups.
Results: we analyzed 104 patients, with a median age of 4.7 years (0-17.4), including 61 boys (59%). Sixty-two patients (60%) had B-cell precursor ALL (all main cytogenetic subtypes represented), 22 patients (21%) had T-cell ALL and 20 cases AML (19%). Our AML series was enriched in infants: the median age was 2.7 (0.08-15.96), included 5 infants (25%) and 8 *MLL* rearranged cases (40%). Median expression level of *LEF1* was 6.44 (0-461), and differed significantly according to lineage (21.6 (0.1-461) in B-ALL; 4.7 (0.01-79.3) in T-ALL and 0.18 (0-13.5) in AML, p<0.0001). In the whole series of patients, *LEF1*^{low} expression was observed predominantly in infants and in patients with *MLL* rearrangement (p=0.043 and p=0.011, respectively). In addition, a trend to hyperleukocytosis was observed in patients with *LEF1*^{low} (p=0.06). After a median follow-up of 4.3 years (0.06-14.96), 14 patients died and 13 relapsed. The overall survival (OS) at 5 years was 94±3% in B-ALL patients, 80±9% in T-ALL and 63±11% in AML patients, p=0.001. In B-ALL subgroup of patients, low expression of *LEF1* was again significantly correlated to infant cases (p=0.025). Interestingly, in T-ALL patients we observed different findings: those cases with high expression of *LEF1* associated with older age (p=0.025) and worse event free survival (EFS, 61±15% *vs* 100%, p=0.02), although the number of patients is low and our results must be taken with caution. Despite the number of patients precluded the finding of statistical significance, in AML cases we observed, as in the whole series of patients, a correlation between *LEF1*^{low} and age<1 year, *MLL* rearrangement and higher WBC count (p=0.08, p=0.055 and p=0.072, respectively).
Summary/Conclusions: in our series of pediatric patients, low *LEF1* expression levels in B-ALL and AML patients correlated to adverse prognostic factors, such as age<1 year, *MLL* rearrangement and hyperleukocytosis. A larger number of patients is needed to confirm our results. GRANTS: PI12/2417, PN I+D+I, ISCIII (SGE, FIS), CIBERER, Fundación AECC, Fundación Sandra Ibarra, Fundación Cris contra el cáncer & "Força Miquel", "Candela" & "Mua" projects.

E869

CHARACTERISTICS OF PONATINIB THERAPY FOR PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ ALL) PATIENTS IN REAL-WORLD CLINICAL PRACTICE COMPARED TO THE PACE TRIAL

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Background: The pivotal phase 2 PACE trial (NCT01207440) studied the use of ponatinib in adult patients with refractory Ph+ ALL and formed the basis of the approval of ponatinib. Prescribing data tracked by the specialty pharmacy exclusively responsible for distribution of ponatinib in the US are available from Jan 2014 onward.
Aims: To compare the PACE clinical trial data *vs* real-world pharmacy data, in order to examine similarities and differences between patient characteristics, regimens and duration of therapy evolving over time.
Methods: We compared PACE data, which enrolled patients Sep 2010 - Oct 2011 (all providing informed consent) to real-world data for Ph+ ALL patients starting ponatinib treatment Jan 2014 - Dec 2015. Real-world data source includes referring physicians, pharmacy intake forms and dispensing records. Data on co-prescribing were available for a small subset of patients. Patient characteristics and dosing were compared using non-parametric tests; average daily dose was calculated, including therapy gaps as "zero" dose. Duration of therapy was assessed using Kaplan-Meier techniques and proportional hazard regression.
Results: PACE enrolled 32 Ph+ ALL patients; 274 US real-world Ph+ ALL patients started treatment with ponatinib over a 2-year period. Demographic characteristics of PACE *vs* real-world patients, including age (median 61.5 *vs* 55.5 years; p=0.589) were similar. Most PACE patients were in their 3rd line of TKI therapy or later (19% 2nd, 44% 3rd and 38% 4th) while most real-world patients appear to be in earlier lines of TKI (29% no prior TKI reported, 32% 2nd line, 24% 3rd, and 15% 4th) (p<0.001). All PACE patients received 45 mg/day of ponatinib as their initial dose; in the real-world, 50% of patients initially received 45 mg/day of ponatinib, 41% received 30 mg/day and 9% 15 mg/day. Average dose was higher in PACE *vs* real-world (39.1 *vs* 27.3 mg/day; p<0.001). PACE only permitted monotherapy ponatinib whereas combination therapy appears to be used in a portion of real-world practice. Median time on therapy was 2.7 months in PACE *vs* 5.5 months in real-world patients (p=0.004), and nearly 50% of real-world patients remained on therapy after 6 months, *vs*<20% in PACE (Table).

Table 1.

Time on therapy in PACE vs real-world Ph+ ALL patients

Population (n)	6	12 (months)	18	24	Hazard ratio [CI] (p-value)
PACE (32)	18.8%	12.5%	3.1%	3.1%	0.573 [0.390 – 0.841]
Real-world (274)	48.2%	21.2%	9.5%	--	(0.005)

Summary/Conclusions: Real-world Ph+ ALL patients appear to be demographically similar to those enrolled in PACE, but there is evidence of ponatinib use earlier (less prior TKI treatment) in real-world patients. Starting dose in real-world reflects 50%<45 mg daily and subsequent lower dose intensity. Median duration of therapy was significantly longer for real-world patients than for PACE, with nearly one-half of real-world patients remaining on therapy at 6 months. Longer median duration of therapy may be related to lower dose intensity, use in earlier therapy lines, and use of combination therapy in real-world clinical practice. Further study of real-world use of ponatinib in Ph+ ALL is needed.

E870

RISK FACTORS IN PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: A COLLABORATIVE STUDY OF THE BIOLOGICAL COMMITTEE OF THE LEUKEMIA GROUP OF THE SPANISH HEMATOLOGY AND ONCOLOGY PEDIATRIC SOCIETY

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Background: Useful biomarkers for risk stratification in pediatric T-ALL are still needed. Several biological variables have been proposed as new prognostic biomarkers, including *NOTCH1, FBXW7, PTEN* & *RAS* mutations, expression of myeloid antigens and presence of copy number alterations (CNAs) of certain genes, but published results are controversial.
Aims: To analyze the prognostic impact of mutations, CNAs and myeloid antigens expression in a series of 56 pediatric T-ALL patients.
Methods: Pediatric patients (0-18 years) diagnosed with T-ALL, treated according to SEHOP (Spanish Hematology and Oncology Pediatric Society) and PETHEMA Cooperative Groups from 2001 to 2015. Mutations of *NOTCH1, FBXW7, PTEN, NRAS* & *KRAS* were screened by Sanger sequencing. CNAs were studied by MLPA technique with SALSA-P383 T-ALL kit (MRC-Holland) and Coffalyser software.
Results: Median age was 7 years (range, 0.3-15.4 years), 72% males. Central nervous system infiltration was present in 3 cases. Median WBC count was 56x10^9/L (range, 1.1-588). Twenty-four cases showed a cortical phenotype and 3 were classified as early T-cell precursor leukemia. Myeloid antigen expression (CD13 and/or CD33) was observed in 5 cases (14%). Twenty-three (41%) cases harbored *NOTCH1* mutations, including 18 single *NOTCH1* mutations (*NOTCH1*^{single}) and 5 cases with more than one mutation, located in different domains (*NOTCH1*^{double}). *FBXW7* was mutated in 9 cases (16%) and *NRAS* in 5 (9%). Overall, we observed *NOTCH1/FBXW7* mutations in 28 cases (50%). Six cases harbored *PTEN* abnormalities (mutations and/or deletions). Thirty-one of 41 analyzed cases (76%) showed ≥1 CNAs: *CDKN2A/B* deletions were the most frequent (28 cases, 60%) and were homozygous in 17 cases (*CDKN2A/B*^{homo}). We also observed *LEF1* deletions (n=4), *MYB* duplications (n=4), *CASP8AP2* deletions (n=4), and deletions (n=2) and duplications (n=1) of *EZH2* gene. *NUP214/ABL* fusion gene was detected in 2 cases. *CDKN2A/B*^{homo} was associated with higher WBC count (p=0.04). After a median follow-up of 4 years (range, 0.09-14.24), 10 patients relapsed and 12 died. The 5 years overall survival (OS) was 70±8%. All patients achieved morphological complete remission (CR) after induction. Minimal residual disease levels (≥0.01%) were detected by flow cytometry in 8 cases (28%). Five patients underwent allogeneic stem cell transplantation. The univariate analysis showed a better OS for patients with cortical phenotype (90±7% *vs* 50±20%, p=0.035) and a worse OS for *CDKN2A/B*^{homo} (44±16% *vs* 78±10%, p=0.048) and mutations in *FBXW7, NRAS* or *PTEN* abnormalities (*FBXW7*^{mut}/*NRAS*^{mut}/*PTEN*^{abn}) (50±14% *vs* 78±8%, p=0.027). In the multivariate analysis, *CDKN2A/B*^{homo} and *FBXW7*^{mut}/*NRAS*^{mut}/*PTEN*^{abn} retained their statistical significance (HR 5.5, p=0.013 and HR 6.4, p=0.006, respectively). For event free survival (EFS), hyperleukocytosis >200x10^9/L and *FBXW7*^{mut}/*NRAS*^{mut}/*PTEN*^{abn} independently predicted a worse outcome (HR 5.3, p=0.034 and HR 8.9, p=0.007, respectively).
Summary/Conclusions: In our series, the presence of *FBXW7*^{mut}/*NRAS*^{mut}/*PTEN*^{abn} predicted a worse OS and EFS, and the homozygous deletion of *CDKN2A/B* genes were independently associated with an inferior OS. Larger series of patients are needed to confirm our results. GRANTS: PI12/2417, PN I+D+I, ISCIII (SGE, FIS), CIBERER, AMPILE, Fundación AECC, Asociación Pablo Ugarte, Fundación Uno entre cienmil, Fundación Sandra Ibarra, Fundación Cris contra el cáncer, "Força Miquel", "Candela" & "Mua" projects.

E871

MANAGEMENT AND OUTCOME OF CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT IN ACUTE LYMPHOBLASTIC LEUKEMİA (ALL): PRE AND POST TRANSPLANT

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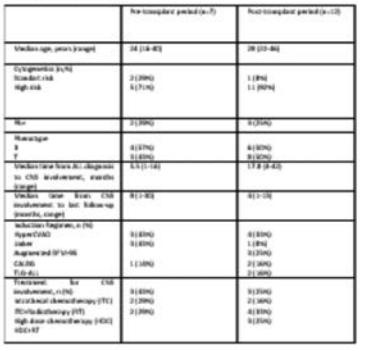
Background: The CNS is most common extramedullary site of involvement in ALL occurs in 4% to 8% of patients under chemotherapy. Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) has emerged the curative treatment of ALL. The data how and which patients respond to CNS involvement treatment in pre and post transplant period remain limited.
Aims: The objectives of this study is to examine the management and outcomes of CNS involvement in pre and posttransplant period.
Methods: This single-center retrospective analysis included 137 ALL patients who underwent Allo-HSCT at Ankara University School of Medicine between 2005 and 2015. CNS involvement/relapse included 1 or more of the following criterias: evidence of leukemic blasts in the cerebrospinal fluid (CSF), cranial nerve palsy or contrast-enhancing brain or spinal mass on imaging.
Results: Pretransplant CNS involvement was diagnoesed in 7 (5%) patients whereas posttransplant CNS relapse was detected in 12 (9%) patients. The patient characteristics are shown in table. All induction regimens included prophylactic intrathecal chemotherapy (Methotrexate, ARA-C). None of the patients with pre-transplant involvement had post-transplant CNS relapse. Isolated CNS relapse was detected in 4 (33%) post-transplant patients. Median time to CNS relapse from Allo-HSCT was 8 months (range, 1-28). Twp pre-transplant (29%) and two post-transplant (16%) CNS involvement patients received reduced intensity conditioning. TBI based conditioning was prefered in 6 pre-transplant (86%) and 7 (58%) post-transplant CNS involvement patients. Peripheral blood was the stem cell source for 17 patients wheras two pre-transplant CNS involvement patients had received bone marrow from their matched relatives. None of the patients received CNS-directed prophylactic therapy after transplantation. All of the pre-transplant involvement patients were died due to disease relapse post-transplant. Two patients who received HDT and RT were alive in post-transplant CNS relapse group.

Table 1.



Summary/Conclusions: The prognosis is poor in CNS relapse detected in ALL patients posttransplant or pretransplant period. HDC and RT seems the most effective treatment strategy.

E872

NOVEL DYNAMIN2 MUTATION IN ADULT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Dynamin 2 (*DNM2*), a GTPase is essential for intracellular vesicle formation and trafficking, cytokinesis, and receptor endocytosis. Recently *DNM2* genetic mutations are identified in a subtype of T-cell acute lymphoblastic leukaemia (T-ALL) termed "early T-cell precursor" (ETP) ALL that comprises up to 15% of T-ALL, and is associated with treatment failure. However, study on *DNM2* genetic mutations in adult ALL is rare.
Aims: To characterize the novel *DNM2* mutations in Chinese adult T-ALL patients.
Methods: Bone marrow (BM) samples from 42 newly diagnosed T-ALL patients [31 male with median age 26 (16-62), 11 female with median age 29 (19-60)] were collected between July 2010 and December 2014 at the First Affiliated Hospital of Nanjing Medical University. The diagnosis of ALL was made according to the morphologic, immunophenotypic, cytogenetic and molecular criteria of WHO Diagnosis and Classification of ALL (2008). All the patients provided their written informed consent in accordance with the Declaration of Helsinki before enrollment in the study. The study was approved by the Institutional Review Board of the Nanjing Medical University. We performed mutational analyses of *DNM2* exons 2-22. Exons in *NOTCH1, FBXW7, PHF6, PTEN, JAK1* and *IL-7R* were also screened. Conventional cytogenetic analysis was performed at the time of diagnosis, using unstimulated short-term cultures according to the recommendations of the International System for Human Cyto-

genetic Nomenclature (ISCN). For each sample, at least 20 BM metaphase cells were analyzed. Immunophenotypic analyses were performed by flow cytometry on fresh BM samples. The cell-surface antigen was defined positive when fluorescence intensity of at least 20% of cells exceeded fluorescence of negative control.
Results: *DNM2* mutations were identified in 4 of 42 T-ALL patients (9.5%). All the four cases were point mutations (c.1081C>T, c.1453T>C, c.1609G>A and c. 1801C>T), which is located in exon 8, 13, 16 and 18. In the four identified mutations, one is nonsense mutation and three are missense mutations. All of the four mutations were lead to amino acid changes (R361X, Y485H, G537S and R601W). The R361X and Y485H are located in middle(MID) domain, while G537S and R601W in pleckstrin homology(PH) domain of *DNM2*. Interestingly, we found that the four patients with *DNM2* mutations co-existed with *NOTCH1* mutations, 3 of the 4 cases co-existed with two *NOTCH1* mutations (point mutations and / or indel mutations), which were located in *NOTCH1* exon 26, 27 and 34. Moreover, 3 of the 4 patients with *DNM2* mutations were also concomitant with *PHF6* mutations which were located in *PHF6* exon 4, 5 and 8. The clinical characteristics of the patients with *DNM2* mutations were further analyzed. It showed that one patient relapsed, one patient had initial high WBC, and two patients had complex karyotype. Importantly, the days for reaching the complete remission after induction chemotherapy were over 4 weeks for all these patients. These data indicated that the patients with *DNM2* mutations in this cohort of adult T-ALL had poor prognosis.
Summary/Conclusions: We identified 4 novel *DNM2* mutations and the co-existence of mutations in NOTCH1/PHF6 in adult T-ALL patients. We also observed the association of *DNM2* mutations with poor prognosis. Our finding suggested the *DNM2* mutations may be involved in the oncogenesis of T-ALL.

E873

LONG-TERM OUTCOME OF RELAPSED ADULT PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH CONTINUOUS IMATINIB PLUS COMBINATION CHEMOTHERAPY
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Background: Treatment outcome with conventional salvage therapy in relapsed Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) is poor and is often associated with significant morbidity. Tyrosine kinase inhibitors (TKI) have changed strategy of Ph+ ALL therapy and improved prognosis of the disease. However, despite the progress associated with TKI therapy for Ph+ ALL, the maintaining remission remains the major challenge.
Aims: We assessed the long-term outcome of relapsed adult Ph+ ALL patients treated with continuous imatinib plus combination chemotherapy.
Methods: Imatinib (600 mg/day orally) was administered continuously with combination chemotherapy, starting from eighth day of remission induction treatment, then through 5 courses of consolidation or until allogeneic hematopoietic cell transplantation (HCT). Patients who were not transplanted were maintained on imatinib for 2 years.

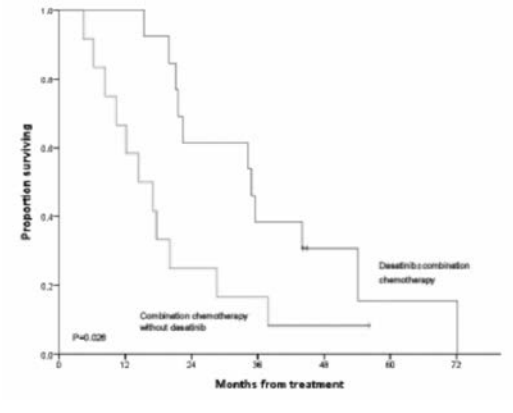

Figure 1.

Results: Between October 2005 and February 2009, total 87 patients, aged 16-71 years, with newly diagnosed Ph+ALL were enrolled. With median follow-up of 5 years among survivors (range: 2.6-8.9 years) and data was frozen up in May 2015. After remission induction, 82 patients (94%) achieved complete remission (CR). Among these patient, 44 patients (54%) experienced leukemia relapse and median time of leukemia relapse-free survival was 13.9 months (range, 0.4-89.5 months). Sites of relapse were bone marrow (79.5%), central nervous system (CNS) (9.1%), lymph nodes (6.8%), testis (2.3%) and pancreas (2.3%), respectively. Sixteen patients (36.4%) relapsed during consolidation treatment, 23 patients (52.3%) relapsed after allogeneic hematopoietic cell transplantation (HCT), and five patients (11.4%) relapsed during maintenance treatment after completion of five consolidation. The relapse rate within one year from CR was 45.5% in total patients and that within one year in patients underwent allogeneic HCT in first CR was 25.0%. Salvage treatments of relapsed Ph+ ALL were combination chemotherapy in 13 patients (29.5%), imatinib in 6 (13.6%), imatinib plus combination chemotherapy in 6 (13.6%), dasatinib in 8 (18.2%), dasatinib plus combination chemotherapy in 5 (11.4%), intrathecal methotrexate in 4 (9.1%) who relapsed in CNS, and to stop immunosuppressant therapy in one who underwent allogeneic HCT. One relapsed patient did not receive treatment due to poor performance state and died of leukemia recurrence. Patients treated with dasatinib±combination chemotherapy were demonstrated a longer survival compared with patients treated with combination chemotherapy without dasatinib (P=0.026). Dasatinib treatment had a tendency of superiority in survival but there was no statistical significance. Nineteen patients (43.2%) among evaluable 43 patients treated salvage therapy achieved a second CR. Two patients of them were received allogeneic HCT after achieving second CR. Eleven patients in twelve evaluable patients who achieved a second CR experienced a second relapse. The median time of survival was 21.3 months (range, 4.4-72.2 months). The causes of death of them were leukemia recurrence (89.2%), and infection (10.8%).
Summary/Conclusions: Inclusion of dasatinib into transplantation strategy allows obtaining sustained remission even in patients relapsed after imatinib treatment.

E874

HEMATOPOIETIC STEM CELLS CAN BE SEPARATED FROM LEUKEMIC CELLS IN A SUBGROUP OF ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS
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Background: Aldehyde dehydrogenase (ALDH) activity which is involved in the metabolism of intracellular aldehydes has been demonstrated to function as a marker for stem cell activity in various tissues including the hematological system. In combination with CD34-positivity and CD38-negativity high ALDH activity ($CD34^+CD38^-ALDH^+$) has been shown to be a reliable tool for the separation of hematopoietic stem cells (HSC) from leukemic cells in a subgroup of patients with acute myeloid leukemia (AML). In B-cell acute lymphoblastic leukemia (B-ALL) successful separation of normal HSC has so far been limited to a subgroup of patients. These isolations were mostly guided by CD19-negativity in combination with the $CD34^+CD38^-$ phenotype. However, in some cases leukemia initiating cells were also detectable in the pool of $CD19^-$ cells indicating that better isolation strategies are necessary.
Aims: In this study we sought to investigate the value of ALDH-activity for the isolation of HSC from leukemic cells in adult B-ALL.
Methods: Between 2011 and 2015 bone marrow (BM) aspirates of 15 ALL patients were collected after written informed consent and stratified in ALDH-numerous (≥1.9% $ALDH^+$ cells) and ALDH-rare (<1.9% $ALDH^+$ cells) cases. The cut-off value of 1,9% was determined by the amount of $ALDH^+$ cells in healthy bone marrow controls. HSC candidates ($CD34^+CD38^-ALDH^+$) and various subpopulations were analyzed for the presence of clonal marker and functionally tested in *in vitro* assays.
Results: In ALDH-rare B-ALL clonal-marker negative HSC could be reliably separated by the $CD34^+CD38^-ALDH^+$ phenotype, whereas this separation was not possible in ALDH-numerous B-ALL. Functional assays confirmed the HSC-potential of isolated cells and showed increased long- and short-term colony-initiating activity. Further analysis showed that in ALDH-rare ALL HSC-potential was uniformly restricted to $CD19^-$ cells. However, addition of ALDH-activity was necessary to further enrich for HSC-activity as $CD34^+CD38^-CD19^-ALDH^-$ cells contained no colony forming potential.
Summary/Conclusions: In summary, we provide a method to separate functionally normal HSC from leukemic cells in a subgroup of B-ALL patients that can be identified by their overall low percentage of cells with high ALDH-activity. This protocol thereby allows comparative analyses of patient matched HSC and leukemic cells in order to improve the understanding of leukemic evolution in adult B-ALL.

E875

DOES TIMING OF CENTRAL VENOUS LINE REPLACEMENT MATTER DURING INDUCTION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA?
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Background: The use of central venous lines (CVL) in children with acute lymphoblastic leukemia (ALL) provides comfortable administration of intensive chemotherapy and blood sampling. The optimal time for insertion of CVL in patients with ALL is controversial.
Aims: The aim of this study is to define the frequency of CVL related complications and also to analyse the frequency of cases of catheter related infection and thrombosis in relation to timing of CVL replacement.
Methods: We reviewed the records of 52 pediatric ALL patients with CVL treated during a 10-year period. Demographics, preoperative blood counts, type of central line, time of CVL replacement, CVL related complications, and blood counts during complications were all noted. All the data were collected from those with the first catheter use.
Results: Fifty two pediatric ALL patients (29 male, 23 female) with a median age of 6,1 years were included in the study. Most of the patients (92.3%) had an internal line (port). CVL was replaced before treatment day 15 (early) in twenty six patients (50%) and after 15 days (late) in 26 patients (50%). Systemic infections occurred in 24 patients (46.2%). Regarding the infection rates, no difference was found between early and late CVL replacement (p=0.09). Most of the infections (68%) were due to coagulase negative *Staphylococcus aureus*. The median absolute neutrophil count during infections was 150/mm^3 (0/mm^3 to 17,700/mm^3). There were three patients with thrombosis and no difference was found between early and late insertion of CVL replacement groups (p=0.35). Other catheter related complications was recorded in seven patients (leakage in one patient, accidental removal of line in one patient, line rupture in one patient, and line occlusion in four patients). CVLs had to be removed in seventeen patients (32.7%) before the end of chemotherapy protocol due to various reasons (catheter related infections in six patients, local skin infections in four patients, and other catheter related complications in seven patients).
Summary/Conclusions: The present study showed no relation between the timing of catheter replacement and occurrence of infection and thrombosis. Our results suggest CVLs can be replaced safely at the time of diagnosis or early period of treatment to provide a comfortable administration of chemotherapy and to decrease painful blood samplings.

E876

TYROSINE KINASE INHIBITORS IN PHILADELPHIA-CHROMOSOME POSITIVE ADULT ACUTE LYMPHOBLASTIC LEUKEMIA IN REAL CLINICAL PRACTICE

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Background: Adult patients with Philadelphia-chromosome positive acute lymphoblastic leukemia (Ph+ ALL) are at high risk of relapse. Allogeneic stem cell transplantation still remains the treatment of choice in spite of the major benefit brought by adding tyrosine kinase inhibitors (TKIs) to the standard chemotherapy. On the other side, TKIs also bear several side effects and some of them require significant dose reductions.
Aims: The aim of this analysis was to focus on treatment strategies in unselected adult Ph+ ALL patients in real clinical practice and how TKI dose reduction may influence their outcomes.
Methods: All adult patients diagnosed with Ph+ ALL and treated with TKIs at our centre between 2005 and 2015 were included into this retrospective analysis. We describe baseline features, treatment options and outcomes. The data were analysed for response and relapse rates and factors affecting survival.
Results: A total number of 41 consecutive patients aged 19 to 82 years (median 58) at the time of diagnosis of Ph+ ALL were included. Leukocyte count was 1.8 to 296 x 10^9/L (median 20) at presentation. Most commonly observed were common-B immunophenotype (73%) and p190 bcr-abl transcript (63%). Thirteen (32%) patients were treated according to pediatric-based intensive protocol, 13 (32%) using a reduced-toxicity protocol, and 15 (37%) in a palliative approach. An allogeneic transplant was performed in 17 (41%) patients. Thirty-three (85%) patients achieved a complete remission (CR) and 56% of them achieved a complete molecular response (CMR). First line TKI was imatinib in all patients, with a median daily dose of 600mg. In 18 (44%) patients the dose had to be reduced to 400, 300 or 200mg due to side effects (haematological 36%, oedemas 32%, gastrointestinal 16%, skin rash 4%). Sixteen (46%) patients in CR eventually relapsed and only 6 (38%) achieved a second CR. Second line TKI was dasatinib (12; 75%) or 800mg imatinib (3; 19%). Dasatinib was given at a median daily dose of 140mg but in a much broader range (35 to 140 mg). This dose was further decreased in 10 (83%) patients, while most common non-haematological side effects were gastrointestinal (17%), oedemas (13%) and effusion (4%). Five (83%) of these patients in CR2 experienced a second relapse. During the follow-up with a median of 12 months (range 1 to 103 months), 27 (66%) patients died; fifteen (56%) of infection, 11 (41%) of disease progression and one (4%) of a secondary malignancy. Fourteen (34%) patients are still alive in complete remission. Five-year overall survival (OS) in the whole cohort was 30%. The survival was not influenced by leukocyte count, immunophenotype nor the type of the bcr-abl transcript. The statistically significant risk factors for longer OS were: age under 55 (5-year OS 57% *vs* 8%, p<0.01), CR (37% *vs* 0%, p<0.01), CMR (54% *vs* 0%, p<0.01), allogeneic transplant (58% *vs* 8%, p<0.01) and relapse (67% *vs* 0%, p=0.01). In non-transplanted patients with imatinib dose reduction the survival was not inferior compared to patients without a reduction. Surprisingly, these patients were surviving even longer (2-year OS 38% with a reduction *vs* 8% without, p<0.01). Whether this finding represents a clinically significant phenomenon or is a mere coincidence remains a question for further investigation.
Summary/Conclusions: Adult patients with Ph+ ALL form a distinct subgroup with a very variable prognosis. The best treatment outcome can be reached in younger patients who undergo allogeneic transplant in first haematologic and molecular remission. According to our analysis, imatinib dose reduction due to side effects is not associated with inferior survival. (Supported by Czech Leukemia Study Group-for Life.).

LB2239

PREDICTIVE VALUE OF IG/TR AND BCR/ABL1 PCR-BASED MINIMAL RESIDUAL DISEASE MONITORING IN PH+ PEDIATRIC ALL TREATED WITH IMATINIB IN THE ESPHALL STUDY

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Background: Several studies showed that minimal residual disease (MRD) detection is a strong independent prognostic factor in childhood acute lymphoblastic leukemia (ALL) including Ph+ ALL treated with conventional chemotherapy. Very few data and of difficult interpretation are available on the predictive value of early MRD response in Ph+ ALL treated with tyrosine kinase inhibitors (TKI)s.
Aims: MRD was detected by real-time quantitative PCR of rearranged immunoglobulin/T-cell receptor (IG/TR) genes and/or BCR/ABL1 fusion transcript in the EsPhALL study to investigate its predictive value in patients treated with imatinib.
Methods: In the EsPhALL study, after induction therapy, patients received BFM high risk ALL therapy; imatinib was given intermittently during the intensive treatment phases for a total of 18 weeks or less, usually 10 weeks, if patients underwent hematopoietic stem cell transplantation (HSCT), indicated for large majority of patients. MRD on the bone marrow (BM) was monitored as an ancillary study after induction therapy (TP1), consolidation Phase IB (TP2), HR Block1 (TP3), HR Block2 (TP4), HR Block3, first and second reinduction, and at end of therapy. Quantitative PCR analysis was performed and interpreted according to Euro-MRD network guidelines for both IG/TR and BCR/ABL1.
Results: MRD negativity increases progressively, both by IG/TR (from 10% at TP1 to 57% at TP4) and by BCR/ABL1 (from 13% at TP1 to 30% at TP4). Conversely, the proportion of patients with highly positive MRD by IG/TR decreases from 78% at TP1 to 14% at TP4 and by BCR/ABL1, from 80% at TP1 to 57% only at TP4. The minority of patients IG/TR MRD negative at TP1 (N=9, 10%) had a very favorable outcome with no relapses, whereas patients with high or low MRD positivity had a similarly high 5-year cumulative incidence of relapse (CIR) (SE) of 35.2 (5.9) and 36.4 (15.4), respectively. Achieving MRD negativity at TP2 is also associated with low risk of relapse (5-year CIR (SE) 14.3 (9.8)), whereas achieving negativity only at TP3 or TP4 is associated with a CIR of 36.4 (15.5) and 42.9 (21.6), respectively, similar to that of patients with low or high positivity at any time-point. BCR/ABL1 MRD negativity (although based on small numbers) at TP1 or TP2 is reached less frequently compared with IG/TR MRD, but is associated with a similar very low risk of relapse (one relapse in 8 patients MRD negative at TP1), while patients with low or high positivity have instead a high risk of relapse, again similar to that observed for positive IG/TR MRD. The overall concordance between the two methods is 69%; for patients with positive MRD by both techniques, the estimated mean differences of BCR/ABL1 *versus* IG/TR results were significantly different from zero, and were consistent according to different TPs with significantly higher positivity by BCR/ABL1. There are also rare cases consistently negative by IG/TR and positive by BCR/ABL1 with a very favorable outcome.
Summary/Conclusions: MRD negativity by IG/TR is obtained more frequently than by BCR/ABL1 and it is highly predictive of a very favorable outcome and the earlier the negativity, the better the prognosis. Further investigations are needed to understand the biology in cases with discordant results, and in particular of those negative by IG/TR and positive by BCR/ABL1. The role of MRD in patients treated continuously with TKI inhibitors and without HSCT needs to be explored in current studies.

LB2240

OUTCOME OF PATIENTS WITH HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA IN HAPLOID-MATCHED *VS* SIBLING-MATCHED ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: The efficacy of allogeneic stem cell transplantation in patients with high risk acute lymphoblastic leukemia (ALL) is unclea, and the relevant prognostic factors are not well-known.
Aims: To explore the efficacy of allogeneic stem cell transplantation in patients with high risk acute lymphoblastic leukemia (ALL) and investigate its relevant prognostic factors
Methods: A total of 69 patients with ALL who were treated with allo-HSCT in our hospital from July 2007 to August 2013, all patients were followed up more than two years. They were divided into haploid-matched (haploidentical allogeneic stem cell transplantation) group (n=42) and sibling-matched(HLA sibling-matched allogeneic stem cell transplantation) *group* (n= 27). Clinical characteristics of two groups were retrospectively analyzed.Survival date were analyzed by the Kaplan-Meier method and the prognostic factors were analyzed with the COX regression model.
Results: 2 years over-all survival(OS) is 63.4% in haploid-matched group, 53.9% in sibling-matched group, difference was not statistically significant (P=0.197); and 2 year LFS(leukemia-free survival) is 59.4% and 53.7% in haploid-matched group and sibling-matched group respectively,difference was not statistically significant(P=0.17). 2 years RR(relapse rate) of sibling-matched group(39.5%) is higher than that of haploid-matched group(19.5%) (P=0.014). In hematopoietic reconstruction, Neutrophils reconstruction of the haploid-matched group later than sibling-matched group(P=0.002), and platelet reconstruction in two group is similar(P=0.072). Grade I-II aGVHD(acute graft-*versus*-host disease) in haploid-matched group is higher than sibling-matched group (P=0.008), then grade III-IV aGVHD and chronic GVHD are similar (P=0.726, P=0.81 respectively) in two group. We collected the early(six months) infection after HSCT, infection rate of haploid-matched group is higher than sibling-matched group(P=0.02). 2 year NRM(non-relapse mortality) rate of sibling-matched group is 11.1%, NRM rate of haploid-matched group is 26.2%, the difference was not statistically significant (P=0.519). The Cox regression analysis showed that disease status before transplant (not CR1) were main risk factors affecting LFS of patients(P=0.001), relative risks is 7.581. limtited GVHD (P=0.013) and shorter time from first diagnosed to transplantation (P=0.012) is protective factors,relative risks is 0.178 and 0.688 respectively.
Summary/Conclusions: In high-risk patients with ALL ,haploid-matched and sibling-matched HSCT are similar in total outcome. haploid-matched donor can be suitable source of allogeneic stem cell transplantation.

LB2241

EXPLORE THE INNER RELATION AMONG THE MORPHOLOGY, IMMUNOPHENOTYPIC, MOLECULAR GENETIC FEATURES IN MIXED PHENOTYPE ACUTE LEUKEMIA

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Background: The mixed phenotype acute leukemia(MPAL) is rare leukemia. The biology of MPAL is unclear.
Aims: To analyze and explore the inner relation among the Morphology, immunophenotypic, cytogenetic, molecular genetic features and prognosis of MPAL.
Methods: MICM classification by morphology and cytochemistry, flow cytometric immunophenotyping,conventional cytogenetic,fluorescence in situ hybridization (FISH) and/or molecular genetic, was used to relrospectively study 2575 patients with AL from January 2008 to March 2015.
Results: The results showed that 30(1.16%) cases were diagnosed as MPAL,fulfilling WHO 2008 criteria. 13 cases of 30 MPALs'Leukemia cells presented the same kind of morphological characteristics,leukemia cells of the other 17 cases has two different morphological characteristics(The two kinds of cells were more than double in volume I. Maxicell: around 16 to 25 um, morphological characteristics similar to myeloid myeloblast and/or monoblast, POX positive II. Cellule:around 8 to 12 um,morphological characteristics similar to lymphocytes,but is not a classic myeloblast,monoblast or lymphoblast, Cytoplasm pseudopodia,burr shape change,drag the caudate,containing A particle or not, folded or irregular nuclei). Based on FAB criteria,12(40%) patients showed AML morphologies, mainly M1 and M5,the dominant subtype. 6(20%) patients were classified ALL,7(23.3%) patients were classified hybrid acute leukemia(HAL). The remaining 5(16.7%) cases resisted classification by morphology and were categorized as acute undifferentiated leukemia. Immunophenotyping data showed that 16 of 30 cases(53.3%) had combined B-lymphoid+myeloid immunophenotype(B+My),12(40%) combined T-lymphoid+myeloid immunophenotype(T+My), 1(3.33%) B+T- lymphoid immunophenotype (B+T), and in the remaining 1(3.33%) cases there was evidence of trilineage concomitant expression [myeloid, B, and T lymphoid(My+B+T)]. In addition, there are 1/16 cases of MY+B with T-lineage marker CD7+ not as trilineage. There were high expression of stem/progenitor cell markers CD123,CD34,CD38 and HLA-DR in MPAL;with the positive rate of 100%,93.3%,90% and 92.6%,respectively. A total of 11 (36.7%) had Ph chromosome and/or BCR-ABL fusion gene by FISH and/or RT-PCR. Median WBC of peripheral blood was $149x10^9$/L ranging between $1.46x10^9$/L and $797x10^9$/L.Compared with Ph-MPAL,it has statistical significance that combined B+My immunophenotype(P=0.001) and the peripheral blood was found to be immature granulocytes (promyelocytic, myelocyte and metagranulocyte) by smears for morphology (P=0.002), at the positive rate of 90.9%(10 of 11 cases) in Ph+MPALs. 7 cases of HAL by morphology associated with the BCR-ABL fusion gene(P=0.01),and these appeared as combined B+My immunophenotype. 22 of 30 patients were treated with at least 2 cycles of chemical drugs,with the complete response(CR) rate of 59.1%(13/22). This study found In these 30 cases of MPALs, according to our set of morphological classification scheme the ALL often appeared as combined B+My immunophenotype that was diagnosed by morphology;the combined T+My immunophenotype cases usually showed AML by morphological classification. While myeloid and lymphoid blast cells were simutalously seen in BM, high leukocyte and high immature granulocytes were seen in PB, and with B+My immunophenotype, it often hint MPAL with Ph chromosome or BCR-ABL fusion gene.
Summary/Conclusions: MPAL is a set of heterogeneous disease that originated in the stage of pluripotent stem cell and have directional differentiation potential,mainly presents the characteristic of myeloid differentiation.

Acute myeloid leukemia - Biology

E877

LPS STIMULATION INDUCES ROS FORMATION, APOPTOSIS, DNA DAMAGE AND OXIDATION OF CYSTEINE RESIDUES IN FLT3-ITD POSITIVE CELL LINES RESULTING IN FLT3-TYROSINE KINASE INHIBITOR RESISTANCE

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Background: Recurrent infections are associated with an increased incidence in the development of malignancies, including acute myeloid leukemia (AML). Inflammatory events directly induce formation of reactive oxygen species (ROS) and cause DNA damage, protein and lipid oxidation. In case of recurrent infections, bacterial peptides, like LPS, can lead to ROS induction. In particular, cysteine residues of proteins can directly be oxidized by ROS causing either loss or gain of function. However, it is still unclear how inflammatory events can induce these protein oxidation and to what extent the oxidation of cysteine residues in a subset of AML, the FLT3-ITD positive AML, causes a change of biological behavior.
Aims: The goal of our study was to characterize the role of LPS-induced oxidative stress in murine and human FLT3-ITD positive cell lines to identify responsible FLT3-ITD- and TLR related signaling pathways. To adress stress-induced cysteine oxidation of the FLT3-kinase, we generated cysteine-to-alanine mutants in murine myeloid 32D cells (FLT3-WT and FLT3-ITD) and investigated the induction of ROS formation, proliferation, cell cycle distribution and the response to TKI treatment (AC220, PKC412).
Methods: 32D cells stably transduced with either FLT3-WT or FLT3-ITD and human FLT3-ITD positive MV4,11 were stimulated with LPS (5-50 ng/ml) for 2h and with 10 ng/ml LPS for different time-points (0-90 min), respectively. ROS formation (H_2DCFDA method) and activation of FLT3-ITD and TLR-related signaling pathways were analyzed by immunoblotting. To analyze effects of chronic inflammation, FLT3-WT and FLT3-ITD positive cells were kept in culture for 12 days with repeated LPS stimulation (10 ng/ml every 48h). To address the role of cysteine oxidation within the FLT3-kinase, we mutated highly conserved cysteine residues C474 (extracellular domain) and C925 (intracellular domain) to alanine by site-directed mutagenesis and performed a retroviral transduction of 32D cells with the respective constructs. Subsequently, cysteine mutants were characterized for proliferation (MTT), ROS formation and their sensitivity to TKI treatment (AC220, PKC412; 5 to 50 nM, respectively) in comparison to standard FLT3-ITD mutant.
Results: LPS stimulation resulted in an increased time- and dose-dependent ROS induction in FLT3-ITD positive cells compared to WT cells (100ng/ml LPS for 2h: 6-fold). In addition, LPS stimulation resulted in a stronger activation of FLT3-ITD- and TLR-related signaling pathways in ITD-positive cells (Stat5, Akt, Erk, NF□B). Long-term LPS stimulation over 12 days induced an enhanced ROS formation, apoptosis and accumulation of □H2AX in both FLT3-WT, as well as in FLT3-ITD positive cells. To address the role of cysteine oxidation, we generated stable cysteine-to-alanine-mutants. However, the FLT3-ITD C925A mutation (intracelullar domain) resulted in an increase of ROS formation compared to the FLT3-ITD C474A mutation (extracellular domain; 25% increase). Furthermore, the C925A mutation resulted in a significant decrease in apoptosis after TKI treatment compared to non-mutated FLT3-ITD and the FLT3-ITD C474A mutant (AC220 50nM: 25%; PKC412 50nM: 40%). In addition, FLT3-ITD C925A mutant showed a reduced decrease in phosphorylation of Stat5 and Akt after short-term (1h) TKI treatment (AC220: 50, 100 nM; PKC412 10, 100nM) compared to non-mutated and FLT3-ITD C474A mutants.
Summary/Conclusions: Our study demonstrates that inflammatory events, like LPS stimulation, induce ROS formation, apoptosis and DNA damage in FLT3-WT and FLT3-ITD positive cells. This might lead to genetic instability and induction of critical mutations, e.g. within FLT3 kinase. Additionally, we could show that cysteine oxidation within the intracellular domain of FLT3-ITD resulted in a resistance to TKI treatment.

E878

ANALYSIS OF SNORNA EXPRESSION AND FUNCTION IN ACUTE MYELOID LEUKEMIA

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Background: Small nucleolar RNAs (snoRNAs) belong to the group of non-protein-coding RNAs with distinct functions in ribosomal RNA (rRNA) modification. snoRNAs are divided into the two major classes: box C/D and box H/ACA snoRNAs. SnoRNAs assembly with small nucleolar ribonucleoproteins (RNPs) and guide those snoRNPs to the rRNA, where they catalyze rRNA modifications. C/D box snoRNAs are involved in 2-O-methylation, whereas H/ACA snoRNAs guide pseudouridylation. Recent studies showed that snoRNAs play a role in cancerogenesis. Hence, we analyzed snoRNAs expression and function in leukemogenesis.
Aims: Investigation of snoRNA expression pattern and function in acute myeloid leukemia.
Methods: To understand the role of snoRNAs in leukemia, we analyzed the snoRNA expression pattern of 63 AML patients at time of diagnosis. Additionally we analyzed snoRNA expression patterns in AML1-ETO or Myc transduced lin$^-$ mouse bone marrow cells. Librarys for small RNA (40-200 nt) specific next generation sequencing (NGS) were prepared with TruSeq Small RNASample Prep Kit. NGS was performed on an Illumina HiScanSQ, using 50 cycles of single read sequencing. For functional analysis of single snoRNAs, we performed single Knockouts of 6 snoRNAs (SNORD14D, SNORD34, SNORD35A, SNORD43, SNORD53 and SNORD104) in Kasumi and MV4-11 cells using CRISPR/Cas9 technology. Mutations in the genomic sequence were confirmed by Sanger sequencing. Subsequently, expression of snoRNAs was analyzed by qRT-PCR. To analyze the effect of snoRNA knockout on colony formation ability of mutated cells, we embedded 300 cells/well in methylcellulose. Colonies were counted after 7-10 days.
Results: Next generation sequencing data revealed 229 C/D box snoRNAs and 90 H/ACA box snoRNAs were expressed in one or more patient samples. In further analyses, we compared clinical data and snoRNA expression pattern. SnoRNA expression patterns were associated with specific risk groups. High levels of snoRNA expression associated with intermediate molecular risk (269 snoRNAs, p≤0.05) and a poor response to chemotherapy (101 snoRNAs, p≤0.05). Further, we could show that snoRNAs are higher expressed in AML patient samples with NPM1 wildtype (124 snoRNAs, p≤0.05). Transduction of the oncogenes AML1-ETO9a or Myc to normal lin$^-$ mouse bone marrow cells induced an increased overall snoRNA expression. We found 93 snoRNAs downregulated and 88 upregulated during ATRA induced differentiation of HL60 cells. The functionally analyzed snoRNAs SNORD14D, SNORD34, SNORD35A, SNORD43 and SNORD53 were downregulated. CRISPR/Cas9 induced knockouts for SNORD14D, SNORD34, SNORD35A or SNORD43 significantly inhibited colony formation of Kasumi-1 cells. Also, loss of SNORD14D or SNORD35A decreased colony formation potential of MV4;11 cells. Loss of SNORD34, SNORD35A, SNORD43 and SNORD104 resulted in a significant reduction of 2´-O-methylation on the respective rRNA modification sides (28S U2824, 28S C4506, 18S C1703 and 28S C1327).
Summary/Conclusions: SnoRNA expression profiles are altered in specific AML subtypes. Further, we identified a functional relevance of snoRNAs in cell growth of leukemic cells.

E879

PERSISTENCE AND EXPANSION OF HEMATOPOIETIC CLONES IN POST CHEMOTHERAPY AML ASSESED BY TARGET SEQUENCING

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Background: Gene variance screening has demonstrated the persistence of pre-leukemic hematopoietic stem cells (HSC) in AML patients during complete morphological remission (CR) (*Corces-Zimmerman et al, Proc Natl Acad. 2014 and Terrence et al, Blood. 2015)* but very little is known about their gene profile, dynamics and/or clinical associations.
Aims: Assess the dynamics of the gene variance profile and its clinical correlation in 20 AML patients who achieved CR after chemotherapy treatment.
Methods: Bone marrows samples from 18 *de novo* AML and 2 secondary AML patients were analysed at diagnosis and monitored for 2 to 34 months post chemotherapy initiation. 19 patients received induction/re-induction chemotherapy including both cytarabine and an anthracycline or purine analog, 1 patient received a non intensive chemotherapy consisting of a low dose cytarabine (LDAC) composed regimen. Targeted exon sequencing of 54 genes was carried out using a commercial panel (*Illumina TruSight, myeloid panel*) with exons fully covered for 17 genes, while a set of specific exonic locations targeted in the other 37 genes. Non-synonymous gene variances with a minimum of 500 reads and ≥5% frequency were reported. Variants were identified using Illumina bio-informatics pipeline augmented by COSMIC, SNP library (dbSNP137), OMIM, 1000 genomes and published data.
Results: Of the 20 patients analysed, 15 patients showed persistence of clonal haematopoiesis with some gene variances reported as 'polymorphic' and 'tolerated', considered to represent CHIP (*Genovese et al., N Engl J Med 2014; McKerrell et al., Cell Reports 2015; Steensma et al., Blood 2015)*. None of the AML 'driver type' variances seen at diagnosis were detected in the CR samples. Certain variances (in order of frequency) in *DNMT3A, TET2, CBLB, ASXL1, ZRSR2, SRSF2, BCORL1, CEBPA, CUX1, CARL, ETV6, CBLC, ABL1* and *HRAS* genes were found to form three distinct patterns in CR: a) expansion of novel variances concurrent to the persistent diagnostic gene profile (10/20), b)

persistence of unique variances seen at diagnosis without novel additions (9/20) and c) no gene variances present after chemotherapy (1/20). Two out of 10 patients from group (a) relapsed during the follow up period with reappearance of the initial *FLT3_ITD* bearing clone or occurrence of a novel *IDH1* variance (p.R132C) respectively, while the rest remained in CR. Interestingly, a patient with a missense *TP53* variance (*Protein var: NP_000537.3: p.Cys238Tyr*) at diagnosis had a clearance of this *TP53* AML clone after being treated with LDAC composed regimen.
Summary/Conclusions: We report an expansion of hematopoietic clonal populations in 10 out of 20 patients analysed by target next generation sequencing. These results indicate that cell populations harbouring unique age related variances may have a competitive survival advantage after chemotherapy although the clinical importance this phenomenon requires further investigations. Accidentally we came across a promising treatment with LDAC and aminopeptidase inhibitor for unfit AML patients harbouring the classical TP53 mutation. Our findings are in alignment with recent studies showing persistence of clonal haemopoiesis in AML during CR, which warrants the need to monitor residual disease using appropriate gene panel.

E880

IDENTIFICATION OF NOVEL PUTATIVE SNORNAS IN AML PATIENT SAMPLES

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Background: Small nucleolar RNAs (snoRNAs) are a large group of non-coding RNAs. Mostly snoRNAs are located in introns of host genes. They are highly abundant in eukaryotes and are divided into two major families, box C/D and box H/ACA snoRNAs, based of common sequence motifs and structural features. The box C/D snoRNAs carry the conserved boxes C (RUGAUGA, R=purine) and D (CUGA) near their 5' and 3' ends, respectively. The H/ACA box snoRNAs consist of two hairpins and two short single-stranded regions, which contain the H box (ANANNA) and the ACA box. snoRNAs guide small nucleolar ribonucleoproteins (snoRNPs) to complementary regions of ribosomal (rRNA) or small nuclear RNAs (snRNA), where the snoRNP complexes catalyze ribosomal modifications. Recent studies showed the relevance of snoRNAs for the pathogenesis of cancer.
Aims: Identification and analysis of unknown snoRNAs in AML.
Methods: To understand the role of snoRNAs in leukemogenesis, we analyzed the snoRNA expression pattern of 63 AML patients, registered in a recent clinical trial (ClinicalTrials.gov NCT00915252). RNA was isolated with Qiagen miRNeasy Kit. Librarys for small RNA specific next generation sequencing (NGS) were prepared with TruSeq Small RNASample Prep Kit and size separation of 40-200 nucleotides. NGS was performed on an Illumina HiScanSQ, using 50 cycles of single read sequencing. Sequencing data were processed with Cutadapt, mapped with Bowtie 2 to the human genome (hg19). Abundant regions were detected with Bedtools genomeCoverageBed and were annotated using snoRNABase (LBME), DASHR, UCSC snoRNA and repeatmasker track. For further analysis reads of discovered regions were calculated as reads per million (RPM). Regions without annotation were analyzed for C/D box motifs to discover putative C/D box snoRNAs.
Results: Next generation sequencing data revealed that 229 of the 269 known C/D box snoRNAs (85%) were expressed in one or more patient samples. For the H/ACA box snoRNAs we could identify 90 of 112 (80%). In addition we found 1396 mapped regions, which could not be annotated as known small noncoding RNAs. *In silico* analysis identified 90 putative C/D box snoRNAs. All of these putative snoRNAs contain the box C/D motifs and were mostly located in introns of known genes. In further analysis we compared clinical data and snoRNA expression pattern. Here we found that expression of the known snoRNAs clustered with specific risk groups. High levels of snoRNA expression are associated with intermediate molecular risk grouped patients (269 snoRNAs, $p \leq 0.05$) and a poor response to chemotherapy (101 snoRNAs, $p \leq 0.05$). Likewise, increased expression of putative snoRNAs was found in patients with an intermediate molecular risk (51 snoRNAs, $p \leq 0.05$) and in patients with a bad response to chemotherapy (20 snoRNAs, $p \leq 0.05$). To investigate putative snoRNAs during myeloid differentiation we treated HL60 cells with 1µM all-transretinoic acid (ATRA) and analyzed their expression (d0 *vs* d6). In the HL60 cells we could recover 83 putative snoRNAs out of 90 we found in the patient samples. The data show that 45 putative snoRNAs were downregulated (<0.7) while 17 putative snoRNAs were upregulated (>1.3) during myeloid differentiation. To analyze the binding of 8 putative snoRNA candidates to the snRNP complex we performed NOP58 and FBL specific RNA immunoprecipitation (RIP) in Kasumi-1 cells. Here, we could show an enriched binding of 2 putative snoRNAs to FBL and NOP58.
Summary/Conclusions: In summary, our data show that snoRNAs may contribute to neoplastic transformation. Further we identified novel unknown snoRNAs, which will be further analyzed.

E881

INHIBITION OF CXCR4 BY THE HIGH AFFINITY ANTAGONIST BL-8040 IN AML DOWNREGULATES BCL-2 THROUGH REGULATION OF MIR-15A/16-1 EXPRESSION

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Background: Acute Myeloid Leukemia (AML) is a heterogeneous group of diseases characterized by uncontrolled proliferation and survival of hematopoietic stem and progenitor cells. The chemokine CXCL12 and its receptor CXCR4 are key players in the retention of AML blasts in the protective bone marrow (BM) microenvironment. The CXCL12/CXCR4 axis is also critical for the survival and maintenance of AML blasts in their stemness state. CXCR4 overexpression is associated with poor prognosis in AML patients. Signaling activated through CXCR4 was shown to be detrimental by increasing survival of tumor cells and promoting resistance to therapy.
Aims: We have studied the effect of the CXCR4-antagonist, BL-8040 on the survival of human AML blasts and investigated the molecular mechanism by which inhibition of CXCR4 signaling leads to leukemia cell death.
Methods: Human AML cell line and human primary AML samples were used for in vitro studies. The in-vivo effect of BL-8040 was tested using MV4-11, U-937, THP-1 and human primary AML cells engrafted in NOD scid gamma (NSG) mice.
Results: We found that the human AML cells MV4-11, THP-1, U-937, and human primary AML cells express high levels of CXCR4. The CXCR4 antagonist, BL-8040 was found to induce apoptosis of the tested AML cells in-vitro and in-vivo. Survival of tested AML cells was found to be dependent on BCL-2 as demonstrated by the ability of the BCL-2 inhibitor, ABT-199 to induce dose dependent apoptosis in vitro. Interestingly, treatment of AML cells with BL-8040 reduced the expression of BCL-2 together with inhibition of the ERK signaling pathway. BL-8040 dependent reduction in BCL-2 expression was associated with increased expression of miR-15a/16-1. In addition to BCL-2, increased expression of miR-15a/16-1 reduced the expression of MCL-1 and cyclin D, its other target genes. Interestingly, these effects were not observed following stimulation with the CXCR4 antagonist, AMD3100. In support of these results overexpression of miR-15a/16-1 in AML cells was shown to induce their apoptosis. In the MV4-11 in-vivo AML model, when BL-8040 was administrated for 7 constitutive days we observed decreased number of AML cells accompanied with apoptosis in BM, Spleen and blood. Following one or two injections of BL-8040 apoptosis of AML blasts was observed together with upregulation of miR-15a/16-1 and decrease of BCL-2, only in the spleen. Importantly, Human AML cells engrafted to the spleen expressed lower levels of BCL-2 as compared to AML cells in the BM suggesting that the BM required prolonged inhibition in order to overcome the high levels of BCL-2 in this microenvironment.
Summary/Conclusions: Our results demonstrate that CXCR4 signaling regulates the expression of miR-15a/16-1 and their target genes BCL-2, MCL-1 and cyclin-D1. Furthermore, these results indicate that the CXCR4 antagonist, BL-8040 may tip the balance toward cell death by down- regulating survival signals through miR-15a/16-1 pathway and inhibition of the ERK signaling cascade in AML cells.

E882

AML ORCHESTRATES MITOCHONDRIAL METABOLISM IN BONE MARROW MESENCHYMAL STROMAL CELLS THROUGH AN INCREASE OF PGC-1A

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Background: Acute Myeloid Leukaemia (AML) is biologically a heterogeneous disease which results from defects in haematopoiesis, characterised by the accumulation of haematopoietic myeloid cells in the bone marrow. Although there have been advances in the treatment of the disease, AML remains incurable for at least 80% of patients. It has been shown that AML cells have an increased mitochondrial mass and higher levels of oxidative phosphorylation (OXPHOS) compared to normal CD34+ cells, suggesting a dependency of OXPHOS for AML survival. Little is known about the mitochondrial activity of bone marrow mesenchymal stromal cells (BM-MSC), which provide a supportive environment for AML cells in the bone marrow.
Aims: Therefore the aim of this study is to determine if there is an increase in mitochondrial respiration in BM-MSCs from AML patients (AML BM-MSCs) compared to non-AML patients (normal BM-MSCs) and to understand how the difference is regulated.
Methods: Primary AML blasts were obtained from patient bone marrow. Primary AML and normal BM-MSCs were isolated from patients bone marrow, with informed consent and under approval from the UK National Research

Ethics Service (LRCEref07/H0310/146), using adherence. BM-MSC were characterised using flow cytometry for expression of CD90+, CD73+, CD105+ and CD45-. Mitochondrial respiration and glycolysis rates were measured using the Seahorse XF24 extracellular flux analyser. RT-qPCR and western blotting were used to determine peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) expression and regulation. Lentiviral mediated knockdown was also used.
Results: Our results show that AML BM-MSCs have an increased ATP production after 3 days compared to normal BM-MSCs. AML BM-MSCs also show an increased oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) compared to normal BM-MSCs. This demonstrates not only an increase in mitochondrial respiration but also increased glycolysis, which correlates with the increased ATP production of the AML BM-MSCs compared to normal BM-MSCs. Mitochondrial respiration and glycolysis increase in AML BM-MSCs after co-culture with primary AML cells. This suggests that exposure to AML cells cause the increased mitochondrial respiration and glycolysis observed in the AML BM-MSCs. There is differential expression of PGC-1α mRNA and protein in AML BM-MSCs after co-culture with AML cells, suggesting regulation of mitochondrial respiration through PGC-1α.
Summary/Conclusions: These results provide evidence of an increased mitochondrial respiration and glycolysis in the AML BM-MSCs compared to the normal BM-MSCs and that the difference is orchestrated by the AML cells. In addition this difference is regulated through PGC-1α, the master regulator of mitochondrial biogenesis. We hypothesise that therapeutic intervention targeting mitochondrial activity in the AML micro-environment would make AML more susceptible to current treatment regimes.

E883

PML-RARA REGULATES AKT THROUGH HSP90 INHIBITION IN APL BLASTS

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Background: HSP90s are molecular chaperons required for conformational stabilization and trafficking of over 400 documented client proteins involved in cell growth, maturation and apoptosis. The HSP90β isoform is constant ubiquitously, whereas α/α1 isoforms are inducible, prone to increase in stress conditions. Functional Hsp90 is required for the stability of the AKT, serine - threonine kinase, which is phosphorylated in response to growth factor stimulation. Activated AKT migrates transducing the signal to over 130 substrates in the cytoplasm and nucleus playing a crucial regulatory role in cell differentiation, cell cycle, transcription, translation, metabolism and apoptosis. In acute myeloid leukemia (AML) higher levels of HSP90s have been associated with poor prognosis.
Aims: The aim of this work is to comprehensively analyze the involvement of HSP90 in pathogenesis of APL.
Methods: Seventeen APL and 22 unselected AML patients were analyzed by quantitative PCR to measure *HSP 90* mRNA expression levels. HSP90 protein levels were also analysed in 21 AML and 20 APL cases using Western Blot. In addition, HSP90 protein levels were studied in: I) PR9 cells, a Zn inducible PML/RARA cell line; II) APL patient primary blasts and PML/RARA expressing NB4 cells after treatment with ATRA. ChIP-qPCR was used to scan HSP90 regulatory sequences for the presence of PML-RARα protein. AKT mRNA and protein levels were analysed in 16 APL and 10 AML patients. *AKT* mRNA and AKT phosphorylation levels were studied in PR9 cells line plus Zn and in NB4 cells treated with 17AAG, ATRA or both. AKT half-life and ubiquitinilation levels were study using Hek293T cells transfected with PML-RARα. Localization of Hsp90 and AKT were analysed using a Confocal microscopy in PR9 cells line plus Zn.
Results: We observed a significantly lower expression of HSP90 and AKT in primary APLs, as compared to other AMLs. In vitro treatment of the NB4 cell line with ATRA upregulated HSP90s and AKT protein levels. Inhibition of HSP90 function by the 17 AAG inhibitor induced a decline of AKT and nullified the effect of ATRA indicating that HSP90 action is necessary for AKT stability. Using an inducible PML/RARα model (PR9 cell line) we observed a decline of HSP90 mRNA and protein starting at 2/4 hours, corresponding to the expression peak of the hybrid protein. AKT protein, but not mRNA, declined to 30% at 24 hours. Using confocal microscopy we observed the translocation to the cytoplasm of co-localized HSP90 and AKT proteins, 8 hours after PML/RARα induction. In Hek293T cells transfected with PML-RARα we observed ubiquitination and down-regulation of AKT. ChIP assays showed the binding of PML-RARα to the HSP90-α and β promoter regions, resulting in downregulation of expression, effect reverted by ATRA. We further detected an increase in acetylation of the DNA promoter region of the two isoforms of HSP90 after treatment of NB4 cells with ATRA, suggesting that PML-RARα inhibits HSP90s expression at the transcriptional level probably through recruitment of the HDAC-repressor complex NCOR.
Summary/Conclusions: In summary, repression of HSP90 expression by PML/RARA is associated in APL cells with loss of specific function in controlling AKT protein phosphorylation, intracellular trafficking and stability.

E884

RUNX1-MUTATED AML SHOW A HIGH FREQUENCY OF TRISOMY 8 AND TRISOMY 13 AND ARE ASSOCIATED WITH MUTATIONS IN ASXL1 AND COMPONENTS OF THE RNA-SPLICING MACHINERY

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Background: Mutations in *RUNX1* have been reported in 5 to 20% of AML. A detailed cytogenetic and molecular genetic analysis of *RUNX1*-mutated AML has not been performed yet.
Aims: 1. Comprehensive cytogenetic and molecular genetic characterization of AML with *RUNX1* mutations. 2. Analysis of potential impact of the respective markers on prognosis.
Methods: The cohort comprised 140 cases of AML with *RUNX1* mutations (95 male, 45 female). Median age was 67 years (range: 18-87 years). All patients were investigated using chromosome banding analysis (CBA). Mutation analyses by amplicon sequencing were performed for *ASXL1, BCOR, CBL, CEBPA, DNMT3A, ETV6, EZH2, FLT3*-ITD, *FLT3*-TKD, *GATA2, IDH1, IDH2, KIT, KRAS, MLL*-PTD, *NPM, NRAS, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1* and *WT1*. Variants of unknown significance were excluded from statistical analysis.
Results: CBA revealed a normal karyotype in 83/140 (59%) and an aberrant karyotype in 57/140 (41%) of patients: 37 (27%) cases harbored trisomies, 20 cases (14%) showed other aberrations. In more detail, +8 was detected in 17 cases (12%), followed by +13 (11 cases, 8%), +11 (4 cases, 3%) and +14 (4 cases, 3%). No other recurrent abnormalities were observed. A complex karyotype (>3 abnormalities) was not detected. The highest mutation frequency was observed for *ASXL1* (42%), followed by *SRSF2* (36%), *BCOR* (21%), *FLT3*-ITD (18%), *TET2* (18%), *IDH2* (17%) and *U2AF1* (16%). Mutation frequencies >5% were also detected for *DNMT3A* (14%), *MLL*-PTD (14%), *NRAS* (13%), *WT1* (12%), *IDH1* (9%), *SF3B1* (9%) and *CEBPA* (5%). Thus, mutations in genes coding for components of the RNA-splicing machinery were detected in 89 patients (64%). No *CEBPA* double mutations were identified. Totally, sequencing revealed 463 mutations in addition to *RUNX1* (mean number of additional mutations per patient: 3). In detail, 3 patients (2%) showed no, 18 (13%) one, 44 (31%) two, 43 (31%) three, 22 (16%) four, 9 (6%) five and 1 (1%) six concomitant mutations. Thus, in 98% of patients at least one molecular mutation additional to *RUNX1* was observed. Correlation analyses between cytogenetic and molecular genetic markers revealed a strong association of trisomies with mutations in splicing factor genes (spliceosome mutations in patients with *vs* without trisomies: 33/37 (89%) *vs* 55/103 (53%), p<0.001). Additionally, cases with +8 correlated with *ASXL1* mutations (*ASXL1*mut in patients with *vs* without +8: 11/17 (65%) *vs* 45/122 (37%), p=0.036). An association was revealed for a normal karyotype (NK) with mutations in *FLT3*-ITD (with NK: 20/83 (24%) with *FLT3*-ITD; without NK: 5/57 (9%) with *FLT3*-ITD, p=0.024). In the total cohort, median overall survival (OS) was 29 months. An aberrant karyotype had no influence on OS. However, mutations in *U2AF1* and *NRAS* both were associated with a significantly shorter OS (median OS, for *U2AF1* mutated *vs* unmutated, 21 *vs* 33 months; p=0.039; for *NRAS* mutated *vs* unmutated, 12 *vs* 31 months, p=0.026) and in patients with ≥3 accompanying mutations (≥3 *vs*<3 mutations, 20 *vs* 57 months, p=0.002).
Summary/Conclusions: *RUNX1*-mutated AML show a NK or specific cytogenetic abnormalities (+8 or +13) and a lack of a complex karyotype. Moreover, they depict a typical pattern of additional molecular mutations with a high frequency of *ASXL1* mutations and mutations in spliceosome genes (especially *SRSF2* and *U2AF1*), which according to Lindsley et al. (Blood 2015) is specific for secondary AML. Further, no entity-defining genetic abnormalities were observed. Thus, *RUNX1*-mutated AML might qualify for a separate entity.

E885

REACTIVE OXYGEN SPECIES (ROS) MODULATES SENSITIVITY TO FLT3-TYROSINE KINASE INHIBITORS IN MURINE AND HUMAN FLT3-ITD POSITIVE AML CELL LINES

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Background: FLT3-ITD positive acute myeloid leukemia (AML) is characterized by an increased concentration of intracellular ROS and high extracellular concentrations of hydrogen peroxide (H_2O_2). Increased ROS levels regulate numerous cellular processes, such as cell cycle, apoptosis, metabolism and drug resistance. Interestingly, in leukemia driven by other oncogenes (e.g. Bcr-Abl) it has been described that modulation of ROS may improve the response to selective tyrosine kinase inhibitor (TKI) treatment. Some pathways, including STAT5/NOX4, have been reported to induce high ROS levels in FLT3-ITD-positive AML. However, it is unclear how FLT3-ITD positive AML cells modulate their cellular functions and/or drug resistance by auto-/paracrine ROS species. In addition, in leukemia driven by other oncogenes (e.g. Bcr-Abl) it has been described that modulation of ROS may improve the response to selective tyrosine kinase inhibitor (TKI) treatment.

Aims: Our goal was to identify the auto-/paracrine effects of hydrogen peroxide on proliferation and cell cycle progression in FLT3-ITD positive murine and human cell lines. In addition, we aimed to characterize the effects of H_2O_2 modulation with respect to the sensitivity to TKI treatement as a possible resistance-inducing factor.
Methods: Murine (32D, BaF3) and human (MOLM-13, MV4-11) FLT3-ITD positive cell lines were analyzed for intracellular ROS levels using H_2DCFD, extracellular H_2O_2 concentration and activities of the scavenging enzymes SOD1, SOD2, catalase, and glutathione peroxidase (GPx). For the analysis of the role of extracellular H_2O_2, cells were incubated directly with H_2O_2 (10 µM) for 2h and assayed for the activation of FLT3-ITD-dependent signaling pathways (STAT5, Akt and Erk) using immunoblotting. For the analysis of the effects of H_2O_2-generating enzyme glucose oxidase and of the H_2O_2-degrading enzyme catalase, cells were cultured for 48h and assayed for proliferation (MTT) and cell cycle progression (PI). Furthermore, the effect of extracellular H_2O_2 concentration on TKI treatment (AC220, PKC412; each 5 to 50 nM) was analyzed by FACS.
Results: Stimulation of FLT3-ITD positive cells using H_2O_2 resulted in a strongly increased time-dependent activation of FLT3-ITD-associated signaling pathways (STAT5, Akt, Erk) as compared to untreated cells. This difference was more pronounced in ITD-positive cells in comparison to WT cells particularly for AKT activation. In addition, stimulation with H_2O_2 also increased the activities of SOD1/2 and catalase. The long-term increase in extracellular H_2O_2 concentration was mimicked experimentally by addition of glucose oxidase to the culture medium (0.75 U/ml). Interestingly, this resulted in enhanced proliferation and cell cycle progression, whereas cultivation with catalase led to decreased proliferation and cell cycle arrest. Combined treatment with catalase and TKIs (AC220, PKC412; 5 and 10 nM, respectively) resulted in a significantly higher rate of apoptosis. Of note, the response to both TKIs was significantly reduced when glucose oxidase (0.75 U/ml) was added to the medium. The decrease in apoptosis was associated with stabilization of the mitochondrial potential (TMRE assay), stable expression of the anti-apoptotic proteins Mcl-1 and Bcl-XL, and a reduced cleavage of caspase 3 and caspase 8.
Summary/Conclusions: Our results strongly suggest that H_2O_2 acts as an auto-/paracrine stimulator of proliferation and cell cycle progression in FLT3-ITD positive cells. Furthermore, we could characterize H_2O_2 as a regulatory element with respect to TKI sensitivity. Our results indicate that combined inhibition of FLT3-ITD and H_2O_2 reducing agents/enzymes, like catalase, may have potential clinical application in treatment of FLT3-ITD positive AML patients.

E886

COMBINING DOT1L INHIBITOR EPZ-5676 WITH SORAFENIB TO TREAT MLL-REARRANGED (MLL-R) PEDIATRIC ACUTE MYELOID LEUKEAMIA (AML)

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Background: About 20% of the childhood AML present MLL gene translocations, that are associated with a very poor prognosis. That is why there is a strong interest in developing novel therapeutic strategies for these patients. As DOT1L is responsible for H3K79 methylation and is associated with MLL-r leukemogenesis, DOT1L inhibitors entered in clinical trials as promising treatments. MLL-r AML also showed an overexpression of FLT3 receptor tyrosine kinase. Therefore, FLT3 inhibitors, such as the multi-tyrosine kinase inhibitor Sorafenib, demonstrated encouraging efficacy in AML.
Aims: To investigate the efficacy of a combination treatment using DOT1L inhibitor EPZ-5676 and Sorafenib to treat MLL-r AML.
Methods: MLL-r (MOLM13, NOMO1, THP1) and non MLL-r (OCI-AML3, HL60 and U937 harboring CALM-AF10 translocation) AML cell lines, and MLL-r primary samples from pediatric AML patients were used. Flow cytometry analyses were performed to assess absolute cell counting and apoptosis (AnnexinV FITC/PI staining). Protein expression and H3K79 methylation were quantified by Western blot. mRNA expression was studied by quantitative Real-Time PCR (Q-PCR).
Results: Firstly, the specific effects of DOT1L inhibition were examined in AML cell lines treated with increasing concentrations of EPZ-5676, up to 18 days. Cell growth was significantly inhibited after at least 8 days of treatment in MOLM13 and NOMO1 cells, but time-dependent apoptosis occurred only in MOLM13, thus suggesting that DOT1L inhibition alone might not be able to induce cytotoxicity. Whereas no effects were observed in HL60, U937 (non MLL-r), or THP1 (MLL-r) cells, both cell growth impairment and apoptosis were detected in non MLL-r OCI-AML3 cells, so that the impact of DOT1L inhibition could not exclusively rely on MLL rearrangements. Repression of DOT1L occurred since the 4th day of treatment, as demonstrated by the complete loss of H3K79me2. To further explore the consequence of this phenomenon, both MLL targets and key component of signaling pathways involved in AML survival (i.e. PI3K, FLT3 and MAPK) were investigated in cells treated with 1 uM EPZ-5676, up to 28 days. Gene expression of *HOXA9*, *MEIS1*, *FLT3* and CDK6 protein (MLL target) decreased in all cell lines, whereas *STAT5* and c-*Myc* mRNAs, along with STAT5 protein expression, were downregulated only in MLL-r cells. Furthermore, in MOLM13 and NOMO1 cells p-Erk was strongly reduced. Finally, p-Akt slightly decreased in nearly every case, whereas a strong induction in p-S6RP was observed only in U937 cells after prolonged treatment, suggesting the possible involvement of PI3K pathway in drug resistance mechanisms. To increase the benefit of DOT1L inhibition, both AML cell lines and MLL-r primary samples were pretreated with increasing concentration of EPZ-5676 for 4/8 days, following 24/48h treatment with Sorafenib. This combination resulted in a synergistic effect in nearly all cases. Importantly, EPZ-5676 pretreatment increased Sorafenib-induced cell growth inhibition in EPZ-5676 refractory cells HL60, U937 and THP1. Moreover, in primary AML samples both EPZ-5676 and Sorafenib showed a limited effect as single agent, whereas their combination induced a drastic increase of apoptosis.
Summary/Conclusions: These results demonstrated that the single administration of EPZ-5676 has a limited antileukemic activity, which is not restricted to MLL-r AMLs. However, the combination of EPZ-5676 with Sorafenib revealed a synergistic effect in both MLL-r and non MLL-r AMLs, paving the way to innovative and more effective treatments for pediatric AML patients.

E887

WHOLE EXOME SEQUENCING IDENTIFIES NOVEL MUTATIONS IN RELAPSED OR REFRACTORY ACUTE PROMYELOCYTIC LEUKAEMIA (APL) UNRESPONSIVE TO ORAL ARSENIC TRIOXIDE

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Background: Arsenic trioxide (As_2O_3)-based regimens induce remissions in >90% of relapsed APL. Further relapses after As_2O_3-induced remissions portend a poor prognosis. With the use of As_2O_3 in frontline treatment of APL, relapses in patients previously treated with As_2O_3 resulting in arsenic resistance is an emerging clinical problem. There is a paucity of information on the molecular mechanisms of As_2O_3-resistance.
Aims: To determine mutations associated with As_2O_3-resistance with whole exome sequencing (WES).
Methods: Seven patients with prior As_2O_3 treatment, who had ≥2 subsequent relapses occurring during or refractory to As_2O_3 treatment, were assigned to the discovery cohort. Serial bone marrow (BM) samples that were arsenic-sensitive (taken before treatment in a relapse responding to As_2O_3) and arsenic-resistant (obtained in subsequent relapses refractory to As_2O_3) underwent WES the illumina HiSeq 1500 platform at an average depth of 100X. Exome data were analysed utilizing bioinformatic pipeline programs designed to identify single nucleotide variants (SNVs) that were present either only or at increased frequencies in the resistant samples. The functional significance of non-synonymous missense mutations was evaluated using SIFT, Polyphen2 and FATHMM programs. Putative SNVs were confirmed by Sanger sequencing. Confirmed SNVs were tested in arsenic-resistant BM samples obtained from a validation cohort of 22 relapsed patients previously salvaged with As_2O_3, who had subsequent As_2O_3-refractory relapse again. Clinicopathologic features, karyotype, *FLT3* and *PML-RARA* mutations were determined in the validation cohort.
Results: In the discovery cohort, serial WES showed following confirmed SNVs of potential functional significance showed increased allele frequency during As_2O_3-resistance: *NOTCH2, FOXD4L5, KCNJ11/18, MADCAL1, CBR3, NSD1, FLG1, CCCDC179, SIGLEC11, ROBO4, CISD2, WT1, PTCH1, KMT2D and MED17*. *PML* B2 domain mutations were not seen. The frequencies of the mutations were validated in a cohort of 22 patients with relapsed or refractory APL following oral As_2O_3-based treatment. The recruited patients comprised 14 men and 8 women with a median age of 44.5 (range:24-76) years at relapse. 1 (5%) patient had microgranular variant of APL while 2 patients (9%) had therapy-related APL. Additional karyotypic abnormalities were seen in 3 (14%) patients.14 patients (64%) had central nervous system (CNS) involvement at relapse. Internal tandem duplication of *FLT3* (*FLT3*-ITD) was detected in 7 (32%) patients. The following recurrent mutations were found in As_2O_3-refractory relapse BM samples: *NOTCH2* (n=17, 77%), *FOXD4L5* (n=22, 100%), *KCNJ11/18* (n=14, 64%), *MADCAM1* (n=15, 68%), *CBR3* (n=17, 77%), *NSD1* (n=17, 77%), *FLG1* (n=20, 91%), *CCCDC179* (n=7, 32%), *SIGLEC11* (n=5, 23%), *ROBO4* (n=3, 14%), *CISD2* (n=3, 14%), *WT1* (n=1, 5%), *PTCH1*(n=1, 5%), *KMT2D* (n=1, 5%) *and MED17* (n=6, 27%).
Summary/Conclusions: Mechanisms other than *PML* B2 domain mutations may account for As_2O_3 resistance. Novel genes regulating the cell signaling and apoptotic pathways, cellular proliferation, histone modification, DNA repair and angiogenesis were frequently mutated in As_2O_3-refractory APL. Further functional validation on the role of these mutations in conferring As_2O_3 resistance is required.

E888

MOLECULAR LANDSCAPE OF PRIMARY AND RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Acute promyelocytic leukemia (APL) with t(15;17)(q22;q12)/*PML-RARA* accounts for ~5% of adult AML. Two therapeutic agents, ATRA and arsenic trioxide (ATO), induce differentiation of promyelocytes in vivo resulting in complete remission. However, some patients still relapse after ATO-ATRA based treatments and the mechanisms associated with resistance to these agents are still poorly understood.
Aims: The evaluation of the mutational spectrum of APL both at initial diagnosis and relapse.
Methods: We analyzed a cohort of 124 de novo APL cases including 14 cases who showed hematologic or molecular relapse. All patients (pts) were proven to have t(15;17)(q22;q12)/*PML-RARA* by chromosome banding analysis (CBA) (n=114), fluorescence in situ hybridization (n=120) and/or RT-PCR (n=124). 69 pts were male and 55 pts were female with a median age of 47 years (range: 16-84 years). All 124 samples were analyzed by next generation sequencing (NGS) using a 24-gene panel targeting *ASXL1, CBL, CSF3R, DNMT3A, ETNK1, ETV6, EZH2, FLT3*-TKD*, GATA2, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1* and *ZRSR2*. *FLT3*-ITD was analyzed by gene scan. Relapse samples were screened for mutations within the *PML-RARA* fusion transcript by direct Sanger sequencing.
Results: Using standard CBA, additional cytogenetic aberrations were observed in 44/114 pts (27%). The most frequent secondary chromosome aberration was +8 (14/44; 32%). Using NGS 77/124 pts (62%) had at least one mutation (mut) in addition to *PML-RARA*, 17/77 (22%) had ≥2 additional mut (maximum: four). Most common were mutations in *FLT3* (*FLT3*-ITD: 47/124, 38%, *FLT3*-TKD: 15/124; 12%) and *WT1* (13/124, 10%). Mutations in other genes (*ASXL1, CBL, DNMT3A, ETV6, KRAS, NRAS, RUNX1, SF3B1* and *TET2)* were found in less than 10% of cases, respectively. Comparing the initial mutational pattern of pts who relapsed during the course of disease with those staying in molecular remission revealed no difference in concomitant molecular mutations. Solely the percentage of pts with concomitant mutations was higher in the relapse group (79% *vs* 60%; n.s.). Survival analysis revealed no influence of concomitant mutations in individual genes on prognosis. Also the amount of additional mutations had no prognostic impact. In selected cases (n=14/124), we compared changes in the patterns of genetic lesions between initial diagnosis and relapse. In 8/14 (57%) pts the initial mutation pattern changed at relapse. Mutations gained at relapse were *WT1* (2/14, 14%) followed by *DNMT3A*, *ETV6*, *FLT3*-TKD, *RUNX1* and *TP53* (each 1/14, 7%). Loss of mutations was observed in *FLT3*-TKD, *WT1* (each 2/14, 14%) as well as in *ASXL1, FLT3*-ITD and *NRAS* (each 1/14, 7%). In 4/14 relapsed pts (29%), mutations within the *PML-RARA* fusion transcript were detected. In none of the cases these mutations were detectable at initial diagnosis and thus all were acquired mutations. Missense mutations in the ligand binding domain of *RARA* were detected in 3 relapsed pts (p.Arg272Gln, p.Arg276Trp and p.Ser287Leu). In one relapsed APL pt, a mutation in the B2 domain of *PML* was observed (p.Ala216His). All mutations occurred in post-ATRA-relapse.
Summary/Conclusions: 1) 62% of APL cases carry additional molecular mutations. 2) The most frequent additional molecular mutation is *FLT3*-ITD (38%) followed by *FLT3*-TKD mutations (12%) and mutations in *WT1* (10%). 3) In 57% of relapsed APL the molecular mutation pattern changes. 4) Acquired mutations within the *PML-RARA* fusion transcript are detected in 29% of relapsed APL patients.

E889

ACTIVATION OF NRF2 IN BONE MARROW MESENCHYMAL STROMAL CELLS MEDIATES SURVIVAL AND CHEMOTHERAPY RESISTANCE IN ACUTE MYELOID LEUKAEMIA BLASTS

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Background: The bone marrow microenvironment and the cells with in it play a critical role in acute myeloid leukaemia. NRF2 regulates a range of cytoprotective transcriptional responses and acts as a master regulator of oxidative and electrophilic stress. We have previously shown that NRF2 forms part of a major cell survival pathway in protecting the AML blasts from chemotherapy induced apoptosis. Here we hypothesise that NRF2 also functions to regulate pro-survival factors in Bone Marrow - Mesenchymal Stromal Cells (BM-MSC) from patients with AML and investigate whether targeting NRF2 in the BM-MSC could enhance the efficacy of cytotoxic chemotherapy in AML.
Aims: In this study we aim to characterise the NRF2 signalling pathway in the BM-MSC and investigate how NRF2 signalling regulates oxidative stress in BM-MSC-AML niche.
Methods: Primary AML blasts and BM-MSC were isolated from AML patients following informed consent and under approval from the UK National Research Ethics Service (LRCEref07/H0310/146). Cytarabine, daunorubicin and hydrogen peroxide (H2O2) were used to induce NRF2 activation in AML blasts and BM-MSC. Annexin V/PI staining was used to determine apoptosis of treated cells. N-acetyl cysteine (NAC) was used to determine the role of glutathione. Cell-Titer Glo assay was performed to determine cell viability. Dichlorofluorescein-H DCFDA (DCF) dye was used to determine levels of reactive oxygen species (ROS). Western blot analysis was used to determine NRF2 expression in drug treated samples.
Results: Results show that AML blasts have increased survival in vitro when co-cultured with BM-MSC. Co-culture with BM-MSC was also shown to confer protection to AML blasts in the presence of daunorubicin, cytarabine and H_2O_2. Lower levels of ROS were observed in the AML blasts after BM-MSC co-culture, as determined by DCF fluorescence. NRF2 in BM-MSC was found to be activated by daunorubicin and cytarabine, as depicted by an increased expression of NRF2 in the nucleus after drug treatment. Moreover depletion of NRF2 in the BM-MSC reduced the protection conferred to the AML cells by the BM-MSC in response to daunorubicin, cytarabine and H_2O_2. Furthermore, the addition of NAC was shown to decrease AML blast apoptosis in culture in these assays.
Summary/Conclusions: These results demonstrate that NRF2 is induced in the non-malignant BM-MSC in response to chemotherapy with the functional consequence of promoting survival in neighbouring AML blasts. We propose targeting the actions of NRF2 in the stromal cells as a novel strategy to increase the tumour cytotoxicity of current drugs used to treat patients with AML.

E890

MICRORNAS HSA-LET-7A AND MIR-142-3P IN T CELLS IN PATIENTS PRESENTING WITH ACUTE MYELOID LEUKAEMIA-A MEANS OF REPAIRING TUMOUR-INDUCED T CELL DEFECTS?

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Background: Our previous work has shown that the absolute number of T cells is increased in the peripheral blood of patients presenting with Acute Myeloid Leukaemia (AML). These T cells can form conjugates with tumour cells but subsequent immune synapse formation and recruitment of downstream signalling molecules is inhibited. We found an altered T cell gene expression profile indicative of aberrant TCR signalling and T cell activation patterns suggesting a blunted immune response. *We now hypothesize that a means by which these alterations in T cell gene expression are effected is via modulation of microRNA (miRNA) expression*.
Aims: To define miRNA deregulation in peripheral blood T cell populations in patients presenting with AML compared to healthy age-matched individuals.
Methods: In a pilot study, CD4 and CD8 T cells were isolated from stored peripheral blood samples taken from AML patients at diagnosis and pre-treatment (n=3) and age/sex matched healthy volunteers (n=3) by FACS (average purity 98%). All AML patients had intermediate cytogenetic risk. Total RNA was hybridised to custom small RNA microarrays (MD Anderson) containing probes for 18694 human miRNA and 30945 other non-coding RNA species. Duplicate normalized probe intensities were averaged and differentially expressed small RNA were determined by LIMMA (linear models for microarray data) taking a FDR p>0.05 and fold change of +/- 1.5. Unsupervised hierarchical clustering and principle component analysis (PCA) were performed in R studio V3.2.2. Supervised clustering was conducted in Cluster V3.0 and Treeview V1.1.6r4. Identification of targeted mRNA was performed using miRTarBase (www.mirtarbase.mbc.nctu.edu.tw/).

Table 1.

microRNA	T cell Fraction	miRNA fold change	Gene Target	mRNA fold change	Target gene Function
hsa-let-7a-5p	CD4	-1.6	CCND2	2.1	Regulates cell cycle progression
hsa-let-7a-5p	CD4	-1.6	CD86	3.7	Negatively regulates CD25+ (IL-2 receptor)
hsa-let-7a-5p	CD4	-1.6	CDKN1A	2.5	P21-Regulates cell cycle progression
hsa-let-7a-5p	CD4	-1.6	DICER1	2.2	miRNA processing
hsa-let-7a-5p	CD4	-1.6	EIF2C4	2.2	Levels of Ago proteins regulate differentiation and activation of T cells
hsa-let-7a-5p	CD4	-1.6	KRAS	2.4	Regulates signaling downstream TCR activation
hsa-let-7a-5p	CD4	-1.6	MAP2	2.2	Induced by KRAS
hsa-miR-19	CD4	-1.5	MDM4	3	Down-regulates PTEN impairing T cell development
hsa-miR-19	CD4	-1.5	NCOA3	2.4	Histone acetyltransferase
hsa-miR-19	CD4	-1.5	NR4A2	6.5	Induces regulatory T cell phenotype
hsa-miR-19	CD4	-1.5	RAB11	2.1	Lymphocyte trafficking and T cell activation
hsa-miR-19	CD4	-1.5	SOCS3	3	Negative regulator of cytokine signaling
hsa-miR-142-3p	CD4	-1.7	MDM2	3.6	T cell development, reduced at activation, Induces regulatory T cell phenotype
hsa-miR-142-3p	CD4	-1.7	MDM4	6.4	Normal T cell development, Induces regulatory T cell phenotype
hsa-miR-142-3p	CD4	-1.7	RORA	3	Represses IL-2 transcription
hsa-mir-147b	CD4	-1.5	KRAS	2.1	Mediates cellular responses to mitogenic signals
hsa-mir-301a	CD4	-1.6	CDKN1A	2.5	P21-Regulates cell cycle progression
hsa-miR-377-3p	CD4	-1.6	PPM1A	2.6	Represses TGF-beta signalling
hsa-let-7a-5p	CD8	-1.7	CD86	3.3	Negatively regulates CD25+ (IL-2 receptor)
hsa-let-7a-5p	CD8	-1.7	EIF2C4	2	Levels of Ago proteins regulate differentiation and activation of T cells
hsa-let-7a-5p	CD8	-1.7	MAP2	2.3	Induced by KRAS Increased in CD8+ CD1 high cells
hsa-miR-195-5p	CD8	1.6	RUNX3	-2.4	Regulates CD8 maturation
hsa-mir-214	CD8	1.5	LTF	-6.2	Granule component in Neutrophils
hsa-mir-214b	CD8	-1.5	KRAS	2	Regulates signaling downstream TCR activation
hsa-mir-337-3p	CD8	-1.6	STAT3	2.1	Inhibits IL-2 production
hsa-miR-377-3p	CD8	-2	PPM1A	3.1	Represses TGF-beta signalling
hsa-mir-429	CD8	-1.7	RORA	2.3	Represses IL-2 transcription
hsa-mir-455	CD8	-1.6	RORA	2.3	Represses IL-2 transcription
hsa-mir-1224	CD8	-1.6	STAT3	2.1	Inhibits IL-2 production

Results: Hierarchical clustering and PCA of all samples, rather than differentiating samples by T cell population, differentiated AML from healthy controls illustrating that *miRNA expression in T cells is altered by the presence of AML blasts.* Supervised clustering demonstrated 39 miRNA were differentially expressed in AML CD4 T cells - 27 down-regulated, 12 up-regulated and 71 miRNA were differentially expressed in AML CD8 T cells - 41 down-regulated, 30 up-regulated. A number of these miRNA have already been shown to have a role in T cell proliferation and cellular migration. To validate this list, this

dataset was correlated with our previously generated mRNA dataset (independent patient and control cohort) (see Figure). Crucially, *this demonstrates 2 independent datasets showing coordinated deregulation of genes critical to T cell function.* In both CD4 and CD8 cells, up-regulated genes are involved in T cell differentiation and activation (EIF2CA and KRAS) but also paradoxically in inhibition of cytokine production (STAT3 and ZEB1 in CD8 cells and SOCS3 and ZEB1 in CD4 cells) supporting our previous hypothesis of aberrant T cell activation in AML. Two candidate miRNAs whose expression may induce the observed T cell defects are hsa-let-7a-5p and miR-142-3p. *Hsa-let-7a-5p is down-regulated in both CD4 and CD8 cells and has recognised functions in the regulation of T cell proliferation via CDK6 and MYC as well as in Th2 differentiation. miR-142-3p is down-regulated in CD4 cells and its reduced expression inhibits T cell proliferation via HOX10A.*

Summary/Conclusions: These data support the hypothesis that T cell miRNA expression is altered by the presence of AML blasts. This microRNA dataset has been validated against an independent mRNA dataset. Potential miRNA of interest are hsa-let-7a-5p and miR-142-3p as they have fundamental roles in normal T cell function. These findings will now be examined in a larger cohort.

E891

TP-1287, AN ORAL PRODRUG OF THE CYCLIN-DEPENDENT KINASE-9 INHIBITOR ALVOCIDIB

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Background: Alvocidib is a potent inhibitor of cyclin-dependent kinase-9 (CDK9) and induces apoptosis in cancer cells by reducing the expression of short-lived, anti-apoptotic protein such as MCL-1. Alvocidib, used in combination with cytarabine and mitoxantrone, is currently in a Phase II clinical trial in younger relapsed/refractory acute myeloid leukemia (AML) patients to evaluate the utility of a patient selection biomarker, dependent on MCL-1. Patients with AML that have a high dependence on MCL-1 are considered more likely to benefit from the alvocidib-containing regimen. Alvocidib is currently delivered by intravenous infusion which, although an effective delivery strategy, does not allow for chronic dosing and scheduling. An oral form or formulation of alvocidib could greatly expand the utility of the drug in combination with other targeted agents by allowing for more prolonged exposure. Alvocidib is highly permeable in CACO-2 monolayers and is soluble at acidic pH's but solubility is strikingly reduced at neutral or basic conditions, which might hamper the development of an oral formulation.

Aims: We hypothesized that a phosphate prodrug of alvocidib would improve solubility under neutral or basic conditions and enable the efficient systemic delivery of alvocidib via oral administration. We planned to investigate the efficacy of an orally delivered phosphate prodrug using various treatment schedules in AML mouse xenograft models.

Methods: We synthesized TP-1287, a phosphate prodrug of alvocidib, in three steps from the parent compound. The solubility of TP-1287, was determined at various pH's, in water. Pharmacokinetic studies were conducted in mice and efficacy was evaluated in AML xenograft mouse models using various dose levels and schedules. MCL-1 was evaluated as a pharmacodynamic biomarker in tumors from xenografted mice, following treatment with TP-1287.

Results: TP-1287 was found to be highly soluble under acidic, neutral, and basic conditions (1.52 mg/mL at pH 2.2; 1.81 mg/mL at pH 4.5; 9.48 mg/mL at pH 6.8 and 9.31 mg/mL at pH 8.7) compared to alvocidib (4.38 mg/mL at pH 2.2; 1.25 mg/mL at pH 4.5; 0.02 mg/mL at pH 6.8 and 0.02 mg/mL at pH 8.7). Pharmacokinetics studies in mice showed that TP-1287 was efficiently converted to the parent alvocidib (C_{max}=1922.7 ng/ml, $t_{1/2=}$4.4 hr) with high oral bioavailability (%F >100%, compared to intravenous alvocidib). TP-1287 demonstrated significant anti-tumor efficacy in an AML mouse xenograft model and produced a more than two-fold inhibition of the pharmacodynamic biomarker MCL-1 in xenografted tumors, demonstrating a wide, 75-fold therapeutic dosing window.

Summary/Conclusions: The phosphate prodrug of alvocidib, TP-1287, is highly soluble over a broader pH range than alvocidib and is efficiently metabolized to the parent compound *in vivo*. Tumor xenograft models and pharmacodynamic studies indicate that oral delivery of TP-1287 is efficacious in mice. Based on these results, we anticipate moving TP-1287, as an orally delivered CDK9 inhibitor, into a forthcoming clinical trial.

E892

POST-INDUCTION MINIMAL RESIDUAL DISEASE ASSESSMENT BY BOTH MULTIPARAMETER FLOW CYTOMETRY AND RT-PCR PREDICTS OUTCOME IN ACUTE MYELOID LEUKEMIA

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Background: Minimal residual disease (MRD) assessment is essential during the follow-up of patients with acute myeloid leukemia (AML). Although it is not yet decisional, except in acute promyelocytic leukemia (APL), its evaluation by RT-PCR in AML has already shown a significant impact on survival in AML. Multiparameter Flow Cytometry (MFC) offers another option to monitor MRD, especially for acute leukemias that lack molecular markers. Furthermore, results can be obtained in a shorter timeframes, which could be of great use in the management of AML patients.

Aims: We aim to study MRD assessment by MFC and/or RT-PCR after induction therapy for AML and its impact on outcome.

Methods: Patients were retrospectively included from a database of 182 patients that had at least one MRD assessment for AML during their follow-up from 2005 to 2013. Patients that were diagnosed with AML, who received intensive chemotherapy, that obtained cytologic complete remission after one or two courses of induction-therapy, and that had their initial follow-up in our center were included. Exclusion criteria included APL patients. MRD was assessed by MFC based on leukemia-associated immunophenotype present at diagnosis, including cross-lineage markers and maturation asynchronies. RT-PCR-based MRD was evaluated by quantification of WT1 or NPM1 expression in either blood or bone marrow samples. MRD data were extracted between post-induction hematologic recovery and first consolidation for further analysis. Concordance between MFC and RT-PCR was evaluated for patients who had an MRD evaluation by both methods at the same time. Overall survival (OS) and relapse free survival (RFS) were estimated according to MRD status. OS and RFS were also evaluated after censoring data at the time of stem cell transplant.

Results: 128 patients were eligible. Median age at diagnosis was 52 (range: 16-73). 36 patients had an initial leucocytosis superior to 50G/L. ELN prognostic groups were represented as follow: 36%, 27%, 22% and 15% of patients were classified in the favourable, intermediate-1, intermediate-2 and unfavourable groups respectively. De novo AML represented 118 out of the 128 patients. 60 patients underwent an allograft, 35 at first remission. 111 patients presented at least one MRD assessment, of whom 75 (68%) by MFC and 84 (76%) by RT-PCR (84 on WT1 and 26 on NPM1 expression). Concordance between both methods was relatively good since results were consistent with both methods for 35 of 44 samples. Interestingly, discordant samples were found in both ways - negative MCF-MRD with positive RT-PCR-MRD or positive MCF-MRD with negative RT-PCR-MRD.The median follow-up was 53 months. 3 years-OS was 61%. 65 patients (51%) relapsed during their follow-up. 3-years RFS was 42%. Patients with negative MRD (either by MCF or RT-PCR) had a better 3-years OS (68% *versus* 41%, p=0.002) and RFS (51% *versus* 27%, p=0.0045) than patients with positive MRD. Censoring data at the time of stem cell transplant did not impact the significativity of the results. For patients with only RT-PCR MRD assessment, the benefit of negative MRD was also significant on OS (p=0.0027) and RFS (p=0.0005). Considering only patients with MCF MRD assessment (75 patients), MRD had no significative impact on RFS (p=0.09) and OS (p=0.52).

Summary/Conclusions: As previously reported, we found that RT-PCR assessment of MRD is associated with OS and RFS in patients with AML. However only few studies have compared monitoring of MRD by both MCF and RT-PCR at post-induction time. We found a relatively good concordance between both methods. Moreover we showed that using MCF in addition to RT PCR for MRD assessment increased the number of patient that could be monitored at post induction time and was still correlated with outcome.

E893

PHARMACOLOGICAL INHIBITION OF THE PI3K/AKT/MTOR AND NF-KB CANCER PROMOTING PATHWAYS FOR TARGETED TREATMENT OF ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Acute myeloid leukemia (AML) develops as a consequence of genetic aberrations in hematopoietic stem and progenitor cells causing a differentiation impairment and an accumulation of AML blast cells in the bone marrow. This results in bone marrow failure as well as depletion of normal blood cells. Standard treatment remains initial high-dose chemotherapy (anthracycline/cytarabine) and subsequent consolidation therapy (allogeneic transplantation), however AML patients retain a poor median 5-year survival rate, thus there is a need for more effective treatment modalities. Furthermore, due to the tremendous genetic and epigenetic heterogeneity of leukemia, it becomes important to define more personalized treatment schemes. One of the most common genetic aberration in AML is the translocation t(8;21), which forms the fusion protein and core-binding transcriptional repressor AML-ETO encoded by the fusion oncogene RUNX1-RUNX1T1. Using our bioinformatics screening of microarray gene expression datasets, aberrant signaling of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin complex 1 (mTORC1) and NF-kB pathways, involved in proliferation and survival advantages, has been identified as a causative agent of malignant transformation in t(8:21) AML patients.

Aims: The aim of this study was to use gene expression signatures associated with the t(8;21) AML subtype in order to define specific targets and evaluate the anti-leukemic activity of selected small molecule inhibitors against these targets, in a personalized treatment strategy. More specifically, the objective was to determine if a combinatorial treatment targeting the PI3K/Akt/mTOR and NF-kB pathways can block leukemic cell proliferation compared to normal cells, using a t(8;21)/TET2$^{-/-}$ AML model. AML-ETO has been shown not to be sufficient to induce leukemia and is often associated with other dominant negative mutations acquired in early progenitors, such as TET2, a methylcytosine dioxygenase, which is represented by our murine model.
Methods: The small molecule inhibitors GDC-0941, NVP-BEZ235, IKK-16, and BAY 11-7082, were used to investigate their effects on cellular proliferation in assays supporting clonogenic growth. For this, GFP^{+}-sorted murine t(8;21)/TET2$^{-/-}$ cells and normal wild type bone marrow cells were plated in semi-solid methylcellulose, in parallel. Colony formation was evaluated after treatment with the inhibitors, either individually or in combination. Furthermore, pathway blockade was evaluated using specific biomarkers, protein phosphorylation and transcription factor translocation.
Results: In our study, we demonstrate that the small molecule inhibitors inactivated their respective targeted pathways, seen through specific biomarker analysis, and inhibited cellular growth and survival of the human t(8;21) leukemic cell line Kasumi-1. Furthermore, the *in vitro* assays supporting clonogenic growth of our murine t(8;21)/TET2$^{-/-}$ AML cells showed that the small molecule inhibitors hindered colony formation in a dose dependent manner, while combinatorial inhibition of PI3K/Akt/mTOR and NF-kB showed synergistic inhibition.
Summary/Conclusions: This study demonstrates that dual targeted therapy against the PI3K/mTOR axis and NF-kB signalling, may represent a novel therapeutic modality for t(8;21) AML. It also showed that identification of specific drivers of AML subtypes can be targeted in a personalized fashion, which could potentially be used in combination with standard chemotherapeutic regimens in order to reduce patient relapse and adapt treatment depending on AML subtype.

E894

NPM1 MUTATION AND FLT3/ITD IN A DOUBLE TRANSGENIC ZEBRAFISH RECAPITULATES FEATURES OF HUMAN ACUTE MYELOID LEUKEMIA
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Background: Acute myeloid leukemia (AML) is a clonal hematologic malignancy that shows great variability with regard to pathogenesis and treatment outcomes. One of the most common mutations associated with AML involves the class III receptor tyrosine kinase, FMS-like tyrosine kinase 3 (*FLT3*), which plays an important role in hematopoiesis. Constitutively activated *FLT3* occurring as internal tandem duplication (ITD) within the juxtamembrane domain is observed in 20-25% of AML patients and refers to the poorest response to current standard treatment. *FLT3*-ITD mutant was reported to be a driver mutation in myeloid leukemogenesis. Zebrafish, sharing similar leukocyte compartment with human beings, recently emerged as a promising animal model for studying diseases and for drug discovery.
Aims: To establish a transgenic *FLT3*-ITD zebrafish line, and further explore their characteristics, on which future drug screening may be developed.
Methods: We established a transgenic zebrafish that is able to express human *FLT3*-ITD or *NPM1*-Mut under the control of a myeloid-specific promoter (5.3 kb *spi-1*). Specifically, we generated two constructs, referred to as *spi1*: *FLT3*-ITD-2A-EGFP/CG2 and *spi1*:*NPM1*-Mut-PA/CG2, respectively.
Results: Cytological analysis of kidney marrow (KM) and peripheral blood (PB) smears prepared from wildtype and *FLT*3-ITD zebrafish were examined at 4, 6 and 9 months. In comparison with wild type fish, the KM from some *spi1*:*FLT3*-ITD-2A-EGFP/CG2 6- and 9-month old transgenic zebrafish had a greater number of myeloid progenitors and an excess of blast cells with focal aggregation. Hematoxylin and eosin (H&E) staining of the 6 month-old *FLT3*-ITD transgenic fish kidney showed mild effacement and distortion of kidney structure as well as increased infiltration of myeloid cells. Further morphological analysis of cytospin marrow showed that AB-wild type fish possessed the normal complement of haematological cells, whereas 2 out of 6 *spi1*:*FLT3*-ITD-2A-EGFP/CG2 zebrafish had an abnormal number of myeloid progenitors and decreased synthesis of erythroid cells at 9 month-old. Finally, leukoerythroblastosis was observed in PB. It is well recognized that the *NPM1* and *FLT3*-ITD mutations occur concurrently in AML patients, and the combined genotype that results from this (*NPM1*/*FLT3*-ITD) is the most powerful indicator of AML prognosis. To better delineate the two-hit model in zebrafish, we also generated *FLT3*-ITD/*NPM1*-Mut double transgenic mutants. In so doing, we found that *FLT3*-ITD and *NPM1*-Mut synergistically promoted myeloid blasts and the expansion of precursor cells in zebrafish as young as six months. Flow cytometric analysis and Liu's stains of the KM and PB smears from 6-month old *FLT3*-ITD/*NPM1*-Mut transgenic fish revealed myeloid hyperplasia with predominance of blast cells and depletion of erythroid series in KM, whereas immature blast cells were able to progressively infiltrate the KM and circulate into the PB. Taken above, we suggested that *FLT3*-ITD and *NPM1*-Mut synergistically promoted myeloid blasts and the expansion of precursor cells in zebrafish.
Summary/Conclusions: In conclusion, *FLT3*-ITD as a common *FLT3* mutation in AML patients could results in expanded myelopoiesis with poor differentiation in adult zebrafish. These transgenic fish could potentially provide a valuable platform to investigate leukemogenesis and screen drugs in the near future.

E895

THE MIR-128A/LIN28A AXIS REGULATES MYELOMONOCYTIC DIFFERENTIATION IN ACUTE MYELOID LEUKEMIA
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Background: Acute myeloid leukemia (AML) is a heterogeneous neoplastic disorder of hematopoietic progenitors. Lin28 is a conserved RNA-binding protein playing an important role in cancer stem cells. Ectopic expression of Lin28 reprograms hematopoietic progenitor cells from adult bone marrow (BM), endowing them to mediate multi-lineage reconstitution which resembles fetal hemotopoiesis. It has been reported the existence of reciprocal regulatory loops between Lin28 and *mir-128*. In particular, this miRNA is expressed in early hematopoietic progenitor cells preventing the differentiation of all hematopoietic lineages. Moreover, increased expression of *mir-128* was associated with high-risk molecular features of AML.
Aims: Since Lin28 and its *mir-128* seem to be important regulators of hematopoiesis, it could be interesting to study their involvement in the induction and maintenance of AML.
Methods: We analyzed, by qRT-PCR, *Lin28A* and *mir-128* expression levels in 37 AML patients, AML cell lines and 13 healthy donors. Lin28 protein expression was evaluated by cytofluorimetric analysis in myeloid and lymphoid precursors of 10 BM healthy subjects and in 9 AML leukemic cells (LC). Overexpression of Lin28A or silencing of *miR-128* were obtained in OCI-AML3 cell line for 24-48 h. Moreover, OCI-AML3 and ME1 cells were treated with PMA for 24, 48 and 72h. Cells were collected and used to perform cell cycle assay, apoptosis assay, caspase 3/7 assay, cytofluorimetric analysis of Lin28A, CD11b, CD45 and CD14, western blot for Lin28A and p21 and qRT-PCR for *EGR2* and *ZFP36*.
Results: Real Time PCR data indicated a down-regulation of *Lin28A* in AML patients ($p<0.001$) and cell lines ($p<0.05$) as compared with controls. To confirm this data, we also analyzed its protein expression in AML LC compared with normal myeloid precursors, showing a significant down-regulation ($p<0.01$). Furthermore, our data showed an up-regulation of Lin28A in normal myeloid precursors with respect to lymphoid ($p<0.001$) and erithroid ones ($p<0.01$). The over-expression of Lin28A in OCI-AML3 induced myeloid differentiation; in fact, we observed a significant increased of CD11b ($p<0.05$), CD14 ($p<0.05$), *EGR2* ($p<0.05$) and *ZFP36* ($p<0.01$). We also observed a significant block of cell cycle in S phase ($p<0.05$), an increased expression of p21 protein and the induction of apoptosis ($p<0.001$). To confirm the involvement of Lin28A in myeloid differentiation of LC, we treated AML cells with PMA. As expected, we observed an increase of myeloid markers (CD11b $p<0.05$, CD14 $p<0.05$, *EGR2* $p<0.01$, *ZFP36* $p<0.01$) and also a significant overexpression of Lin28A ($p<0.05$). Moreover, PMA treatment confirmed a significant block of cell cycle in G2 phase ($p<0.01$), an increased expression of p21 and the induction of caspase-dependent apoptosis ($p<0.01$). Literature data indicate that *miR-128* regulate *Lin28A*; in fact, we demonstrated by qRT-PCR its up-regulation in AML patients ($p<0.05$) and cell lines ($p<0.01$). Furthermore, its silencing in OCI-AML3 induced a significant increase of Lin28A, p21 and myeloid markers as *EGR2* and *ZFP36* ($p<0.001$), confirming their involvement in myeloid differentiation and in cell cycle arrest.
Summary/Conclusions: Our data indicate *mir-128*/*Lin28A* axis as a potential regulator of myeloid differentiation, suggesting its involvement in AML development.

E896

DEEP SEQUENCING OF 23 GENES DESIGNATED BY TCGA STUDY IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE
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Background: In the study carried out by The Cancer Genome Atlas Research Network (TCGA), 99% of patients with acute myeloid leukemia (AML) presented at list one mutation over 23 genes (*NPM1, FLT3, ASXL1, BCOR, CEBPA, DNMT3A*, *EZH2, IDH1, IDH2, KDM6A, KIT, KRAS, NRAS, PTPN11, RAD21, RUNX1, STAG2, SMC1A, SMC3, TET2, TP53, U2AF1* and *WT1*). However, there are not studies focused on these 23 genes to evaluate their incidence and clinical role within independent cohorts of patients lacking chromosomal aberrations, which represents nearly 50% of patients (NKAML).
Aims: To establish the frequency and prognostic influence of mutations and their interactions in the 23 genes designated by TCGA study in the context of other prognostic clinical and molecular markers in a cohort of *de novo* NKAML patients.
Methods: The study includes 112 adult patients with *de novo* AML (excluding acute promyelocytic leukemia) with normal karyotype. Available DNA sample at diagnosis was the only limiting criterion. DNA samples from Hospital Universitari i Politècnic La Fe were provided by Biobank La Fe. We carried out targeted gene sequencing (Ion Torrent Proton System–Life Technologies) using a 23 genes custom panel including all coding regions (*NPM1, FLT3, ASXL1, BCOR, CEBPA, DNMT3A*, *EZH2, IDH1, IDH2, KDM6A, KIT, KRAS, NRAS, PTPN11, RAD21, RUNX1, STAG2, SMC1A, SMC3, TET2, TP53, U2AF1* and *WT1)*. Selected variants were annotated using IonReporter® Software for clinical reporting. Non-pathogenic variants were filtered out by excluding synonymous, intronic and polymorphic variants (major allele frequency (MAF) ≥0,01 and/or included in dbSNP). *FLT3-ITD* mutations were assessed as previously reported[1].
Results: After filtering procedure we identified a total of 314 high-confidence variants, with an average of 3 mutations per sample, (range 0-7). Mutations in at least one of the genes were found in 103 out of 112 patients (95%). The most commonly mutated genes were *DNMT3A* (n= 43, 39%), *NPM1* (n= 37, 33%)*, FLT3*-ITD (n=33, 29%)*, IDH* (n=31, 28%)*, TET2* (n=30, 27%)*, RUNX1* (n=19, 17%)*, ASXL1* (n=17, 15%)*, KDM6A* (n=14, 13%), *WT1* (n=11, 15%) and *PTPN11* (n= 9, 12%). Of the 112 samples, 91% contained at least one mutation in one of eight categories defined according to biologic function with a putative role in AML pathogenesis: DNA-methylation–related (71%), encoding nucleophosmin (*NPM1*) (33%), activated signalling (37%), chromatin-modifying (27%), myeloid transcription-factor (25%), cohesin-complex (20%), tumor suppressor (16%), and spliceosome-complex (*U2AF1*)(4%). In addition, we identified a strong pattern of mutual exclusivity between *ASXL1* mutations and three other genes, in an independently manner, *U2AF1* (*P*=0.011), *TET2* (*P*=0.032) *and DNMT3A* (*P*=0.03). In addition, we found a significant co-occurrence between mutations in *DNMT3A* and *NPM1*, (*P*=0.001) and *TET2* and *IDH2* or *NRAS* (*P*=0.004; *P*=0.039, respectively). In univariate analyses, NKAML patients carrying indels in *TET2* or in *ASXL1* showed a significantly worse outcome (5-year OS, wt34% *vs*. mut 7%, *P*=0.025; 5-year OS, wt 30% *vs.* mut 12%, *P*=0.001, respectively). Further results will be presented in the meeting
Summary/Conclusions: Over 23 genes described by TCGA, 91% *de novo* NKAML patients showed at least one mutation. The most frequently mutated functional category was DNA-methylation (71%), which might trigger the process of leukemogenesis in NKAML. This work was supported by: Fundación Española de Hematología (FEHH), PI12/01047, RD12/0036/0014, PIE13/00046, PI13/01640, PI13/02387, PT13/0010/0026, PI14/01649 and PROMETEOII/2014/025.

Reference

1. Moreno I, *et al.* Haematologica 2003;88 1: 19-24.

E897

CD97 EXPRESSION MEDIATES MIGRATION AND ADHESION OF FLT3-ITD POSITIVE ACUTE MYELOID LEUKEMIA CELLS AND MODULATES THE BONE MARROW STROMAL MICROENVIRONMENT

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Background: Bone marrow niches are specialized microenvironments that facilitate homing and survival of normal hematopoietic stem and progenitor cells (HSPCs) as well as leukemic stem cells (LCSs). Targeting the niche is a new strategy to eliminate persistent and drug-resistant LSCs. However, defined mechanisms mediating interactions of acute myeloid leukemia (AML) cells with the microenvironment remain largely unexplored. We have recently demonstrated that expression of the adhesion GPCR CD97 is elevated in blasts of FLT3-ITD positive AML patients.
Aims: Here, we investigated the underlying mechanisms in more detail and the impact on mesenchymal stromal cells (MSCs) as a main cellular component of the bone marrow niche *in vitro*.
Methods: FLT3-ITD MV4-11 and FLT3 wildtype OCI-AML3 AML cells were used for the *in vitro* experiments. CD97 knock-down was achieved by lentiviral transduction of plko1.6/shRNACD97. Primary MSCs were isolated from bone marrow aspirates of healthy donors and co-cultured with AML cell lines in direct or indirect manner. Expression analyses were carried out by flow cytometry and Western blot. Trans-well migration was analysed in a Boyden chamber assay and the deformation capacity of the AML cells was investigated by real-time deformability cytometry (RT-DC).
Results: CD97 knock-down by lentiviral transduction of plko1.6/shRNACD97 in MV4-11 leukemic cells caused inhibited trans-well migration towards FCS and LPA which is at least in part Rho-ROCK pathway dependent. Adhesion to a stromal layer was significantly decreased within two days and immunoblotting revealed inhibited Akt phosphorylation in the CD97 knock-down cells. The expression of the lysophosphatidic acid (LPA) receptor correlated with CD97 levels in FLT3-ITD MV4-11 but not in FLT3 wildtype OCI-AML3 cells indicating a physical interaction of these receptors. Changes in size as well as the deformation capacity after retroviral transduction of leukemic cells suggested effects on actin-myosin cytoskeleton dynamics. In the second part of the study, we tested the impact of leukemic cells and their CD97 expression on the MSC phenotype. FACS analysis was performed after three days of MSC incubation with leukemic cell lines or their conditioned medium, respectively. Interestingly, CD90 and CD146 expression levels were increased by about 50% after coculture with MV4-11 wildtype cells but declined to the basic level after incubation with CD97 knock-down cells. In contrast, the expression levels of CD73 were increased by MV4-11 and even further elevated by CD97 knock-down cells. Comparable results were observed after MSC culture with conditioned medium of MV4-11 cells.
Summary/Conclusions: In summary, our data suggest a modulation of the bone marrow microenvironment by FLT3-ITD positive leukemic cells expressing CD97 in association with LPA receptor rendering it a potential new theragnostic target.

E898

REPOSITIONING OF QUINACRINE FOR TREATMENT OF ACUTE MYELOID LEUKEMIA - SYNERGIES AND IN VIVO EFFECTS

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Background: We have previously reported that quinacrine, formerly extensively used as an antimalarial drug, may have repositioning potential for treatment of acute myeloid leukemia (AML)[1].
Aims: The aim of this study was to further evaluate the potential of quinacrine for a clinical trial in AML by investigating its possible synergistic effect with other antileukemic compounds as well as evaluating its efficacy in vivo in a mouse model.
Methods: Cytotoxic activity of quinacrine in combination with one of 9 different drugs (daunorubicin, cytarabine, azacitidine, decitabine, sorafenib, geldanamycin, All-Trans Retinoic Acid (ATRA), vorinostat, and arsenic trioxide) in AML cell lines was evaluated using the fluorometric microculture cytotoxicity assay (FMCA). Assessment of combinatorial effects and possible synergy was performed using conventional Bliss independence analysis based on results from all combinations of 4 different concentrations (including zero concentration) of each drug. Activity of quinacrine in vivo was investigated in a model with AML-PS cells injected intravenously in female SCID mice (at Accelera S.r.l). After two days, groups of ten mice were randomized to treatment with quinacrine or vehicle control. Quinacrine was administered by oral gavage at the dose of 100 mg/kg three times a week for two consecutive weeks; control animals were treated with vehicle (iv) only (twice a week for two weeks). Mice were monitored daily for mortality and clinical signs, body weights were evaluated twice a week. Blood aliquots were collected on days 30 and 31 to determine the percentage of circulating leukemic cells by FACS analysis. BD Cellquest software was used for data collection and analysis. Median survival time (MST) was calculated and the log-rank test was used to evaluate the statistical significance of differences between the control group *vs.* the quinacrine-treated group.

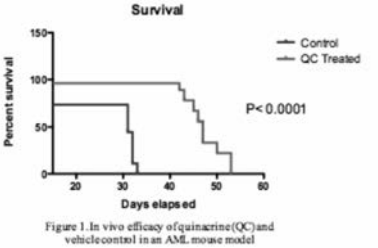

Figure 1.In vivo efficacy of quinacrine (QC) and vehicle control in an AML mouse model

Figure 1.

Results: In the in vitro drug combination analysis, several promising synergies were observed after studying duplicated experiments, for instance when combining quinacrine with geldanamycin, ATRA, cytarabine, hypomethylating agents or sorafenib. In the AML mouse model, evaluation of circulating leukemic

cells detected in blood samples (in percent of white blood cells) showed 72% human tumor cells in the control mice, whereas in mice treated with quinacrine, this was only 2.2%. In agreement with these data, the MST of control mice was 34 days whereas it was 46 days in quinacrine-treated mice ($p<0.0001$). At the tested dose, quinacrine did not decrease the body weight of treated mice.
Summary/Conclusions: These results strengthen the repositioning potential for quinacrine in AML, suggesting in vivo efficacy as well as promising synergies in combination with different agents, including geldanamycin, conventional chemotherapeutics as well as the hypomethylating agent azacitidine. These results provide further support for evaluation of the anti-leukemic effect of quinacrine in a clinical trial in AML.

Reference

1. Eriksson A, Osterroos A, Hassan S, *et al.* Drug screen in patient cells suggests quinacrine to be repositioned for treatment of acute myeloid leukemia. *Blood cancer journal* 2015; 5: e307.

E899

PRIMARY ACUTE MYELOID LEUKEMIA CELLS WITH OVEREXPRESSION OF EVI-1 ARE SENSITIVE TO ALL TRANS RETINOIC ACID

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Background: Acute Myeloid leukemia (AML) is a heterogeneous myeloid proliferative disease that can be classified based on morphology, cytogenetics, molecular aberrations, gene expression and methylation profiles. Aberrant expression of the transcriptional regulator ecotropic viral integration site-1 (EVI-1) occurs in ~10% of adult AML patients and is associated with particularly aggressive disease and a very poor outcome. Up to 95% of EVI-1 positive patients have an overall survival of less than 1 year. Therefore, there is an urgent need for novel treatment strategies to improve the survival of EVI-1 positive AML patients. For decades, most AML patients, including the group with EVI-1 overexpression, are treated with a chemotherapy combination consisting of cytarabine-arabinoside and an anthracycline. Importantly, EVI-1-positive AML patients have an very poor initial response to this currently used combination chemotherapy; 39% of EVI-1 positive patients do not achieve a complete remission after induction therapy as compared to 18% of patients in the other AML subgroups. To date, only a few alternative treatments have shown to be slightly more effective than this combination of chemotherapeutics. The exception to this is the treatment of acute promyelocytic leukemia (APL) patients with all trans retinoic acid (ATRA). ATRA treatment has significantly increased the survival chances for APL patients and has turned APL from a poor prognostic leukemia into a curable disease.
Aims: As treatment of APL patients with ATRA is very successful, we sought to determine the response to ATRA of EVI-1 positive AML patients.
Methods: Here we investigated the response of EVI-1 positive AML to ATRA (n=14) and compared this to the response of EVI-1 negative AML cases (n=7). We studied the effect of treatment with ATRA on myeloid blast differentiation, clonogenic capacity and on the *in vitro* and *in vivo* survival of AML cells.
Results: Like induction of CD11b expression on APL cells, we observed increased CD11b expression on the cell membrane of 69% of EVI-1-positive AML cases (9/13) after 7 days of incubation with ATRA. In contrast, EVI-1 negative AML cases had no induction of differentiation after ATRA treatment. In several of the EVI-1 positive AML cases, treatment with ATRA induces apoptosis of the leukemic blasts. Moreover, pre-incubation of patient EVI-1-positive AML cells with ATRA results in enhanced sensitivity to doxorubicin. Besides low complete remission rates after initial treatment, the extreme poor prognosis of EVI-1-positive patients is because of high relapse rates. This relapse is due to chemotherapy resistant leukemic progenitors or leukemic stem cells (LSC). Eradication of these LSC is crucial to improve the outcome of EVI-1-positive patients. We observed a significant reduction in colony forming capacity of leukemic progenitors/stem cells in primary AML after treatment with ATRA but most importantly we demonstrated that *in vivo* ATRA treatment of primary EVI-1 positive AML cases leads to a significant reduction in leukemic engraftment in the bone marrow and spleen (Figure 1).

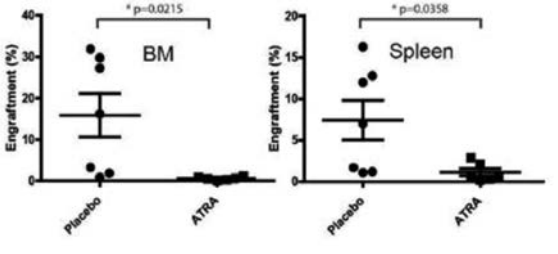

Figure 1.

Summary/Conclusions: This study is the first to show that a considerable part of the primary EVI-1 positive AML patient samples are sensitive to ATRA treatment, suggesting that combining ATRA with conventional chemotherapy might be a promising treatment strategy for this poor prognostic subgroup of AML patients.

E900

GENOME-WIDE ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISM (SNPS) IN PATIENTS WITH DE NOVO ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE

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Background: Although recurrent chromosomal alterations are the main diagnostic and prognostic markers in acute myeloid leukemia (AML) at the time of diagnosis, nearly 50% of patients have an apparently normal karyotype (NKAML), displaying an intermediate risk for survival and relapse. SNP array (SNP-A) allow us to study variations in the number of copies (CNV) and regions of loss of heterozygozity (LOH) in myeloid neoplasms. Using systematically matched germline samples might ascertain the somatic nature of each lesion.
Aims: To establish the role that may play some cryptic abnormalities present in NKAML patients through the frequency, recurrence and any possible clinical association of CNVs and LOHs.
Methods: A total of 218 de novo NKAML patients were analyzed. Among them, 25 patients were enrolled in the consecutive multicenter PETHEMA trials at the Hospital Universitari i Politècnic La Fe and Hospital 12 de Octubre, with available DNA sample at diagnosis and at complete molecular remission were selected for this study. DNA from Hospital Universitari i Politècnic La Fe were provided by Biobank La Fe. We expanded the cohort with other AML series with publicly available SNP-A data to a total of 181 cases (n=49 del TCGA,[a] n=53 de Kronke J, *et al.*,[b] n=30 de Tadayuki A, *et al.*[c] y n=49 Koren-Michowitz M, *et al.*[d]). Samples were genotyped using Human CytoScan HD (n=37) and Genome-Wide Human SNP 6.0 (n=181) according to manufacturer's protocol (Affymetrix Santa Clara, C.A., U.S.A.). DNA copy number and paired LOH analyses were performed using the Genotyping Console and the Chromosome Analyses Suite (ChAS) software (Affymetrix). Filters applied for segment Copy Number Abnormalities (CNA) detection were ≥20 consecutive markers in a region of at least 100Kb, and for regions of Copy Neutral Loss of Heterozygocity (CN-LOH), ≥100 markers in at least 500Kb. All abnormalities found in the remission sample were ruled out and assume as non-somatic. Besides, every potential abnormality was checked in the Database of Genomic Variants (http://projects.tcag.ca/variation). Size, position, and location of genes were identified with UCSC Genome Browser (http://genome.ucsc.edu/). The human reference sequence used for alignment was the GRCh37/hg19assembly
Results: A total of 304 abnormalities were found as an acquired event in 143 NKAML patients (66%), resulting in 2.1 abnormalities/case. These consisted of 151 heterozygous deletions, 99duplications and 54 CN-LOH. Cryptic chromosomal aberrations were more frequents in chr 1, 2, 5, 6, 7, 11, 13, 16 and 19. The most common CN-LOH was in 13q (being *FLT3* involved in all them), 1p y 7p. In 19p (chr19:1-16300000) we detected more deletions, while in chr 1, 4, 5 and 20 more insertions. Likewise, patients harbored cryptic aberrations (mainly deletions or CN-LOH) involving *KMT2A,*(n=5), *RAD21* (n=4), *RUNX1* (n=4), *ETV6* (n=3), *EZH2* (n=3), *WT1* (n=3), *PTPN11* (n=2), *TET2* (n=2), *U2AF1* (n=2), *IDH1* (n=1) and *NPM1* (n=1). Further results will be presented.
Summary/Conclusions: This study has delineated recurrent abnormalities that may act as driver event and could explain leukemogenesis in up to 66% of NKAML cases. This work was supported by the following grants: Fundación Española de Hematología (FEHH).

E901

A NON-FUCOSYLATED FULLY HUMAN MONOCLONAL ANTIBODY AGAINST IL-3RA, KHK2823, SHOWS PROMISING PHARMACOLOGICAL CHARACTERISTICS IN NONCLINICAL STUDIES

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Background: Human interleukin-3 receptor alpha (IL-3Rα, CD123) is highly

expressed in myeloid malignancies, including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), and is considered to be an attractive target molecule for antibody targeting. We generated KHK2823, a non-fucosylated fully human IgG1 monoclonal antibody against human IL-3Rα, which is armed with ADCC-enhancing POTELLIGENT® technology.
Aims: KHK2823 is currently in Phase 1 study in the United Kingdom to investigate safety and efficacy on AML and MDS patients (NCT02181699). Here, we describe nonclinical profiles and binding characteristics of KHK2823 as a potential therapeutics against AML and other myeloid malignancies.
Methods: Binding profiles of KHK2823 were analyzed by primary cells derived from patients in AML, MDS and B-ALL, and cell lines by using flow cytometry. Epitopes were analyzed by overexpressing IL-3Rα-GM-CSF-R chimeric proteins on 293F cells. Antibody-dependent cellular cytotoxicity was measured by ^{51}Chromium release assay using AML cells as target and peripheral blood mononuclear cells as effector Effects of IL-3 signal were assessed by using IL-3-dependent TF-1 cell line. Nude rats subcutaneously transplanted with MOLM-13 cells and cynomolgus monkeys were used for evaluating pharmacological activities *in vivo*.
Results: KHK2823 bound to various hematological malignant cells and leukemic stem cells, including AML, MDS and B-ALL. ADCC assay showed high antibody-dependent cellular cytotoxic activity in patients' malignant cells. A IL-3Rα antibody 7G3 but not KHK2823 inhibited the growth of TF-1, a IL-3-dependent cell line, in the presence of human IL-3 in vitro, suggesting that KHK2823 does not interfere IL-3R to bind IL-3. For further characterization of KHK2823, we screened epitopes of IL-3Rα antibodies. Three dimensional structure of IL-3Rα protein was predicted from the homology modeling of IL-4Rα protein (PDB: 3BPNC). Three structural domains of the extracellular region of IL-3Rα, designated as A-, B- and C- domains from the N-terminal, were predicted and replaced by GM-CSFRα. For further precise epitope analyses, six regions with 7-amino acid in IL-3Rα were substituted to the equivalent regions of GM-CSFRα protein. The analyses showed that KHK2823 recognizes the epitope distant from the critical region for IL-3 signals. The data coincided with that KHK2823, in contrast to 7G3 antibody, did not interfere with the binding of IL-3 to IL-3R. The epitope analysis also unraveled that KHK2823 but not 7G3 could recognize a isoform of IL-3Rα without A-domain predicted in public database. Several in vivo studies were conducted to evaluate the nonclinical profile of KHK2823. The nude rat xenograft model showed pharmacological effects. The pharmacodynamics and the toxicology profiles of KHK2823 were assessed in cynomolgus monkeys administered by i.v. infusion, once weekly for 4 weeks. KHK2823 was found to be well tolerated in the monkey study at a maximum dose of 100 mg/kg. IL-3Rα-positive cells in the peripheral blood were depleted in all the KHK2823-administered monkeys tested.
Summary/Conclusions: The anti-IL-3Rα antibody KHK2823 possesses promising pharmacological and toxicological profiles analyzed on the animal studies and in vitro studies, and recognizes the distinctive epitope, which does not interfere with the IL-3 signals and also does not lose recognition to the isoform. These data suggest that KHK2823 might possess safer and more efficacious characteristics to show promising profiles in the future.

E902

HETEROTYPIC SIGNALLING BETWEEN BONE MARROW STROMAL CELLS AND BLASTS PROVIDES INSIGHT INTO THE CELLULAR COMMUNICATION IN ACUTE MYELOID LEUKAEMIA

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Background: The bone marrow stromal microenvironment (BMSM) has a well-established role in the pathophysiology of acute myeloid leukaemia (AML). This is demonstrated by primary AML blasts dependence on stromal conditioned media to survive in culture. Although it is clear that there are components of the stromal secretome that augment AML blast survival, we are currently only aware of a fraction of these factors and the precise dynamics of the stromal-blast interactions are not fully understood. In this study we were interested in dissecting the secretome and identifying novel secreted proteins that may contribute to AML cell survival outside of the current understanding (e.g. SDF-1).
Aims: The ultimate aim of this project is to determine proteins secreted by AML and bone marrow stromal cells that may mediate communication between AML blasts and the stromal environment. Further, we wanted to identify secreted proteins in AML cells harbouring different karyotypes to understand how these cells illicit heterotypic activation or inhibition of signalling pathways in cells across the different AML-subtypes.
Methods: To assess the feasibility of this approach, the secretome of 4 human AML cell lines and 1 mouse bone marrow stromal cell line were characterised by harvesting the conditioned media from each cell line individually and in co-culture (9 conditions) in triplicate (n=3). The secretomes were then purified from the conditioned media before undergoing global quantitative proteomic analysis using Liquid Chromatography Tandem Mass Spectrometry. MASCOT searches against both mouse and human proteomes allowed for discrimination between the stromal and AML proteins during bioinformatic analysis. In parallel the viability of these cell populations was recorded using a Beckman Coulter Vi-Cell cell viability analyser, functionally assessing the supportive capabilities of the BMSM after the AML cells were subjected to 24 hours of serum starvation.
Results: Secretomic analysis of the chemical crosstalk identified 520 bone marrow stromal proteins (including Osteopontin, Fibronectin, ECM proteins) and 293 AML blast proteins that satisfied identification criteria across the samples. 98 of such proteins have known functions as cytokines/growth factors. Analysis of the stromal secretome revealed a set of 20 stromal proteins whose secretion was modulated as a result of AML-stromal co-culture. These putative extracellular signalling proteins have known roles in cell adhesion, migration or microenvironment remodelling. In addition, the modulations in the composition of the AML secretome are suggestive of reactive crosstalk with the BMSM leading to stromal cell complicity. Finally, we display how serum starvation only leads to a drop in viability for AML cells cultured in isolation. It appears the BMSM is able to maintain if not increase basal AML cell viability under the same conditions. These findings are complimented by the reduction in extracellular markers of stress in the co-cultured secretomes.
Summary/Conclusions: This proteomic approach has allowed for the identification of a panel of proteins secreted by the stromal cells that can affect cell signalling and therefore behaviour of AML blast cells. Interestingly there are a number of novel signalling proteins secreted by the blasts that are indicative of orchestrating the BMSM. Analysis is now underway to fully map the progression of signalling in AML blasts that is initiated by the binding of these stromal signalling proteins. When complete this study will provide insight as to how the stromal microenvironment helps propagate leukaemic blasts across the different AML-subtypes.

E903

ONO-5390556 AS POTENTIAL TARGETED THERAPY FOR ACUTE MYELOID LEUKAEMIA WITH ETV6-NTRK3 FUSION GENE

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Background: Chromosomal rearrangements that target *NTRK1*, *NTRK2* and *NTRK3* are rare but recurrent abnormalities observed in some types of cancer. Fusion variants for each *NTRK* gene have been described in cancers that give rise to constitutive activation of kinase domains that are believe to drive the oncogenic process. Translocation of *NTRK3* and Ets family transcription factor 6 (*ETV6*), *ETV6-NTRK3* appears with the greatest frequency across many cancer types, including acute myeloid leukaemia (AML), radiation-associated thyroid cancer, high grade gliomas, ductal carcinoma, fibrosarcomas, congenital mesoblastic nephroma and secretory breast carcinoma. Patients bearing *ETV6-NTRK3* show resistant to chemotherapy, indicating that targeting active *NTRK* may be effective in the treatment of patients with this fusion.
Aims: ONO-5390556 is a highly potent and selective NTRK inhibitor with an IC50 in the sub-nmol/L range. We have examined the activity of ONO-5390556 against *ETV6-NTRK3* bearing cell line, IMS-M2 models both in vitro and in vivo.
Methods: Response was assessed by oral administration of ONO-5390556 once daily for 26 days in mice bearing IMS-M2 cells that were subcutaneously injected into female SCID mice. To investigate the regulation of signaling pathways by ONO-5390556 in IMS-M2 cells, western blotting was performed.
Results: IMS-M2 cell lines showed reduced proliferation (IC50: 0.8 nmol/L) compared with Ba/F3 cells (IC50 not reached at 1000 nmol/L). In the IMS-M2 xenograft model, tumour growth inhibition at the final treatment day was 62.7% in the 0.6 mg/kg treatment group and 100% both in the 2 mg/kg and 6 mg/kg treatment groups respectively (All treatment groups: $P<0.001$ v.s. Vehicle). The treatment of ONO-5390556 was well tolerated in IMS-M2 bearing SCID mice with no body weight loss. Western blotting demonstrated a dose-dependent reduction in phosphorylation of the NTRK and the downstream signaling of NTRK pathway, ERK, AKT and PLCg1.
Summary/Conclusions: The dramatic and sustained response to NTRK inhibition in IMS-M2 cells provides a rationale for the mechanisms underlying the response. Our in vivo study therefore demonstrate that ONO-5390556 has significant activity against cells containing *NTRK* rearrangement and shows promise for the treatment of patients with *ETV6-NTRK3* fusion gene positive AML.

E904

ASSESSMENT OF THE ALLELIC RATIO OF DNMT3A R882 MUTATIONS IN ACUTE MYELOID LEUKEMIA BY DIGITAL DROPLET PCR

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Background: In acute myeloid leukemia (AML) the allelic ratio (AR) of internal tandem duplication in the *FLT3* gene (*FLT3*-ITD) has been shown to impact clinical outcomes. *DNMT3A* mutations (mut) occur in approximately 20% of AML patients (pts) & the majority is found in amino acid R882. These mut arise early in leukemogenesis & their presence is associated with worse prognosis in some AML subgroups. To date the biological & clinical impact of *DNMT3A*mut AR has not been investigated. Simultaneous absolute quantification of mut & wild type

(wt) copies with the highly sensitive & specific digital droplet PCR (ddPCR) may represent an excellent tool for *DNMT3A* R882mut AR assessment.
Aims: To evaluate the feasibility of *DNMT3A* R882mut AR assessment by ddPCR at diagnosis & during disease course in AML pts.
Methods: AML pts treated at our institution between 2000 & 2015 with *DNMT3A* R882H or R882C mut & eligible diagnostic (n=33) &/or follow-up material (n=20) were analyzed by ddPCR. cDNA was applied to a duplex assay measuring *DNMT3A*mut & wt simultaneously. Absolute copy numbers of *DNMT3A*mut/ *DNMT3A*wt defined the AR. Samples with an AR<0.0001 or<3 positive droplets were rated negative according to the manufacturer's recommendations. Mut in *NPM1*, *CEPBA* & presence of *FLT3*-ITD & -TKD were assessed at diagnosis. For pts receiving hematopoietic stem cell transplantation (HSCT) data on bone marrow chimerism at day 28 & 56 after HSCT were collected.
Results: 35 pts (median age 63 years [y], range 29-87y) were identified to have a *DNMT3A* R882H (71.4%) or R882Cmut (28.6%). All mut were determined to be heterozygous by visual inspection of the Sanger sequence traces. At diagnosis 31.4% of the AML pts had a *DNMT3A* R882mut AR>1 (median AR 0.92, range 0.0002-2.3). Pts with an AR>1 had more often secondary AML by trend (36.4% *vs* 8.3%, *P*=.06), were less frequently *NPM1*mut (18.2% *vs* 65.2%, *P*=.03), had fewer platelets by trend (*P*=.08) & more blasts in peripheral blood (*P*=.04). A *DNMT3A* R882mut AR>1 at diagnosis associated with shorter overall survival (Figure 1A, *P*=.005). 27 pts received HSCT with 15 having available samples pre-HSCT. *DNMT3A* R882mut AR declined in all pts pre-HSCT (AR range 0-0.57, median 0.14; median AR reduction 67.3%, range 30.6-100%, *P*<.001). The majority of patients remained *DNMT3A* R882mut positive (86.7%) pre-HSCT. At day 28 after HSCT five pts had material available to assess *DNMT3A* R882mut AR. Four pts were positive for *DNMT3A* R882mut (AR range 0.0001-0.51) of whom three experienced relapse. One patient was negative for *DNMT3A* R882mut at day 28 & is in continuous remission. Figure 1B displays an exemplary AR course of one patient who experienced relapse after HSCT. Notably, the *DNMT3A* R882mut AR increased by two log-levels between day 28 & 56 after HSCT while the bone marrow total chimerism remained at 100%.

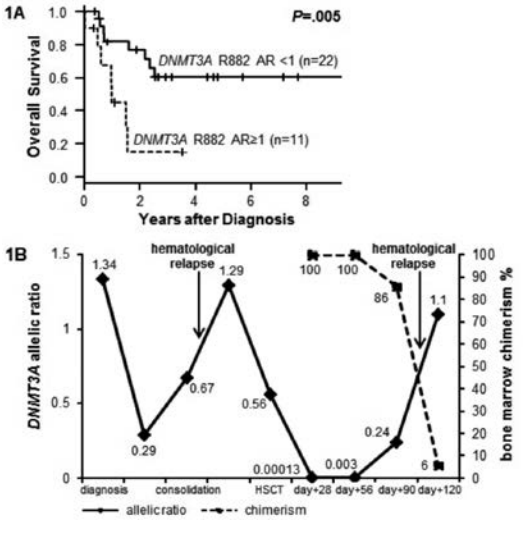

Figure 1.

Summary/Conclusions: ddPCR is a feasible method to assess *DNMT3A*mut AR during disease course. In spite of the limited number of pts, our data suggest that the *DNMT3A* R882mut AR at diagnosis may associate with biological & clinical features & outcome of AML pts. *DNMT3A* R882mut AR may be useful for monitoring minimal residual disease (MRD) after HSCT. Assessment of *DNMT3A* R882mut AR by ddPCR will be included in future clinical trials to validate its prognostic impact & its feasibility as MRD marker after HSCT in AML.

E905

USE OF NGS MULTIGENIC PANEL IN MOLECULAR DIAGNOSTIC OF MYELOID MALIGNANCIES TO STRATIFY PATIENTS FOR PERSONALIZED THERAPIES

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Background: In the last years, next generation sequencing (NGS) technology resulted to be a new effective strategy in identifying genetic aberrations in myeloid neoplasms. Molecular mutation information became essential for biological subclassification, risk stratification and therapeutic decisions; the mutational status of several genes became important for understanding the complex interactions among different pathways in leukemogenesis.
Aims: Characterization of myeloid neoplasms using a multigenic panel of NGS sequencing in order to identify important alterations in a shorter time than traditional molecular methods and with a higher sensitivity. This will be helpful in a prematurely detection of small clones, important for monitoring disease progression and the inclusion in target therapy protocols.
Methods: We performed 15 runs with the TruSight Myeloid Panel of Illumina, for a total of 118 patients analyzed at diagnosis: 95 AML/sAML, 15 MPN, 3 CML, 3 MDS and 2 CMML. 25 patients (21,2%) had a normal karyotype, 25 (21,2%) presented one or two alterations, 26 (22%) had a complex karyotype, while for 42 patients (35,6%) no information about the karyotype was available. The panel is a next-generation sequencing platform to screen somatic variants in 54 genes relevant in myeloid diseases: 15 full genes (exons only) and oncogenic hotspots of 39 additional genes, for a total of 568 amplicons.
Results: The output data were then analyzed with the Illumina's software Variant Studio and the results were filtered by a coverage of minimum depth of 500 and allele frequency >3%. Variants already classified as SNP were removed. Only non-synonymous mutation were considered. The mean coverage was 3662 with a mean of 24 alterations per patient. 57,5%, 3.9% and 38.6% of the alterations had a Variant Allele frequency (VAF)<10%, between 10-30% and >30%, respectively. Three patients resulted wild-type, while 10 patients carried only one mutation. The most mutated genes were *ASXL1*, *BCOR*, *TET2*, *BCORL1*, *KDM6A*, *STAG2*, *DNMT3A*, *NOTCH1*, *ATRX*, *EZH2*, *RAD21*, *TP53*, *ETV6* (p<1e-05). We also detected 66 short deletions and 11 short insertions (max length 23 bp and 25 bp respectively). Then we proceeded with the validation of the mutations >10% of *NPM1*, *DNMT3A*, *TP53*, *FLT3*, *JAK2*, *MPL*, *SETBP1*, *IDH1* and *IDH2* with conventional molecular methods available in our laboratory (Sanger Sequencing and dPCR) and all the 145 mutations were confirmed. We also validated the alterations<10% of *CEBPa*, *TP53*, *RUNX1*, *IDH1*, *IDH2*, *CALR* and *FLT3* with the Roche GS Junior 454 and we obtained >90% of concordance. Moreover 18 AML samples were also analyzed by WES and 97,5% of the mutations were confirmed.

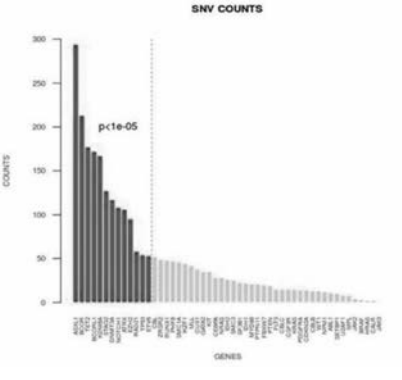

Figure 1.

Summary/Conclusions: These data suggest that a NGS multigenic panel is an effective strategy in myeloid neoplasms characterization and stratification in light of the development of novel personalized therapies (IDH-inhibitors: AG120 and AG221, MEK-inhibitors: GDC-0973, JAK-inibitors: Ruxolitinib and FLT3-inhibitors: Sorafenib, Midostaurin, AC220, ASP2215). Moreover, this approach is cheaper and time-saving and can also reveal alterations with a higher sensitivity than conventional methods. For this reason, we think that this approach could be strongly recommended for all new diagnosis/relapse myeloid neoplasm in order to obtain a more complete and premature characterization of the disease that will give advantages in term of therapeutic approach and OS of the patients. *Akcnowledgments: work supported by ELN, AIL, AIRC, Progetto Regione-Università 2010-12 (L.Bolondi), FP7 NGS-PTL project, Illumina inc.*

E906

BIOLOGICAL MARKERS OF RELAPSE IN ELDERLY PATIENTS WITH AML IN CR AFTER INDUCTION-CONSOLIDATION CHEMOTHERAPY AND MAINTENANCE WITH 5-AZACITIDINE

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Background: Acute myeloid leukemia (AML) is biologically complex due to the continuously evolving clonal architecture that marks remission and relapse. Though biological targeted therapeutics are changing clinical practice, there is still a lack of epigenetic markers of prognosis.
Aims: To identify biomarkers of relapse in elderly patients with AML in CR after conventional induction ("3+7") and consolidation chemotherapy.
Methods: In a phase III, prospective, randomized, open-label, multicenter trial, designed to assess the efficacy of post-remission treatment with 5-Azacitidine *versus* best supportive care (BSC), patients are included if: they are >60 years of age; have newly diagnosed "de novo" AML or evolving from myelodysplastic syndrome; >30% bone marrow blasts; no contraindications for intensive chemotherapy; and an ECOG performance status<3. Induction chemotherapy consists of two courses of "3+7" (Daunorubicin 40 mg/m^2 daily days 1-3 and cytarabine 100 mg/m^2 daily continuous IV infusion days 1-7). Patients in complete remission (CR) receive consolidation therapy (cytarabine 800 mg/m^2 3 hour infusion bid days 1-3) after which they are randomized 1:1 to receive BSC or 5-Azacitidine maintenance therapy up to 4 years and six months until AML relapse. Bone marrow (BM) samples were obtained from patients at diagnosis before treatment and at CR before randomization. Genomic DNA was obtained from BM mononuclear cells. Prognostic somatic mutations were evaluated by the Human Hematopoietic Neoplasm qBiomarker Somatic Mutation PCR Array (QUIAGEN) that includes DNA sequence mutation assays designed to detect the most frequent, functionally verified, and biologically significant mutations in human hematopoietic neoplasms, such as: FLT3, NPM1, IDH1, IDH2, CEBPA, KIT, KRAS, WT1, ASXL1, EZH2, DNMT3A and TP53. Various univariate logistic regression models were constructed. Each model included the treatment allocation, the dichotomous variable, "Relapse Yes/No" as the dependent variable, and each somatic mutation (mutated/wild-type) as independent variables. The median time to relapse will be estimated by the Kaplan Meier method.
Results: At interim analysis, 31 patients (14 males), mean age 69 SD±6 years achieved a CR and were randomized to receive BSC (N=13) or 5-Azacitidine (N= 18). At the time of the present report, 21 of the 31 patients have relapsed (10 in BSC arm, 11 in 5-Azacitidine arm), while the remaining 10 are alive in CR. Median time to relapse was 296 days, 95% CI 73 -519. Amongst biomarkers assessed, only TP53 (NM_000546 p179Y) at diagnosis was associated with AML relapse (p=0.007, Table 1A). Specifically, 15 out of 17 patients with mutated TP53 at diagnosis relapsed compared to 6 out of 14 patients with wild-type TP53. Of the latter 6 patients, 4 were on 5-Azacitidine. Finally, we built a logistic regression model adjusting the TP53 variable for the effect of the random allocation, which did not represent a confounder for relapse. Median time to relapse in TP53 mutant patients was 147 days, 95% CI 51-242 *versus* 389 days, 95% CI 353-425.in TP53 wild-type patients (p=0.022, Figure 1).

Table 1.

Table 1A

Variable	Unit	Odds ratio	CI (95%)	P
TP53_RP179Y (crude)	0 = Wild-type 1 = Mutated	6.177	1.627 – 62.462	0.013
TP53_RP179Y (adjusted for treatment allocation)	0 = Wild-type 1 = Mutated	9.721	1.559 – 60.622	0.015

Figure 1

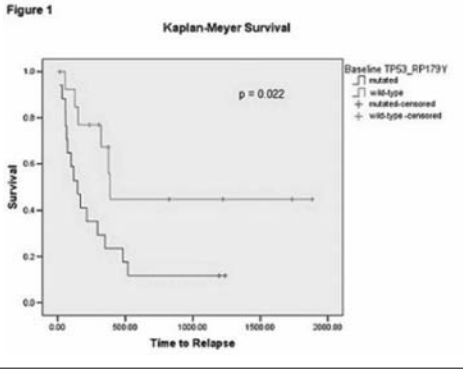

Figure 1.

Summary/Conclusions: The presence of TP53 mutation at diagnosis in elderly "fit" patients with AML undergoing induction-consolidation chemotherapy seems to confer an independent adverse clinical course which is not abrogated by post-remission treatment.

E907

PATTERNS OF INTERACTION BETWEEN MULTIPOTENT MESENCHYMAL STROMA CELLS (MSC) AND ACUTE MYELOID LEUKEMIA (AML) CELLS IN VITRO AND IN VIVO

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Background: MSC are a key factor within the bone marrow (BM) niche by regulating hematopoietic stem cells (HSC) within the endosteal and perivascular region. Moreover, MSC are suspected to support the aberrant hematopoiesis in a similar manner.
Aims: In our study we sought to investigate the effect of MSC on AML cells *in vitro* and *in vivo*.
Methods: MSC from AML patients (LD) and donors without BM disorder (BD) were obtained from BM aspirates. Phenotypic and functional criteria of MSC were tested subsequently. Liquid culture assays were performed from LD and BD samples to compare expansion capacity. Co-cultures of AML cells (LC) on MSC were initiated and compared to those on mouse fibroblasts (MS5). Additionally, the effect of blocking CXCR4 and TGF-b_1 on proliferation of LC was tested in these co-cultures with and without cytarabine supplementation. Ultrastructural intercellular communication of MSC and LC was investigated by electron microscopy (ECM). Gene expression profiles of cultured LD-MSC *vs* BD-MSC were established by using Affimetrix gene chip. Finally, homing and engraftment of MSC, LC and combinations thereof in NSG mice were investigated.
Results: MSC were successfully harvested from BM aspirates and showed a typical differentiation pattern and surface marker profile. LD-MSC showed significant decreased expansion capacity compared to BD-MSC. Co-culture of LC on MSC resulted in a higher proliferation than on MS5. Blocking TGF-b_1 but not CXCR4 increased proliferation of LC and enhanced chemo-sensitivity towards cytarabine. ECM analysis showed no evidence for junctional bridging between LC and MSC. Gene analysis revealed a marked overexpression of stanniocalcin-1 (STC-1) in LD-MSC compared to BD-MSC. mRNA and western blot analysis as well as ELISA and immunohistological findings confirmed this observation. *In vivo* experiments emphasized that HSC and LC exhibited significant higher homing efficiency than MSC, the latter being mainly trapped within the lung. Mice treated (transplanted) with MSC showed decreased cytarabine sensibility as compared to control mice without MSC.

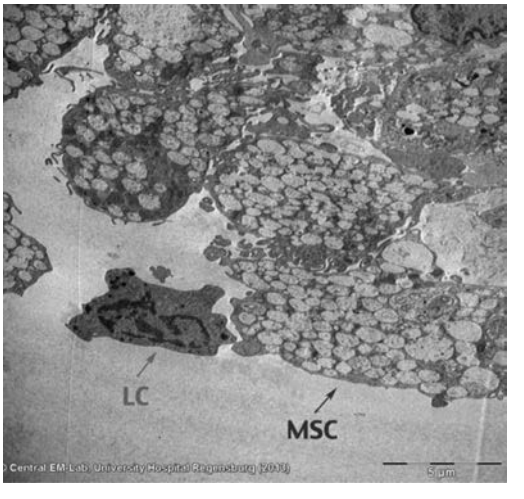

Figure 1.

Summary/Conclusions: MSC derived from patients with AML show distinct differences to MSC from BD. MSC support LC *in vitro* and *in vivo*. TGF-b_1, CXCR4 and STC-1 signaling may play an important role within the leukemic niche.

E908

THE ROLE OF THE NUCELOPHOSMIN-1 SPLICE VARIANT R2 IN ACUTE MYELOID LEUKEMIA DEVELOPMENT

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Background: A close cytogenetic and molecular analogy between *de novo* MDS and AML of elderly people indicates a common pathogenic mechanism for these conditions. The frequent mutations of the splicing machinery in MDS

suggest that alternative splicing of certain molecules of the crucial signaling pathways might drive progression to AML. Recently, we found that high expression of the *NPM1* splice variant R2 may provide prognostic value for CN-AML patients.
Aims: Assuming the common origin of MDS and AML we aimed to characterize the *NPM1* R2 splice variant expression in groups with MDS, sAML and AML patients. Moreover, NPM1 stabilizes the ARF and interacts with the tumor suppressor p53, regulates the increase in stability and transcriptional activation of p53, thus contributing to modulating growth-suppressive pathways. Therefore, we characterized expression pattern of *ARF, MDM2*, *TP53* genes with additional downstream molecules (p21, *miR-34a*) in AML, s-AML and MDS patients.
Methods: For 128 samples (58 AML, 62 MDS and 8 samples with sAML) expression levels of *NPM1* R2, *ARF, MDM2*, *TP53* and p21 were assessed by qRT-PCR. We also measured expression levels of miR-34a, miR-34b and miR-34c in CD33+ cells from 20 AML patients samples. To investigate whether R2 might disrupt localization of the NPM1 wild type protein, we used constructs with GFP-tagged NPM1-R2 and NPM1-wt under cytomegalovirus promotor to transfect WI-38 fibroblasts and performed immunohistochemistry analysis for NPM1 in 23 AML bone marrow smears.
Results: The expression of *NPM1* R2 was significantly higher in AML, s-AML and MDS groups compared to HVs (median 0.023 *vs* 0.005, p<0.001, 0.025 *vs* 0.005, p<0.001 and 0.017 *vs* 0.005, p<0.001, respectively). *NPM1* R2 positively correlated with *TP53* expression in AML (r=0.77, p<0.001) and MDS (r=0.68, p<0.001). We observed elevated expression of miR-34c in HVs group compared to AML (0.11 *vs* 0.07, p<0,001). No differences were found in miR34a, miR-34b and miR-34c expression between groups with high or low R2 expression. Transfections analyses showed that GFP-tagged NPM1-R2 was detected in nucleoplasm, whereas the GFP-NPMwt was detected in the nucleoli. However, the IHC stainings for AML samples revealed that in cases with high R2 expression we were able to determine a cytoplasmic localization of NPM1 even in the absence of its concomitant mutation. Therefore, we provide further evidence that the cytoplasmic localization of NPM1 might depend not only on its mutational status, but might be influenced by the distribution of its splice variants.
Summary/Conclusions: In our study we found that the expression levels of R2 were elevated in AML, sAML and MDS groups compared to HVs suggesting that R2 might play a role in the process of the tumorigenesis not only in AML cases but also in early stages of development of this disease. As the *NPM1* R2 splice variant represents a truncated form of *NPM1* gene, our transfections analyses confirmed that this isoform mostly localizes in the nucleoplasm, and thus might also have a biological impact in the malignant cells by interaction with other proteins. Moreover, strong positive correlation between R2 and *TP53* expression was found in AML and MDS groups suggesting biological link between these transcripts. In summary, the expression of *NPM1* R2 might be of biological importance for AML as well as for sAML and MDS patients. This work was supported by National Centre for Science Grant HARMONIA (UMO-2013/10/M/NZ5/00313).

E909

THE ROLE OF ADENOSINE DEAMINASE AND DNA METHYLATION IN ACUTE MYELOID LEUKEMIA

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Background: Genes coding for epigenetic regulators are frequently mutated in acute myelogenous leukemia (AML). As epigenetic marks are reversible, drugs that target these events may have an important impact on AML survival rates. The objective of this study is to investigate the importance of a metabolic enzyme, Adenosine Deaminase(ADA), whose gene is upregulated by abnormal DNA methylation during AML induction. Analysis of the mouse microarray gene expression data showed that the *ADA* gene is highly upregulated in NRASD12/BCL2 MDS mouse models, which was validated by Q-PCR (Tekin and Padua, unpublished data). If the expression of different subtypes of AML samples is assessed, *ADA* levels are highest in the subgroup carrying inv(16) or t(8;21) chromosomal translocations. Notably, these subgroups of patients also have a conserved DNA methylation pattern (Figueroa et al. 2010).
Aims: Based on the observations, the following hypothesis has been formulated and was tested using human AML cells: *Is ADA a key enzyme regulating the hypermethylation status in AML and contributing to disease progression?*
Methods: To address the hypotheses, two approaches were taken: 1) To silence the ADA gene using shRNAs against *ADA* and to investigate its impact on DNA methylation 2) To overexpress ADA using a lentiviral vector expressing the *ADA* cDNA, and similarly investigate altered DNA methylation patterns. In initial experiments, three human cell lines were chosen: ME-1 AML cells carrying the inv(16), Kasumi-1 AML cells with t(8;21) and K562 t(9;22) cells, which was used as a control group. The cell lines were manipulated to overexpress or repress ADA by transduction with lentiviral vectors. As a control and comparison, azacitidine (AZA), a powerful demethylation agent, was also used. After transduction with lentiviral vectors and treating with AZA, RNA and DNA were extracted and proteins were purified. To investigate if inhibiting ADA mimics the effect of AZA, DNA methylation levels of Kasumi cells were measured by gene expression of the specific genes (*CDKN1A*, *ITGA2B*, *LY86)*. Bisulphite conversions of the DNA samples were carried on for the evaluation of the methylation status for the future studies.
Results: ADA protein levels were determined via western blot to ensure the success of the *ADA* gene manipulation by lentiviral transduction. Expression of *ADA* and two related genes encoding downstream enzymes from the methylation pathway DNA methyltransferase 1 (DNMT1) and S-adenosylhomocysteine hydrolase (SAHH) were investigated by Q-PCR. *ADA* gene expression levels do not impact on *SAHH* and *DNMT1* gene transcription, but we concluded that it does impact at the protein level. Interestingly, an inverse correlation between ADA and DNMT1 protein levels were determined between the cell lines. While ME-1 cell line has the highest ADA level and lowest DNMT1 level, K562 showed the highest DNMT1 levels with lowest ADA protein levels. In Kasumi cells a correlation between the expressions of methylated genes and ADA silencing was observed, which was similar to that observed upon AZA treatment. While AZA treatment resulted in >20 fold increase in the expression of methylated genes, silencing ADA increased the expression of methylated genes >2,5-10 fold.
Summary/Conclusions: The analysis of this study showed that silencing ADA can partly mimic the effect of AZA. To confirm the impact of ADA levels on DNA methylation, global gene analysis using bisulfite converted DNA samples, followed by promotor array or sequencing will be performed.

E910

NEW DELETION OF JAK2 DETECTED BY SNP ARRAY IN ACUTE MYELOID LEUKEMIA: A ROLE IN OVERALL SURVIVAL

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Background: In Acute Myeloid Leukemia (AML), a hematologic malignancy that originates in hematopoietic stem and myeloid progenitor cells, there is a strong need to develop new diagnostic and therapeutic options. Age and karyotype have been recognized as the most prominent prognostic factors in AML patients. Novel array-based technique-single-nucleotide polymorphism (SNP) microarray can detect cytogenetic lesions involve mostly structural alterations with losses or gains of chromosomic material. Those chromosomic abnormalities are predictive of response and are very important to define therapeutic strategies. SNP microarray can detect copy-neutral loss of heterozygosity (CN-LOH), which plays a role in oncogenesis by duplicating oncogenes, inhibiting tumor suppressors, and stimulating improper epigenetic programming.
Aims: Our objective is to evaluate the prognostic impact of these genetic alterations on clinical outcome.
Methods: We analyzed 310 AML patients (pts): 218 performed by SNP Array 6.0 (Affymetrix) and 92 performed by Cytoscan HD Array(Affymetrix) and also by Next Generation Sequencing (NGS)-WES HiSeq 2000 (Illumina). SNP Array data were analyzed by Nexus Copy Number™ v7.5 (BioDiscovery).

Table 1.

Pt	Call Start-End	Call Lenght (Bps)	Zygosity	Probe Count	From Exon	to Exon
1	5,065,391-5,084,407	19,016	Heterozygous	45	10	19
2	5,049,001-5,075,904	26,903	Heterozygous	72	9	19
3	5,074,734-5,071,809	7,075	Heterozygous	21	15	19
4	5,080,280-5,098,786	18,506	Heterozygous	28	17	22
5	5,080,280-5,098,786	18,506	Heterozygous	28	17	22
6	5,070,280-5,113,117	42,837	Heterozygous	70	17	23
7	5,070,940-5,078,433	7,493	Heterozygous	14	13	16
8	5,083,622-5,140,230	56,608	Heterozygous	65	20	24
9	5,080,280-5,083,622	3,342	Heterozygous	13	17	19
7 bis	5,083,622-5,140,230	56,608	Heterozygous	65	20	24

Results: The median age of our cohort of pts was of 52 years and they had a median age at diagnosis of 51,3 years with a female/male ratio of 51% and 49%. Copy Number Alterations (CNAs) were detected in all patients affecting all the chromosomes, in particular our cohort of pts present a percentage of CNA, divided as follow: 42% of UPD (Uniparental Disomy), 19% of Copy Number (CN) gain, 39% of CN loss. By SNP array analysis we found that several genes were preferentially deleted, for example ADAM3A (62,2% pts), TP53 (15% pts), HRAS (8,6% pts), ETV6 (7,8% pts) and JAK2 (7% pts), while genes preferentially involved in amplifications are: FLT3 (42,5% pts), KIT (36,5% pts),

KRAS (34,2% pts) and SIRPB1 (32,5% pts). We focused on a particular heterozygosis loss of JAK2, detected in 8% of pts, which goes from 5,049,001bp to 5,140,230p, involving the second tyrosine-kinase domain (figure 1). We showed that the group of patients which present this deletion had an overall survival rate better than the group with an amplification of the gene (p-value<0,01). None CN loss in this region of JAK2 had been described in hematopoietic tissue before.

Summary/Conclusions: By SNP arrays we have identified CNAs involving important cancer genes in AML and we showed that a new deletion in JAK2 may play a role in the overall survival. Future prospectives will be to confirm and correlate other cancer genes aberrations and mutations with the prognosis of AML, in order to identify new biomarkers relevant for the disease.

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LB2242

PROTEOMIC ANALYSIS OF RELAPSE AML IDENTIFIES OPPORTUNITIES FOR THERAPEUTIC INTERVENTION IN INDIVIDUAL PATIENTS

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Background: A poor outcome in patients with acute myeloid leukaemia (AML) is usually related to chemorefractory disease or relapse after an initial response. Standard therapy generally includes days 1-3 daunorubicin (D) and days 1-7 cytarabine (A) followed by several similar courses should complete remission be achieved. Resistance of AML blasts to such treatment can be attributed to the activity of pro-survival enzymes, some of which can be inhibited by pharmacological inhibitors, therefore could potentially be repurposed for relapsed AML in a case by case basis. However, finding the right inhibitor for the right patient presents a challenge due to the plethora of possible causes of resistance in each case. Liquid chromatography – tandem mass spectrometry (LC-MS/MS) proteomics is a powerful discovery technology enabling quantification of global protein expression and enzymatic activity in samples. Hence this approach may be an effective way to identify suitable drug targets in biopsies and better understand the biochemistry of cells following chemotherapy.

Aims: Our primary aim was to identify biochemical pathways modulated in AML blasts and cell lines as a result of treatment with standard chemotherapy. We also aimed to test pharmaceutical alternatives to personalise AML treatment following thorough investigation into the expression and activity of protein drug targets in AML blasts and cell lines before and after chemotherapy.

Methods: We used LC-MS/MS proteomics and phosphoproteomics to investigate global protein expression and kinase activity in 19 matched diagnosis and relapse AML biopsies, as well as in 3 AML cell lines before and after chemotherapy. Briefly, we collected frozen biopsy specimens from Barts and the London tissue bank. After thawing the AML blasts were incubated in FBS containing media for 2 hr at 37ºC. Cell lines (HL60, MV411 and p31Fuj) were treated±D and/or A (2, 6, or 24 hr). After incubation, cells were centrifuged and washed in PBS, then proteins extracted in urea lysis buffer. For proteomics, proteins were digested with trypsin, and resulting peptides analysed directly by LC-MS/MS. For phosphoproteomics, phosphorylated peptides were first enriched using TiO_2 prior to analysis. Commercial (Mascot) and in-house (Pescal, KSEA) software were utilised to identify and quantify proteins, determine kinase activities and investigate intracellular signalling. Cell Viability of blasts±treatments were recorded using the Guava ViaCount Reagent and Cytometer.

Results: On average we identified >3000 proteins and >9,000 phosphorylation sites per sample. We observed that AML blasts showed high expression and activity of enzymes involved in DNA repair (e.g. PARP1, ATR and PRKDC) at diagnosis and several increased significantly after relapse (e.g. PLK3 and APEX1). Pro-survival signalling pathways and those regulating apoptosis and metabolism were also modulated after relapse but these were patient specific. AML cell lines were more sensitive to D than A. HL60s were most sensitive while p31Fuj cells were least sensitive. Chemotherapy induced significant increase in activity of ATM, ATR, PRKDC and CHEK2. Simultaneous inhibition of ATM and ATR significantly reduced cell viability±A.

Summary/Conclusions: We identified the most abundant and active protein drug targets in AML primary samples and cell lines. Treating AML cell lines with chemotherapy uncovered biochemical pathways involved in DNA repair and survival that determined sensitivity or resistance to these drugs. Proteins significantly modulated in expression and activity after relapse include those involved in DNA repair and pro-survival but these were patient specific, suggesting that targeted therapies will have to be personalized.

LB2243

IDENTIFICATION OF A NOVEL RNA GIANT NUCLEAR BODY IN MYELOID LEUKEMIA

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Background: Constitutive synthesis of oncogenic mRNAs is essential for maintaining the uncontrolled growth of myeloid cells. However, little is known about how these mRNAs are exported from the nucleus to the cytoplasm. Cancer cells, unlike normal cells that only divide a finite number of times before they enter into a state of growth arrest or die, are able to maintain uncontrolled proliferation. The cell nucleus, which houses much of the genome and the machinery needed for its replication, maintenance, and expression, is crucial for the survival and proliferation of cells. However, no nuclear bodies have been definitively linked to the uncontrolled proliferation of cancer cells so far. EIF4E nuclear bodies were initially reported two decade ago and thought to be involved in the exporting of nuclear mRNAs associated with cell proliferation. studies have shown a positive correlation between increased eIF4E phosphorylation and cancer cell proliferation as well as tumorigenesis. Consistently, highly phosphorylated eIF4E (p-eIF4E) was frequently observed in a variety of human cancers. Therefore, we hypothesized that cancer-associated nuclear bodies as detected by p-eIF4E antibody may exist in the nuclei of cancer cells.

Aims: To identify the putative cancer-associated nuclear body, we initially stained two human leukemia cell lines KG-1 (acute myeloid leukemia, AML) by immunofluorescence staining with antibodies against phosphorylated eIF4E (p-eIF4E) or total eIF4E (t-eIF4E); To reveal the morphology and structure features of GNB; To determine whether RNA-GNB abundance was associated with the hyperproliferative phenotype in human cancer.

Methods: Cell lines and culture. Myeloid cell lines were used in this study.Hematopoietic malignant cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum (FCS) at 37°C in a 95% air, 5% CO2 umidified incubator. Normal human blood samples and human leukemia cell samples. Normal blood cells and primary leukemia cell samples were isolated from healthy volunteers or leukemia patients with their informed consent in accordance with the Declaration of Helsinki. All experiments were approved by the ethics committee of Hangzhou First People's Hospital. Immunofluorescence staining. Cells were fixed with freshly prepared 3.7% paraformaldehyde in PBS (pH 7.2) for 20 min at room temperature on slides. Cells were then blocked and permeabilized with PBS containing 10% FBS and 0.1% Tween-20 for 30 min at room temperature. Staining of cells with primary antibodies was performed overnight at 4°C, and then with a FITC or rhodamine-conjugated secondary antibodies for 1 h at room temperature. After three washes with PBS, the slides were mounted in Vectashield with DAPI and sealed. Immunopurification of GNBs from leukemia cells. GNBs were purified from leukemia cells as centrifugation at 2,000 rpm for 5 minutes at 4°C. After washed three times with PBS, cells were resuspended in PBS containing protease inhibitors and transferred to a 7 mL Dounce tissue homogenizer for dounce homogenization. The homogenized suspension was collected for removing intact cells and undisrupted nuclei by centrifugation at 1,000 rpm for 5 minutes at 4°C. Separation of GNBs from nucleoli on the cell nuclei. Cells were collected by centrifugation at 2500 rpm for 5 minutes at 4°C. After removing the supernatant, the cell pellet was lysated with 0.5ml of buffer A for 5min and then centrifuged at 700 rpm, 5min at 4°C. The supernatant was carefully removed and the nuclei were washed with 1.5ml of Buffer A without NP40 by centrifugation at 700 rpm, 5min at 4°C.

Results: We report the identification of a RNA giant nuclear body (RNA-GNB) that is abundant in acute myeloid cells but rare in normal cells. The RNA-GNB contains a RNA core surrounded by a protein shell. We identify 782 proteins from acute myeloid-associated RNA-GNBs, 40% of which are involved in the nuclear mRNA trafficking. RNA-GNB is required for cell proliferation, and its abundance is positively associated with tumor burden and outcome of therapies.

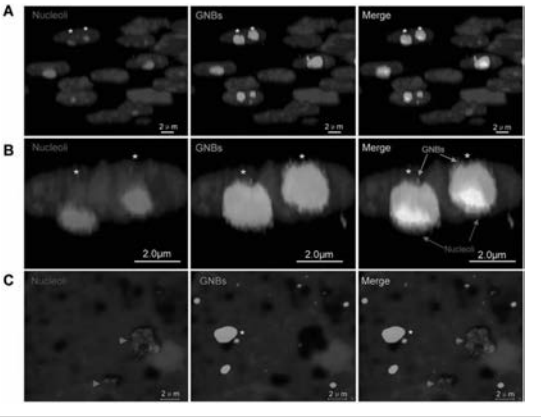

Figure 1.

Summary/Conclusions: Our studies have identified RNA-GNB as a potential nuclear mRNA trafficking organelle. Further studies will be required to characterize the components of proteins and mRNAs in RNA-GNB and reveal their functions. This will help elucidate how RNA-GNBs form and regulate the uncontrolled proliferation of cancer cells.

Acute myeloid leukemia - Clinical

E911

PHASE 1B/2 STUDY OF VENETOCLAX WITH LOW-DOSE CYTARABINE IN TREATMENT-NAÏVE PATIENTS AGED ≥65 YEARS WITH ACUTE MYELOGENOUS LEUKEMIA

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Background: Treatment options for older patients (pts) with acute myelogenous leukemia (AML) unfit for intensive chemotherapy are limited. Expected complete remission rates for low-dose cytarabine (LDAC) are about 10% in this population. Targeting the pro-survival molecule BCL-2 has demonstrated clinical efficacy as a therapeutic strategy in various hematologic malignancies. Venetoclax (VEN), a selective BCL-2 inhibitor, shows synergy with cytarabine in several AML cell lines and primary samples.
Aims: The primary objectives of the study include evaluating the safety of VEN administered with LDAC and preliminary estimates of efficacy.
Methods: This is a non-randomized, open-label phase 1/2 dose-escalation/expansion study of VEN+LDAC, in treatment-naïve AML pts ≥65 years not eligible for intensive chemotherapy. Pts receive oral VEN once daily (QD) on days 1-28 and subcutaneous LDAC 20mg/m^2 QD on days 1–10 of each 28-day cycle. VEN dose escalation follows a 3+3 design; dose-limiting toxicities (DLTs: grade 4 toxicity, platelet count <25,000/μL, or absolute neutrophil count <500/μL within 14 days of last VEN dose) are assessed during cycle 1, up to day 42.
Results: As of Oct 1 2015, 18 pts (66.7% male; median age 74 years) have received LDAC+VEN in the phase 1 portion (VEN 600 mg target dose [n=8]; VEN 800 mg target dose [n=10]). Median time on study is 127.5 (range 30–272) days; 9 pts (50%) remain on study. DLTs of Grade 4 thrombocytopenia lasting >42 days without evidence of residual leukemia occurred in 2 pts in the VEN 800 mg dose group. The recommended phase 2 dose is 600 mg. Adverse events (AEs; ≥30% prevalence) were nausea (77.8%), anemia (55.6%), febrile neutropenia, neutropenia, fatigue (each 38.9%), vomiting, diarrhea and hypokalemia (each 33.3%). The most common serious AE was febrile neutropenia (33.3%). No clinically significant tumor lysis syndrome was observed. The overall response rate in phase 1 was 44% (complete remission, n=4; complete remission without complete marrow recovery, n=4; resistant disease, n=8; death before evaluation, n=2).
Summary/Conclusions: Initial findings suggest that VEN+LDAC has acceptable tolerability and promising clinical activity in older, treatment-naïve AML pts. Available phase 2 updates will be presented.

E912

CLINICAL OUTCOME AND EFFECT OF ALLO-SCT IN ADULT PATIENTS WITH DE NOVO OR AML-RELATED MYELOID SARCOMA. RESULTS FROM AN ITALIAN MULTICENTER SURVEY

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Background: Myeloid Sarcoma (MS) is a rare hematologic myeloid neoplasm that can involve any site of the body. It can occur as isolated "*de novo*" extramedullary form or it can be associated with an acute myeloid leukemia (AML), a chronic myeloproliferative neoplasm (MPN) or a myelodysplastic syndrome (MDS) at onset or at relapse (secondary MS).
Aims: The rarity of MS does not enable prospective clinical trials and therefore a specific multicenter register can be very useful for the clinical and biological studies of this rare disease.
Methods: We report the clinical characteristics and outcome of 53 histologically confirmed MS, diagnosed and treated in 9 Italian Hematological Centers in the last 10 years (2005-2015).
Results: The median age of these patients (pts) was 47 years (range 15-82) and 30 (56.6%) were male. There were 9/53 *de novo* extramedullary MS, 24/53 primary AML-related MS and 20/53 were secondary MS (the median time to the onset of MS from the previous haematologic disease was 34.5 months, range 4–94). Histologic and biologic data are available in all cases. The most common extramedullary anatomic sites of disease were: skin, lymph nodes, soft tissues, bone and testis. Treatment: 46/53 pts (86.8%) underwent a program of intensive chemotherapy (combined with radiotherapy in 16/46 cases) including FLAI, HDAC-IDA, HyperCVAD and MEC schemes, with a CR Rate of 43.5% (20/46). Twenty-four (52.2%) pts underwent Allo-SCT, 9 from an HLA-identical sibling donor, 2 from an haploidentical donor and 13 from a MUD. The median OS of the whole population (53 pts) was 16.7 months with no differences between *de novo* extramedullary MS and AML-related MS (p=0.71). The OS probability at 5 yrs was 33,8%. The survival was significantly better in the pts that underwent an intensive therapeutic program (median OS: 18.3 mths *vs* 5.4 mths, P=0,006). Furthermore, among the intensively treated pts, the survival was better in those pts that underwent Allo-SCT (median OS not reached *vs* 10.6 mths, P=0,001), in pts with *de novo* or primary MS (median OS 20.4 mths *vs* 10.6 mths of the secondary MS, P=0,012) and in the pts that achieved a CR after induction chemotherapy (median OS not reached *vs* 14.6 mths, P=0,07) without differences between *de novo* extramedullary MS and AML-related MS (p=0.76). In multivariate analysis, Allo-SCT and Response to Induction therapy, were the only significant variables in predicting survival (P=0,002 and P=0,037, respectively). The median post-transplant OS of the Allo-SCT recipients was not reached after a median follow-up of 17.5 months and we observe a survival advantage in the patients who achieved a pre-transplant CR (P=0,042) and in the patients who developed a chronic GvHD after Allo-SCT (P=0,065).
Summary/Conclusions: The pts with isolated MS result to have a similar unfavourable outcome than the patients with AML-related MS. These data outline the need of underwent an intensive therapeutic program that includes Allo-SCT, whenever possible, both in isolated extramedullary MS and in AML-related MS. The outcome after Allo-SCT is positively influenced by the development of chronic GvHD suggesting a Graft *versus* MS effect.

E913

OVEREXPRESSION OF ADAM28 ENHANCES ACUTE MYELOID LEUKEMIC CELL MIGRATION AND INCREASES THE GROWTH OF XENOGRAFT TUMOURS IN MICE

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Background: Acute myeloid leukemia(AML) is fatal as a result of primaryrefractoriness, relapse, or treatment-related mortality. Cytogenetics is the most powerful predictor of prognosis in AML, and integrating additional genetic data may further improve predictive capabilities. A disintegrin and metalloproteinases (ADAMs) are involved in various biological events, including cell adhesion, cell fusion, membrane protein shedding, and proteolysis.Our previous study has demonstrated that the expression level of ADAM28 is significantly elevated in relapsed ALL patients and is regulated via a PI3K/Akt signalling pathway.We hypothesized that overexpression of ADAM28 might contribute to invasion and metastasis in adult AML, promoting thetumourigenicity potential andproliferation of AML cells.
Aims: To investigate whether overexpression of ADAM28 was associated with relapse in de novo AML patients, we performed this prospective clinical study and explored the functions and regulation of ADAM28 overexpression in vitro.
Methods: The mRNA expression of ADAMs in the bone marrow of de novo AMLpatients was measured by qPCR. ADAM28 expression levels in BM, serum and cerebrospinal fluid were also measured. Twenty-three healthy donors as controls and 200 AML patientswere included in our group. The cumulative incidence of relapse (CIR),overall survival (OS) and event-free survival (EFS) after a 3-year follow-up were used to evaluate prognosis.Primary AML cells and different AML cell lines were cultured to examine the expression of ADAM28 in vitro. ADAM28-specific cDNA and siRNA were designed and established. Leukemic cell migration was measured using modified Boydenchambers.A nude mouse model was established to measure the effect of ADAM28 on thetumourigenicity of leukemic cells *in vivo*. The expression of Ki-67 and PCNA was detected to measure proliferation.
Results: In general, mRNA expression of ADAM28 displayed the most significant differences between patients and healthy donors among the ADAMs detected.The expression of ADAM28 was significantly increased in AML patients compared with healthy donors. Patients suffering relapse exhibited significantly higher expression levels of ADAM28 compared with those who remained in complete remission. Patients with CNSL also demonstrated significantly increased ADAM28 expression in the CSF and serum compared with non-CNSL patients. The Kaplan-Meier survival curve showed that EFS was shorter andCIR was higher in patients with high expression levels of ADAM28.The results showed that age, karyotype and ADAM28 level are inde-

pendent risk factors for OS and EFS.In vitro, the expression of ADAM28 was relatively higher in primary blast cells and SHI-1 cells, which were selected forsubsequentprocedures. Overexpression of ADAM28 promoted the proliferation and invasiveness of SHI-1 cells and primary blast cells transfected with ADAM28 cDNA; in contrast, knockdown of ADAM28 expression inhibitedproliferation and invasiveness.Following subcutaneous injection into nude mice,ADAM28 knock-down cells displayed an inhibited tumourigenicity potential, whereas ADAM28-overexpressingcells presented largerxenograft tumours.
Summary/Conclusions: Our findingsdemonstrate that overexpression of ADAM28 in AML patients is correlated to relapse; patients with higher levels of ADAM28 have a higher CIR. Overexpression of ADAM28 can enhance cell proliferation and migration, as well as tumourigenicity. These data suggest that ADAM28 may be a novel biomarker for predicting the prognosis of AML, potentially providing a new therapeutic target.

E914

PROLONGED SURVIVAL IS ACHIEVABLE FOR A QUARTER OF PATIENTS IN PRIMARY INDUCTION FAILURE FOR ACUTE MYELOID LEUKEMIA, BUT DOES NOT RELY ON REPEATED SALVAGE THERAPY-A MONOCENTRIC RETROSPECTIVE STUDY

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Background: Response to first induction chemotherapy is a strong prognostic factor in patients with newly diagnosed acute myeloid leukemia (AML) eligible for intensive treatment. Primary induction failure (PIF) leads to an escalation of therapeutic means, the benefit of which remains controversial.
Aims: Allogeneic hematopoietic stem cell transplantation (HSCT) appears to be the most appropriate consolidation treatment to lower relapse risk but determining its best timing is still a challenge: disease-free (DFS) and overall survivals (OS) are known to be better if allogeneic HSCT is done in complete remission (CR), but earlier transplant is usually associated with a better outcome. These two conditions seem to pull in opposite directions in PIF AML.
Methods: We therefore conducted a retrospective monocentric study analyzing the outcome of all adult patients with newly diagnosed AML in Strasbourg University Hospital between 2002 and 2014 and failing to achieve remission after first induction.
Results: Of 704 AML patients, 394 (56.0%) received intensive chemotherapy, and 90 (22.8%) were considered PIF. Sixty-three of these patients (70.0%) received further intensive treatment, enabling a median OS of 418 days, compared to 65 days for patients on palliative care and 253 days for patients receiving hypomethylating agents only (p<0.001). Salvage chemotherapy led to CR in 40% of patients, and those having received an allogeneic HSCT while still on CR reached a median OS of 2068 days, with a three-year survival of 57.4%. Patients in CR after salvage therapy but who were not subsequently transplanted had a median OS of only 551 days. Patients receiving more than one salvage chemotherapy before being transplanted had a median OS of 418 days, compared with 467 days for patients who were transplanted while refractory without or after only one salvage therapy (p=0.7). Among the 23 patients still refractory at transplantation, allogeneic HSCT eventually led to remission in 20 patients (87%), but with a high subsequent relapse rate. Allogeneic HSCT in refractory patients led to a median OS of 467 days, with three-year survival reaching 25.6%. On the whole, the three-year survival rate of the 38 patients who received an allogeneic HSCT was 39.5% compared with 2% for the 52 patients who did not undergo transplantation.
Summary/Conclusions: Continuing intensive treatment of AML after primary induction failure is still the better therapeutic option in terms of survival for fit patients: we have observed that it can result in prolonged survival, and perhaps cure, for roughly a quarter of them, supporting this approach. However, considering our data, receiving more than one salvage chemotherapy doesn't appear to lead to a better outcome and may impair the rate of allogeneic HSCT. This might eventually affect the outcome of refractory patients, since allogeneic HSCT is a crucial step in their management. Although survival is consistently lower for patients lacking CR, allogeneic HSCT for refractory patients still offers prolonged survival to a significant proportion of PIF AML patients in spite of their initial dismal prognosis.

E915

IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS OF ABCB1 GENE UPON THE EFFECTIVENESS AND TOXICITY OF INDUCTION CHEMOTHERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: The intake of anthracyclines in blast cells could be affected by efflux pumps of *ABC* family.
Aims: Previous studies suggested that single nucleotide polymorphisms (SNPs) of *ABCB1* may influence anthracycline effectiveness in acute myeloid leukemia (AML) induction therapy, although their impact in induction death and anthracycline-related organ toxicity.
Methods: The SNPs of *ABCB1* gene (rs1128503, rs1045642, rs2032582 and haplotype) were evaluated in 225 adult patients at initial diagnosis from AML using a Sequenom (iPLEX) mass spectrometry–based multiplex genotyping assay (Sequenom, San Diego, CA). All patients received induction chemotherapy consisting of idarubicin plus cytarabine (PETHEMA-LMA 99, 2007 and 2010 trials). Efficacy of first induction cycle was evaluated comparing complete remission (CR) *vs* partial remission (PR) or resistance, excluding these patients dying during induction. Induction death was defined as patients dying during induction against CR, excluding these patients with PR or resistance. Based on WHO grading scale, toxicities were grouped as binary variables (grade 0-1 *vs* grade 2-4). The grade of toxicity assigned to an organ group was the maximum grade of all the specific toxicities within that group. Genotypes were studied with a co-dominant model. Association between variables was assessed using linear and logistic regression adjusting for age, gender, cytogenetic risk, ECOG, leukocyte and platelet count, hemoglobin, creatinine, bilirubin, albumin and LDH level at diagnosis (R® version 3.1.2).
Results: The median age of patients was 51.1 years (16-78 years). There were no statistically significant differences in CR. Nevertheless, induction death was associated to *ABCB1* triple variant haplotype (40.0% *vs* 17.2%; OR: 0.2; 95%CI: 0.05-0.8; P=0.017). Besides, the *ABCB1* triple variant haplotype was related to more nephrotoxicity than the other genotypes (OR: 3.6; 95%CI: 1.3-10.4; p=0.016). The variant alleles of *ABCB1* rs1128503, rs2032582 and recessive haplotype were also related to hepatotoxicity (OR: 6.9; 95%CI: 2.2-21.2; p= 0.001; OR: 2.9; 95%CI: 1.01-8.1; p=0.049; OR: 6.0; 95%CI: 2.1-16.3; p<0.001; respectively). Regarding hematologic toxicity, time to neutropenia recovery was delayed with variant allele of *ABCB1* rs2032582 (OR: 3.0; 95%CI: 1.1-10.1; P=0.047).
Summary/Conclusions: The variant alleles of *ABCB1* polymorphisms have been related to lower anthraclycline clearance and higher tissue exposure. This could be the reason of the higher toxicity and induction death observed. This study shows a prognostic impact of *ABCB1* polymorphisms in adult AML patients regarding induction chemotherapy toxicity. Further studies with larger population are needed to validate these associations, which could be useful biomarkers in clinical practice. Study supported by grants from the "Instituto Carlos III" (PIE13/00046), "Instituto Investigación Sanitaria La Fe" (2013/0331) and the Cooperative Research Thematic Network (RTICC), Grant RD12/0036/014 (ISCIII & ERDF). Samples have been managed by the La Fe Biobank, licensed as required by Spanish Royal Decree 1716/2011 of 18 November (Ref.: PT13 / 0010/0026).

E916

CLEARANCE OF NPM1 MUTATION AT FIRST COMPLETE REMISSION AFTER INDUCTION THERAPY DID NOT PREDICT THE SURVIVAL IN FAVORABLE RISK GROUP WITH NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA

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Background: The presence of minimal residual disease is known to be an independent prognostic factor for the duration of remission and survival in acute myeloid leukemia (AML). However, since AML has a molecular heterogeneity, the routine assessment of minimal residual disease (MRD) has not been adopted in the current practice of patients with AML.
Aims: Herein, we evaluated the *NPM1* mutation as a marker of MRD at first complete remission (CR) after induction therapy in normal karyotype AML (NK-AML) with favorable molecular risk group.
Methods: A total of 413 patients were included in the present study who met the following eligibility criteria: 1) age ≥15 years; 2) a diagnosis of NK-AML

confirmed by conventional cytogenetic analysis; 3) treatment with induction chemotherapy using a standard protocol (a 3-day course of anthracycline with a 7-day course of cytosine arabinoside). Analysis of *NPM1* mutations were performed using targeted resequencing by SureSelect capture and Illumina platform technology. The 184 patients (44.6%) showed *NPM1* mutation at diagnosis and, 99 patients were *NPM1* mutated and *FLT3*-ITD negative (favorable molecular risk by ELN). We have focused on the prognostic impact of *NPM1* MRD esp. in the group with favorable risk by ELN risk classification. The 52 out of 99 favorable risk patients were available the analysis of *NPM1* mutations at CR samples.

Results: In 52 patients, 37 patients (71.2%) were negative (*NPM1*$^{MRD-}$) and, 15 patients (28.8%) persistently observed the *NPM1* mutations (*NPM1*$^{MRD+}$) in CR samples. There was no difference in variant allele frequencies of *NPM1* mutations at diagnosis between *NPM1*$^{MRD-}$ *vs* *NPM1*$^{MRD+}$ (32.7% [range, 6.85-43.9] *vs* 36.8% [range, 25.0-48.7], p=0.168). There was no difference in gender (p=0.754) or age (p=0.545). However, WBC count in *NPM1*$^{MRD-}$ group (median: 15.3 x 10^9/L, range: 0.9-142.0) was lower than in the *NPM1*$^{MRD+}$ group (median: 81.1 x 10^9/L, range: 1.7-224.6.0), (*p*=0.014). In 15 out of 52 patients received allogeneic stem cell transplantation (SCT), there was no difference of proportion of patients receiving allotransplant between *NPM1*$^{MRD-}$ *vs* *NPM1*$^{MRD+}$ group (21.7% vs.20.0%, p=0.897). In the survival analysis according to the MRD of *NPM1* mutation, there was no difference between *NPM1*$^{MRD-}$ *vs* *NPM1*$^{MRD+}$ group in terms of 5-year overall survival (OS) (*NPM1*$^{MRD-}$ *vs* *NPM1*$^{MRD+}$, 66.1% vs.72.7%, p=0.699) and 5-year event free survival (EFS) (*NPM1*$^{MRD-}$ *vs* *NPM1*$^{MRD+}$, 57.1% vs.61.1%, p=0.596). There was no difference in relapse risk at 5-year (*NPM1*$^{MRD-}$ *vs* *NPM1*$^{MRD+}$, 36.4% vs.32.2%, p=0.509). Regardless of clearance of *NPM1* mutations, allogeneic SCT substantially reduced the relapse risk (p=0.007). However, allogeneic SCT did not confer to overall survival benefit due to higher non-relapse mortality (p=0.046).

Summary/Conclusions: In this cohort, clearance of *NPM1* mutation at first CR in favorable risk group of NK-AML was not replicated as a prognostic marker to predict the survivals or relapse risk. Even in patients with *NPM1* positive at first CR in favorable molecular risk group, the most of patients demonstrated long term survival even with consolidation chemotherapy without allogeneic transplantation. To clarify the role of clearance of *NPM1* mutations, further analysis after consolidative therapy will be needed.

E917

VALIDATING THE PATIENT'S "FITNESS" CRITERIA PROPOSED TO GUIDE TREATMENT DECISION IN ELDERLY AML: A MULTICENTER STUDY ON A SERIES OF 699 PATIENTS BY THE NETWORK "RETE EMATOLOGICA LOMBARDA"

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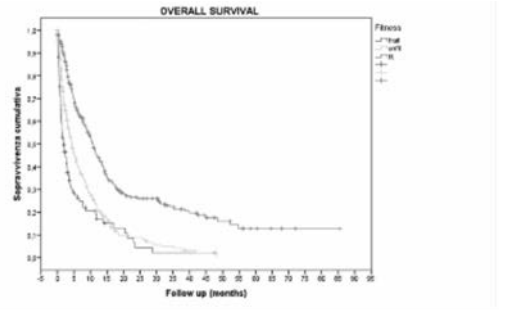
Background: Treatment of elderly patients (pts) with acute myeloid leukemia (AML), is still controversial. In 2013 an Italian panel of experts proposed a set of objective criteria to define pts fit or unfit to conventional intensive chemotherapy (iCT) or non intensive therapy (niT) (Ferrara et al, Leukemia, 2013). Since such criteria derived from experts opinion, they need to be validated in the clinical setting to become an useful tool for therapy decision making.

Aims: Fitness criteria were applied to a population-based series of pts with AML (not M3), to retrospectively evaluate their actual applicability, their concordance with the treatment actually given, and the outcome of pts according to "fitness", leukemia biology and treatment.

Methods: We evaluated 699 pts aged >65 y, diagnosed between 2008 and 2015, at 8 Centres of the Hematological Network of Lombardy (REL). AML was de novo in 419 pts and therapy-related or secondary to myeloid neoplasms (s-AML) in 280 pts; median age was 74 (range 65-96). The categorization according to "fitness" criteria was carried out retrospectively by physicians who had followed pts and through medical files. Pts were defined as fit to iCT (FIT), unfit to iCT (UNFIT), or unfit even to niT (FRAIL). ELN prognostic criteria could be applied to 117 (27.9%) de novo AML [12.2% favorable-risk (fav) (CBF 4.6%), 32.5% intermediate-1 (int1), 14.2% intermediate-2 (int2), and 36.4% adverse (adv) risk]. Karyotype (K) was adverse (ELN) in 193 (36%) of 537 evaluable AML. According to physicians decision, 274 pts (39.2%) received iCT, 134 pts (19.2%) niT, including low-dose ara-c, hypomethylating agents or experimental non-myelotoxic drugs, and 291 pts (41.6%) best supportive care (BSC).

Results: Fitness criteria were not applicable in 13 pts (1.9%), because of insufficient data. Among 686 evaluable pts, 292 (42.5%) were FIT, 289 (42.1%) UNFIT, and 105 (15.3%) FRAIL. Median age was 69, 78 and 76 y, respectively (p<0.0001). Median overall survival (OS) of FIT, UNFIT and FRAIL pts was 10.9, 4.2 and 1.8 months (m), respectively (p=0.000) (Figure 1). According to ELN risk, median OS was 17.5, 14.1, 8.0, 9.8 m, in fav, int1, int2 and adv risk de novo AML, respectively (p 0.008). Median OS was 12.6 and 5.9 m in not-adv and adv K, respectively (p 0.002).Overall concordance between "fitness criteria" and the treatment actually received by pts was 79.4% (76% in FIT, 82.7% in UNFIT and 80% in FRAIL pts). There was a significant interaction between disease biology and treatment actually given. FIT pts receiving niT had a higher proportion of adv K (66%) than pts receiving iCT (19%). In FIT pts median OS was best with iCT, being significantly longer than in pts actually "undertreated" (11.6 m with iCT *vs* 7.6 m with niT or BSC; p 0.006). In UNFIT pts median OS was 8.7, 9.2, 2.5 m with iCT, niT and BSC, respectively. It was significantly worse in patients actually "undertreated" with BSC only (p 0.0001), but "overtreating" UNFIT pts with iCT did not improve OS. Among FRAIL pts, "overtreatment" using niT didn't improve OS compared to BSC (2.9 *vs* 1.5 m, p:ns).



	FIT	UNFIT	FRAIL	TOT
	292 (42.5%)	289 (42.2%)	105 (15.3%)	686
iCT	222 (76%)	50 (17.3%)	2 (1.9%)	274 (40%)
niT	39 (13.4%)	76 (26.3%)	19 (18.1%)	134 (19.5%)
BSC	31 (10.6%)	163 (56.4%)	84 (80%)	278 (40.5%)

Figure 1.

Summary/Conclusions: The "fitness criteria" proposed were easily applicable even retrospectively and in a multicenter setting. Overall concordance between "fitness criteria" and treatment actually received by pts was high (79.4%). Fitness was significantly related to patient's survival. The "fitness criteria" could correctly identify patients most likely to benefit from different treatment intensities or BSC. Knowing biologic risk profile may further refine treatment decisions.

E918

CLINICAL CHARACTERISTICS AND OUTCOME OF PEDIATRIC ACUTE MYELOID LEUKEMIA WITH DEL(5Q): A REPORT FROM THE JAPANESE PEDIATRIC LEUKEMIA/LYMPHOMA STUDY GROUP

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Background: Deletion of chromosome 5q (5q-) confer a poor prognosis in adults with acute myeloid leukemia (AML) and associated with higher white blood cell count at diagnosis. However, since this chromosomal abnormality is rare in pediatric patients with AML, the clinical characteristics and prognostic significance are not clear.

Aims: To clarify AML with 5q- in terms of hematological and clinical characteristics, and prognosis.

Methods: Between November 2006 and December 2010, 485 consecutive patients aged<18 years with suspected AML excluding acute promyelocytic leukemia, Down syndrome, secondary AML, myeloid/natural killer cell leukemia, and myeloid sarcoma were registered in AML-05 conducted by the Japanese Pediatric Leukemia/Lymphoma Study Group. The diagnosis according to the 2008 WHO classification was determined centrally by integrating morphologic, cytogenetic, immunologic, molecular and clinical parameters. We stratified patients after the second induction course to either of the three risk groups according to the cytogenetics and *FLT3*-ITD status at diagnosis and the response to induction chemotherapy. Allogeneic hematopoietic transplantation (HSCT) was planned for the patients allocated for the high risk group including 5q- after the third or fourth chemotherapy course.

Results: Of the 485 patients registered, 32 patients were excluded because

of misdiagnosis or refusal by the guardians. Of the 453 eligible patients, a total of 11 patients (2.4%) were identified as AML with 5q-. The table 1 shows cytogenetic data for the 5q- patients. All patients with 5q- had unrelated chromosomal abnormalities >3 and were therefore diagnosed as AML with myelodysplasia-related changes. Median age at diagnosis was 1.9 years (range, 0.2-13.8) and median WBC count at presentation was 28.1×10^9/l(range, 2.1-50.7). Any significant differences were seen in age and WBC count compared to those without 5q-. Of the 11 patients, 7 (64%) had a complete response (CR) to induction chemotherapy. Of the 4 patients with induction failure, 2 were treated with HSCT and were alive. Of the 7 patients achieving CR, bone marrow relapse was recognized in 5, 2 of whom were alive after HSCT. Excluding patients with core binding factor leukemia, the 3-year event-free survival for patients with 5q- was significantly lower than for those without 5q- (18% *versus* 56%, P=0.02). However, the 3-year overall survival was not significantly different between patients with 5q- and those without 5q- (54% *versus* 70%, P=0.07).

Table 1.

Table 1 Cytogenetic Data of 11 patients with 5q-

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Summary/Conclusions: Our study suggests that AML with 5q- has a poor prognosis, but that intensive therapy including HSCT may improve outcome in children with AML with 5q-.

E919

DOSE ESCALATION STUDY OF CRENOLANIB IN COMBINATION WITH HIGH DOSE CYTARABINE/IDARUBICIN SALVAGE CHEMOTHERAPY IN MULTIPLY RELAPSED FLT3 POSITIVE AML

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Background: FLT3 inhibitors usually provide transient clinical benefit to patients with FLT3-mutated AML. FLT3 inhibitor-based combination therapy has been suggested to enhance such benefit. Crenolanib is a novel, type I, oral pan-FLT3 inhibitor with high single-agent activity against multiply relapsed FLT3-ITD and tyrosine kinase domain (TKD) mutant AML. We here report interim data from a Phase I trial to evaluate the safety and efficacy of salvage therapy with high dose cytarabine/idarubicin followed by crenolanib in multiply relapsed FLT3+ve AML.
Aims: Dose escalation study to determine the maximum tolerated dose (MTD) of crenolanib when combined with high dose cytarabine+idarubicin.
Methods: Pts >18y with refractory/relapsed FLT3 ITD and/or TKD+ve AML are eligible. Pts receive salvage therapy with high dose cytarabine (1.5 g/m^2/day over 3 hours for 4d or for 3d if >60y) along with idarubicin (12 mg/m^2 for 3d). Daily crenolanib starts from d5 and is continued until 72hrs prior to next chemotherapy regimen. The starting dose cohort of crenolanib is 60 mg TID and is then escalated in subsequent cohorts to 80 mg TID and 100 mg TID. Pts may receive consolidation with cytarabine (750mg/m^2 for 3d) and idarubicin (8 mg/m^2 for 2d) followed by daily crenolanib. Crenolanib may be administered for maintenance for up to 365 days.

Table 1.

Age	Sex	FLT3 Status	Prior chemo regimens	Prior Investigational agents	Crenolanib dose	Response
43	F	ITD	2	sorafenib	60 mg	CRi
67	M	D835	1		60 mg	CR
21	M	ITD	4+SCT	sorafenib, E6201	60 mg	No Response
31	M	ITD+ D835	1		80 mg	ETP-ALL
51	M	ITD	8	AG221, BGB324	80 mg	NE
30	M	D835	9+SCT	sorafenib, AG221, E6201	80 mg	NE
58	M	ITD+ D835	3	quizartinib	80 mg	CRi
57	M	ITD+ D835	2	sorafenib	80 mg	No Response
70	M	D835	3	AMG330	100 mg	pending
19	M	D835	3+2 SCT		100 mg	pending
24	M	ITD+ D835	3	quizartinib	100 mg	pending

Results: To date, 11 pts (10 males, 1 female) have been enrolled with a median age of 43 (range 19-70). Pts were heavily pre-treated with a median of 3 prior systemic treatments (range: 1-9). 8/11 pts had received prior investigation agents including 6 with prior FLT3 inhibitors. Three pts had undergone allogeneic SCT. Besides FLT3, multiple other leukemia associated mutations were present at baseline: NPM1 (40%), DNMT3A (40%), WT1 (30%), TET2 (30%), NRAS/KRAS (30%), IDH2 (20%), NOTCH1 (20%), MLL (20%), RUNX1 (10%), ASXL1 (10%) and EZH2 (10%). All 3 pts treated with cytarabine/idarubicin followed by crenolanib 60 mg TID tolerated the regimen well with no DLTs. Two achieved a CR (including one who became FLT3 negative after reinduction). Both these patients have undergone an allo SCT. 5 pts received cytarabine/idarubicin followed by crenolanib 80 mg TID. One of the three evaluable pts in this cohort achieved a CRi and has undergone SCT. The patient with ETP-ALL had no response. No pt required dose reduction in the 80mg TID cohort. All 3 pts in crenolanib 100mg TID cohort have now completed cytarabine/idarubicin salvage and are receiving crenolanib (but are too early for assessment of response).
Summary/Conclusions: Crenolanib can be safely administered with HiDAC/idarubicin salvage chemotherapy in multiply relapsed AML. 3 patients have achieved a CR/CRi which allowed them to be bridged to an allo SCT. Accrual to this trial will continue at the crenolanib 100 mg TID dose.

E920

QUANTIFICATION OF COMMON AND RARE NPM1 SUBTYPES USING A SINGLE MASSIVELY MULTIPLEX DIGITAL PCR ASSAY FOR MINIMAL RESIDUAL DISEASE IN AML

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Background: Acute myeloid leukemia (AML) is a fatal disease with poor outcomes. Despite initial remissions, most patients relapse and ultimately succumb to their disease. The presence of minimal residual disease (MRD) in patients has been shown to be predictive of relapse and thus is likely to better inform therapeutic decision-making. Improved assessment methods are therefore urgently needed. Molecular assessment of MRD has been accomplished using a number of markers including NPM1 mutations (NPM1mut). The vast majority of NPM1 mutations are four nucleotide insertions in exon 12, the most common of which is type A (c.860_863dupTCTG), found in approximately 75% of NPM1 mutated AML patients. The remaining patients often have rarer subtypes with differing and often patient-specific insertion polymorphisms with over 50 such polymorphisms being reported. Currently, real time quantitative PCR (RQ-PCR) is used to assess mutant NPM1 percentages. However, RQ-PCR requires custom assays and development of plasmid standards tailored to each subtype. RQ-PCR assays have been adapted to digital PCR (dPCR) to eliminate the need for plasmid standards, but custom primers and/or probes are still needed. Moreover, in diagnostic cases where NPM1mut diagnosis is made by capillary electrophoresis without sequencing, subtype information is absent and MRD cannot be assessed reliably with standard approaches. To overcome these obstacles, we demonstrate a massively multiplex dPCR assay that operates on >95% of NPM1 polymorphisms without requiring prior knowledge of the specific polymorphism and is robust to NPM1 evolution and mixtures of NPM1 mutant clones.
Aims: To determine if a single assay can consistently quantify NPM1mut percentages in a manner that is robust to NPM1 subtype.
Methods: AML samples and synthetic reference standards with varying NPM1 mutations were quantified as NPM1 mutant copies per ABL1 copies (NPM1mut/ABL1). Subtype-specific assays were compared to massively multiplexed dPCR assay. Agreement between NPM1mut/ABL1 measurements was assessed in parallel using both methods.
Results: NPM1 mutant copies were quantified using massively multiplexed dPCR assay alongside subtype-specific assays. The massively parallel dPCR assay demonstrated excellent correlation with subtype-specific assays (r >0.95; Pearson's correlation), including rare subtypes such as NPM1-DD1. We additionally assessed mixtures of subtypes alongside standard assays. In such mixtures, our massively multiplexed assay correctly ascertained NPM1mut/ABL1 ratios whereas standard subtype-specific assays underestimated NPM1mut/ABL1 ratios, demonstrating the robustness and accuracy of this assay in samples with mixtures of NPM1 mutants. Sensitivity was comparable with standard subtype-specific assays without cross-reactivity to wild-type NPM1.
Summary/Conclusions: Massively multiplexed dPCR inexpensively allows a single assay to detect the vast majority of NPM1 mutations both common and rare. The quantification is largely impervious to NPM1 evolution/polyclonality and greatly diminishes the need for subtype-specific assays with custom primer sets and plasmid standards. It is therefore a useful strategy for the follow up of AML patients diagnosed with NPM1mut AML in MRD assessment.

E921

A SIMPLE 3-GENE MOLECULAR SCORE RETAINS HIGH PROGNOSTIC VALUE IN NON HIGH RISK ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH AIDA 2000 PROTOCOL: A RETROSPECTIVE STUDY FROM A REGIONAL REGISTER

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Background: Non high risk acute promyelocytic leukemia (APL) patients, as

defined by WBC count at diagnosis, have nowadays a very good prognosis when treated with ATRA plus chemotherapy based protocols, with high rate of complete molecular remission after consolidation therapy. However, a small portion of patients (roughly 10%) will eventually relapse, despite the achievement of molecular CR. In the past years, some groups showed that relapse risk could be predicted by testing for some gene alterations, such as FLT3-ITD, or by break point cluster region (BCR) analysis. However, those results were not confirmed in prospective trials.

Aims: We recently showed that a simple gene-expression panel including WT1 and BAALC expression levels analysis has high prognostic value in MDS patients. The aim of the present study was to evaluate if the application of a similar panel to APL patients at diagnosis could be predictive of relapse risk and survival.

Methods: We retrospectively applied a simple 3 gene based molecular panel including WT1 and BAALC expression levels at diagnosis, FLT3-ITD mutational status (molecular profile), alongside with BCR analysis, to a cohort of de novo 66 non high-risk acute promyelocytic leukemia patients, treated in our institution between January 1st 2004 to June 30th 2015. All patients have been treated according to the Italian age-adapted AIDA protocol. Median age was 47 years (range 16-88), 17 patients were older than 60 years. Median follow up was 58 months. BCR analysis was available in all patients, 43 patients showed BCR1/2, whereas 23 patients had BCR3 (35%). Molecular profile was available in 44/66 patients (66%). Cut-off values for WT1 (25000/Abl x 10^{-4}) and BAALC (450/Abl x 10^{-4}) were arbitrary chosen after pre-analysis and comparison with our published data. Seven patients had FLT3-ITD mutation (17%), 18 (45%) patients had high WT1 expression levels and BAALC was overexpressed in 9 (23%) patients.

Results: Sixty-three patients survived induction, all of them achieved CR. Among patients who had completed the whole therapeutic program at the time of the analysis 2 showed molecular persistence of disease and therefore received further therapy achieving molecular CR. Ten patients experienced disease relapse (15%). Pre-analysis showed that FLT3-ITD, not over-expressed WT1 levels, high BAALC levels but not the presence of BCR3 were associated with higher relapse risk. None of the molecular alterations reached statistical significance if taken alone. Therefore a molecular score including those 3 genes was built: 18 patients had no risk factor, 20 had one risk factor and 6 had two risk factors. Relapse risk was influenced only by the presence of at least one molecular risk factor ($p<0.05$). Disease free survival (DFS) duration was significantly higher in patients who had no molecular risk factor when compared to patients with at least one (3-years DFS of 100% and 85%, respectively, $p<0.05$). Overall survival (OS) analysis led to similar results: patients with no molecular risk factors had a very good outcome when compared to patients with at least one alteration (3-years OS of 100% and 79% respectively, $p<0.03$). Furthermore, we observed that both in DFS and OS analysis, the presence of two molecular factors leads to a worse outcome compared to the presence of only one alteration ($p<0.05$).

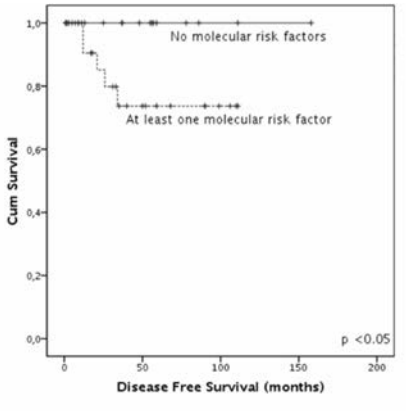

Figure 1.

Summary/Conclusions: Even in a small cohort, we demonstrate how a simple molecular profile, including FLT3-ITD and levels of expression of BAALC and WT1 could identify a subset of standard APL patients at higher risk of relapse. Sub-stratification of patients could allow us to recognize patients who may take benefit from an intensified consolidation therapy, as it is currently applied to high risk patients, in order to reduce risk of relapse.

E922

ACUTE MYELOID LEUKEMIA (AML) IN ELDERLY PATIENTS (≥70 YRS): SEMI-INTENSIVE INDUCTION AND CONSOLIDATION WITH MAINTENANCE TREATMENT (1-YR) IN OUTPATIENT BASIS

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Background: The results of AML treatment in patients' ≥70 years are disappointing. Finding tolerable and efficacious treatments remains a major challenge. Intermediate dose cytarabine schedules in combination with other drugs allow treating patients in an outpatient basis.

Aims: The CETLAM04LAM70 semi-intensive protocol (Eur J Haematol. 2015;95:576-82) aimed to achieve a significant proportion of durable remissions in elderly (≥70 years) patients with AML based on reduction of the toxicity of full dose cytarabine. Despite this, toxicity and deaths in consolidation were of concern. In the current protocol (CETLAM11LAM70) intensive consolidations were substituted by 1 year of 5-azacitidine treatment. The aim of this study was to evaluate the tolerability and efficacy of the CETLAM11LAM70 protocol and to compare the outcomes with those from CETLAM04LAM70 protocol.

Methods: Eligible patients: 70 years of age or older with a newly diagnosed AML excluding t(15;17), biphenotypic leukemia with t(9;22) or blast crisis of chronic myeloid leukemia, ECOG 0-3 and able to attend weekly hospital visits at baseline. Treatment regimen: Induction-1 with FAG (fludarabine 25 mg/m^2 PO days 2-5, cytarabine 100 mg/m^2 SC days 2-8, filgrastim 300µg SC days 1-8), induction-2 or consolidation with IAG (idarubicin 20 mg/m^2 PO days 2-4, cytarabine 100 mg/m^2 SC days 2-8, filgrastim 300µg SC days 1-8) followed by 12 courses of AZA (5-azacitidine 75 mg/m^2 SC days 1-5 every 28 days). The main differences with the CETLAM04LAM70 protocol were the cytarabine dose (200 mg/m^2 *vs* 100 mg/m^2), the number induction/consolidation cycles and the 12 months maintenance.

Results: From January 2011 to June 2015, 44 patients were included (target 50 patients). Median age 74[70;87] years, 23 males (52%). AML characteristics: poor-risk cytogenetics 15 (34%), trilineage dysplasia 14/42 (33%), *FLT3 ITD* 6/33 (18%), mutation of *NPM1* 9/31 (29%). Outcomes: induction-related deaths 8 (18%), overall response to induction-1 26 (59%)(11/44 CR and 15/44 PR), consolidation-related deaths 3 (12%) and deaths during AZA treatment 2 (14%). Fourteen patients (32%) completed the scheduled treatment. The 1 and 2-yr (95%CI) OS, DFS and response duration are summarized in Table 1. Toxicities: Median duration of thrombocytopenia (<20x10^9/L) and neutropenia (<1x10^9/L) were 3 weeks (range 1-6) without differences between induction and consolidation. Fever in induction was observed in 30 patients (68%) and 34 (77%) were hospitalized; the median hospital stay was 15 days (1-45). Ten patients (23%) during induction and 8 (31%) during consolidation could be managed completely as outpatients. No toxicities grade >2 were observed during azacitidine treatment. No differences in OS, CR duration and DFS were observed on comparison of the two protocols (Table 1).

Table 1.

	CETLAM04LAM70 (%)	CETLAM11LAM70 (%)
1 yr OS (95%CI)	30 (14-46)	40 (25-55)
2 yr OS (95%CI)	23 (12-35)	24 (10-38)
1 yr response duration (95%CI)	50 (23-77)	67 (46-88)
2 yr response duration (95%CI)	41 (14-68)	38 (13-63)
1yr-DFS (95%CI)	35 (12-58)	43 (24-62)
2yr-DFS (95%CI)	29 (5-47)	21 (5-37)

Summary/Conclusions: The CETLAM11LAM70 protocol for elderly AML patients ≥70 years is tolerable, feasible and effective with reasonable response and induction mortality rates. Despite azaciditine was less toxic than previous consolidation strategies, the modification of the protocol did not help to improve OS. Supported: grants PI10/01417 and PI14/00450 from FIS, RD12/0036/0029 from Instituto Carlos III and 2014SGR225(GRE) from Generalitat de Catalunya, Spain.

E923

COMBINATION OF RNA- AND EXOME-SEQUENCING EFFICIENTLY ELIMINATES FALSE-POSITIVE SOMATIC POINT MUTATIONS AND INDELS-EXEMPLIFIED BY CASES OF CN-AML

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Background: Thorough annotation as a means of detecting highly relevant

mutations, and aberrated genes, is becoming more feasible as the evidence of biological pathways underlying malignant transformation compiles. However, there is a continuous risk of misinterpretating both true and false positive observations, which is closely coupled to the sheer number of observations in NGS projects. Thus, there is still a need to find ways to efficiently filter data ab initio, with little knowledge added from disease aetiology and other clinical findings in order to gain new insights.

Aims: We aimed at finding an efficient method to pinpoint true positive and known causal mutations, decreasing the need of downstream interpretation of variants to a minimum.

Methods: DNA and RNA were extracted from lysated bone marrow aspirate to a mean concentration of 35 ng/µl for both groups and 20 samples in total (Fig.). In order to streamline the alignment and variant calling workflow, we implemented CLC Biomedical Workbench (Qiagen, Aarhus, DK) to keep all dataprocessing in one simple workflow. Only regions covered by the Nextera Rapid Capture Exome kit (Illumina, CA, USA) were included and common variants excluded (dbSNP, NCBI, MD, USA). Alternative GATK and MuTect de facto standard workflow was used to survey the quality of CLC Biomedical Workbench alignment and single nucleotide variant (SNV) detection.

Results: Exome sequencing yielded an average of 9.1*10^7 sequence reads with read lengths of 100 and over 99% mapped in each sample. The combination of whole exome and RNA sequencing efficiently reduced the number of detected mutations to a median number of 6 [3, 21] somatic nucleotide variants (see figure). 6 new mutations, reported to be mutated in at least one other case of haematological neoplasms (COSMIC, Wellcome Trust, Sanger Institute, Cambridge, UK), *i.e.* not detected by routine laboratory assays at the department. Detection of NPM1 mutations confirmed results from Sanger sequencing, but were found in one additional sample. Likewise, IDH1 census mutation confirmed positive lab results, one additional mutation previously undetected and added IDH2 mutation. CLC Biomedical Workbench did not prove successful in detecting *FLT3* internal tandem repeats. The cytogenical status was confirmed by assessment of allelic frequencies from NGS (not shown).

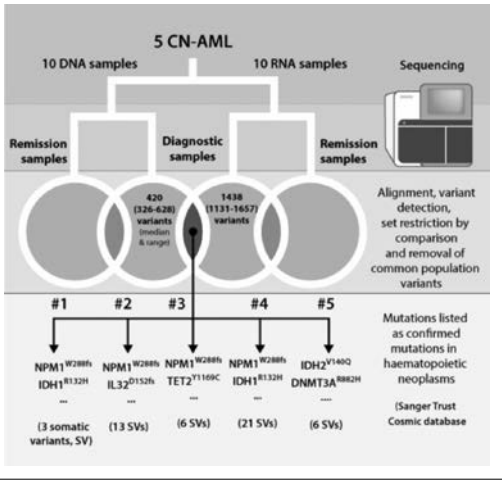

Figure 1.

Summary/Conclusions: Removal of the substantial number of false positives and irrelevant mutations are a high-priority issues when performing NGS. As CLC Biomedical Workbench, and other tools such as VarScan2, generally detects a large number of somatic variants in raw comparison of paired samples it is crucial to apply stringent and optimal filtering. We show that the inclusion of RNA sequencing in the workflow, not only provides information on malignant expression profiles excluded here, but importantly help to capture the, often very few somatic mutations of myeloid leukaemia. As signature indels, such as found in *NPM1*, *TET2* and *FLT3,* are important drivers the practicality of DNA-RNA combination is evident, as we demonstrate here-with the exception of *FLT3*-ITD aberration, which was not detected properly. This small research study not only shows how careful the large number of variants from NGS must be approached-it is also in agreement with previously reported relatively low number of somatic mutations, concluded by several studies. Although we use remission samples in both cases for added stringency, a clinical practical counterpart would be the combination of RNA and DNA malignant sample and skin biopsy or sorted control, DNA only, in whole exome or even targeted panel sequencing.

E924

A BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM-LIKE PHENOTYPE IDENTIFIES A SUBGROUP OF NPM1-MUTATED AML PATIENTS WITH WORSE PROGNOSIS

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Background: NPM1-mutated acute myeloid leukemia (NPM1-mut AML) is considered by WHO classification of myeloid neoplasms a distinct entity, representing about 30% of de novo AML in adults. Isolated NPM1 mutations display a strong positive prognostic value since they are associated with high complete response rate to induction therapies, reduced relapse risk and increased overall survival. A certain degree of clinical heterogeneity is however evident among NPM1-mut patients. The prognostic relevance of NPM1-mut AML immunophenotype (IF) is unclear. NPM1-mut blasts are usually CD34 negative, CD33 and CD13 positive. "Myeloid IF" or "monocytic IF" can be distinct by the expression of monocytic antigens as HLA-DR, CD64, CD14, CD11b.

Aims: The aim of the present study was to identify leukemia-associated immunophenotypes (LAIP) in a cohort of previously untreated NPM1-mut AML patients and to possibly disclose the prognostic role of antigens-expression patterns.

Methods: We retrospectively evaluated a cohort of 38 young, de novo NPM1-mut AML patients who have been intensively treated in our institution between 2006 and 2014. All patients were treated with a fludarabine containing induction. Multi-color immunophenotypic analysis is routinely performed in our centre by analysing erythrocyte-lysed whole BM blasts collected at diagnosis to identify relevant antigen aberration patterns and the pathological leukaemia phenotype for future minimal residual disease assessment.

Results: By citofluorimetric analysis three different subgroup of patients could be identified: 16/38 patients displayed a myeloid IF [CD33/CD13/CD38/CD117/MPO (+), CD16/ CD123 (-)]; 7/38 patients displayed a moncytic IF [CD33/CD64/cyLys/CD11b/CD15 (+) with 3/7 patients CD13+]; the third group included 10 patients who displayed a "myelo-monocytic IF" [CD33/CD13/CD38/CD117/MPO/CD64/cLys/CD11b/CD15 (+)]. Differently from what reported, in our experience HLA-DR displayed a heterogeneous expression and was not associated with monocytic differentiation. No statistically significant differences in relapse free survival (RFS) and overall survival (OS) were detectable among the three groups. FLT3-ITD mutation incidence was significantly higher in the monocytic group, however this did not translate in a worse outcome; as well, the expression of CD34 did not negatively affect RFS and OS. Interestingly we found that a peculiar immunophenotype CD56/CD123/CD4 (+) was recurrent in 6 patients, it was not significantly associated with one of the previously described cytofluorimetric subgroups, and was significantly associated with worse disease free survival (p<0.01, Fig. 1). Since these markers represent part of the typical immunophenotypic pattern of blastic plasmacytoid dendritic cell-neoplasm (BPDCN) we called this phenotype "BPDCN-like". BPDCN-like phenotype was not significantly associated with the concomitant presence of FLT3-ITD mutation; moreover, median values of WT1 m-RNA expression levels were significantly lower in this subgroup of patients in comparison with the whole cohort of patients (2200 WT1/ABL copies *vs* 16090 WT1/ABL, p<0.05).

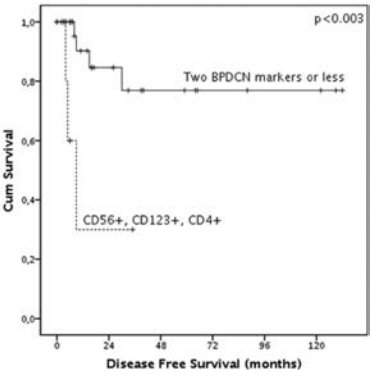

Figure 1.

Summary/Conclusions: We found a peculiar BPDCN-like immunophenotype among NPM-1 mut AML patients associated with significantly worse outcome. The biological explanation of this finding is not clear; further gene-expression profiling studies could contribute do understand our findings. We explained the lack of negative prognostic impact from FLT3-ITD mutation as consequence of the intensive fludarabine containing-induction.

E925

MFC -MRD ASSESSMENT MAY DRIVE POST INDUCTION CONSOLIDATION IN INTERMEDIATE RISK AML. IS IT TIME FOR MRD-DRIVEN DECISION MAKING?

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Background: Since the induction therapy for acute myeloid leukemia (AML) has not changed in the last decades the optimization of post remissional therapeutic strategies is fundamental to improve patients outcome. Allogeneic

hematopoietic stem cell transplantation (BMT) offers the greatest chance of cure for most AML patients, however in the heterogeneous group of intermediate cytogenetic risk (int-risk) patients who achieve complete remission (CR) after induction courses, the role of BMT in 1st CR is not well defined.
Aims: The aim of the present study was to evaluate the prognostic role of minimal residual disease (MRD) assessment through multiparameter flow cytometry (MFC-MRD) after 1st and 2nd induction courses in int-risk patients undergoing or not BMT in 1st CR.
Methods: Fifty-five consecutive int-risk AML patients, according to MRC classification, achieving CR after induction therapy, with available post induction MFC-MRD evaluation were retrospectively included in this study. All patients were uniformly treated with a fludarabine-containing induction. Median age was 49 years. BMT in 1st CR was scheduled if an HLA identical sibling donor or, for selected patients, if an haploidentical donor was available. The remaining 34 patients proceeded to high dose cytarabine consolidation and underwent BMT in 2nd CR from any donor if available (6 patients). A positive MFC MRD was defined by the presence of no less than 25 clustered leukemic cells/10^5 total events at four-color flow-cytometry. Twenty-three and 30 patients achieved MFC MRD negativity after one or two induction cycles, respectively. Median follow up was 44 months. Relapse-free survival (RFS) was calculated from the time of diagnosis until last follow-up or documented leukemic relapse.
Results: Fifty-five consecutive int-risk AML patients, according to MRC classification, achieving CR after induction therapy, with available post induction MFC-MRD evaluation were retrospectively included in this study. All patients were uniformly treated with a fludarabine-containing induction. Median age was 49 years. BMT in 1st CR was scheduled if an HLA identical sibling donor or, for selected patients, if an haploidentical donor was available. The remaining 34 patients proceeded to high dose cytarabine consolidation and underwent BMT in 2nd CR from any donor if available (6 patients). A positive MFC MRD was defined by the presence of no less than 25 clustered leukemic cells/10^5 total events at four-color flow-cytometry. Twenty-three and 30 patients achieved MFC MRD negativity after one or two induction cycles, respectively. Median follow up was 44 months. Relapse-free survival (RFS) was calculated from the time of diagnosis until last follow-up or documented leukemic relapse.

Table 1.

		Num.(%)	Relapse (%)	median RFS	3-year RFS (%)	p (univ.)	p (univ.)
ALL PATIENTS		55	16 (29)	69	71.4	-	-
MFC MRD after 1st cycle negative	BMT 1st CR	9/23 (39)	1 (11)	62	100	0.748	0.003
	No BMT/2nd CR	14 /23 (61)	2 (14)	NR	83.6		
MFC MRD after 1st cycle positive	BMT 1st CR	12/32(38)	1 (8)	NR	87.5	0.048	
	No BMT/2nd CR	20/32 (62)	12 (60)	36	45.2		
MFC MRD after 2nd cycle negative	BMT 1st CR	9/30 (30)	2 (22)	62	87.5	0.790	0.098
	No BMT/2nd CR	21/30 (70)	6 (29)	NR	77.9		
MFC MRD after 2nd cycle positive	BMT 1st CR	8/19 (42)	0 (0)	NR	100	0.023	
	No BMT/2nd CR	11/19 (58)	7 (64)	15	33.3		

Summary/Conclusions: MFC-MRD assessment after induction therapy is a strong predictor of relapse risk and could improve risk stratification for intermediate patients. A deeper evaluation of MRD through MFC may overcome morphologic CR assessment becoming a powerful tool to delineate the therapeutic iter. Early BMT may improve the outcome of patients with persistence of MRD.

E926

Abstract withdrawn.

E927

ZYGODACTYLY IS STRONGLY ASSOCIATED WITH ACUTE MYELOID LEUKAEMIA

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Background: Zygodactyly is a subtype of syndactyly characteristed by bilateral partial cutaneous webbing of the second and third toes without hand involvement. It is a failure in separation of developing toes during organogenesis. The incidence of zygodactyly is approximately 4 in 10,000 men and males are affected twice as frequently as females[1]. In Northern Ireland there are 3.3 per 100,000 new cases of Acute Myeloid Leukaemia (AML) each year[2]. Podiatry assessment of two haematology ward patients with AML confirmed zygodactyly. Given its relatively low incidence in the general population we decided to inspect all our inpatients.
Aims: To assess if there is a correlation between patients with zygodactyly and the development of acute leukaemia.
Methods: On the 18th January 2016, all the inpatients with acute leukaemia were examined by a physician and a podiatrist for presence or absence of zygodactyly. There were 17 patients in total (7 male, 10 female).
Results: 14 out of 17 had partial or full zygodactyly and AML (figure 1). In the unaffected group one patient had Acute Lymphoblastic Leukaemia (ALL), two had AML (one of whom had Acute pro-myelocytic leukaemia). This ward based observational case series interestingly shows a higher frequency of Zygodactyly amongst patients with AML. It also demonstrated no particular sex predominance which one usually attributes to Zygodactyly. This raises the question of way subtle 2nd/3rd toe syndactyly is higher amongst patients with acute myeloid leukaemia?

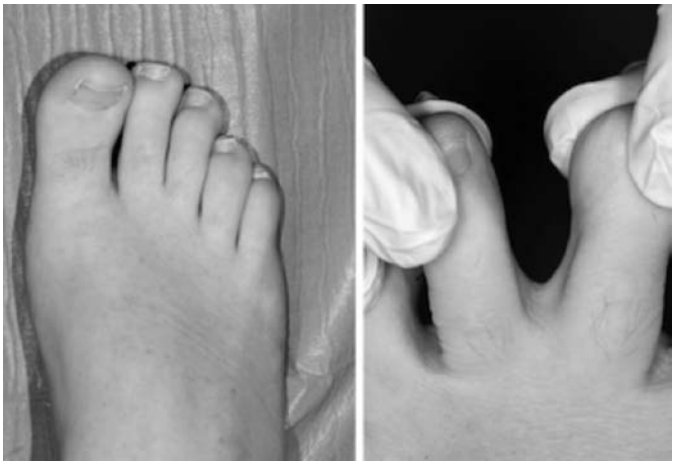

Figure 1.

Summary/Conclusions: It is not unusual for physical abnormalities to be associated with an increased risk of developing AML. Congenital bone marrow failure syndromes demonstrate multisystemic abnormalities and a risk of AML in later life. Diamond Blackfan anaemia has physical abnormalities (~50%) including webbed neck, malformed thumbs and 5% develop AML. Over 50% of Fanconi anaemia cases have skeletal abnormalities especially the radial ray - 10-20% develop Myelodysplastic syndrome/AML over a 13 year period. Dyskeratosis Congenita is characteristed by the association of dyskeratotic nails and bone marrow failure with a signifcant increase in AML[3]. Case reports show these syndromes are occasionally diagnosed in the 3rd and 4th decades as clinical features are occasionally subtle. AML arises from genetic alterations in a stem or progenitor cell that result inuncontrolled growth. The hematopoietic microenvironment has been implicated in the pathogenesis of hematologic malignancies. Hematopoietic stem cells live in a highly specialized complex microenvironment, known as a niche[4]. The structures common to bone marrow environment and cutaneous tissues could help explain the link between physical abnormalities and the risk of AML. Zygodactyly subtype 1 (ZD1) has been linked to chromosome 3p21.31 so genes in this region may be associated with AML or may modify the niche. Further studies are needed to determine the exact causes of this interesting finding.

E928

CLINICAL DECISION SUPPORT SYSTEM (CDSS) BASED ON PATHWAYS CONSTRAINED SOMATIC MUTATIONAL DATA INTEGRATION IN ACUTE MYELOID LEUKEMIA PERSONALIZED MEDICINE

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Background: Personalized medicine will strengthen the prognosis and provide better, patient-specific drug identification. This type of clinical approach is based on high throughput analysis technology available today. Cancer could benefit from targeted therapy because of high number and heterogeneity of DNA alterations. Acute Myeloid Leukemia (AML) is characterized by several type of known point mutations and chromosomal aberration. Massive parallel sequencing showed that a number of new discovered single nucleotide variants (SNV) could affect patients survival time and drug resistance. In this clinical settings next generation sequencing (NGS) is becoming a useful tool to investigate patients genome giving a detailed picture of which mutations occurred and hence an improved diagnosis.
Aims: To exploit these informations for prognosis and personalized therapies there is the need of easily available tools for clinicians. We propose a statistical model learned from data provided by NGS technology, patients cytogenetics predictive risk and clinical outcomes, which could be useful to predict drug sensitivity and prognosis for new diagnosed patients.
Methods: DNA from a cohort of 65 AML patients from S. Orsola University Hospital, Bologna, Italy, were analyzed by Whole Exome Sequencing (WES). Different computational tools were assembled in an in-house pipeline in order to achieve an optimal detection lowering false positive rate. Briefly, pre-processing was carried out following GATK guidelines and re-calibrated alignment files were analyzed by MUTECT and VARSCAN2 algorithms in order to detect single nucleotide variants (SNV) and indels. An additional AML dataset containing WES data from 200 AML patients were downloaded from TCGA database in order to collect somatic variants and clinical data. A training set (60 percent of

cases) was built stratifying patients by age and sex in order to maintain the proportion of the original entire dataset. Other 30 percent of cases were employed as testing set. SNV for each patients were mapped to Reactome and Pathways Commomns. Binary matrix factorization was applied to the patients/pathways matrix in order to find pathways signatures capable to cluster patients. To model pathways constrained mutational data toward event free survival or drug resistance we employed a generalized linear model with binary outcome, including also cytogenetics risk and presence/absence of cancer genes driver mutations from AML biomedical literature. Regression model was applied with 10-fold cross validation to find statistically significant explanatory variables.

Results: Whole exome sequencing detected 4783 somatic mutations of which 2441 labeled as non-silent. Non-silent mutations were mapped to pathways databases and binary matrix factorization. Five pathways signatures were obtained allowing clustering of patients in three groups. Recurrent driver genes involved in AML onset were found with different frequencies in patients and included in the model. Regression analysis provided a model which allow classification of patients in responder/non-responder to standard chemotherapy and toward event free survival.

Summary/Conclusions: In the complex task of interpreting NGS data collected from AML patients, this tool could be useful to guide clinician in the selection of a standard therapy *versus* experimental therapy: when poor prognosis or drug resistance is more likely associated with patients mutational data, latter could be preferable.

Acknowledgements: ELN, AIL, AIRC, PRIN, Progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

E929

MOLECULAR RESPONSE IN ACUTE MYELOID LEUKEMIA WITH WT1 OVEREXPRESSION HAS A STRONG PROGNOSTIC IMPACT IN PATIENS TREATED WITH CHEMOTHERAPY ONLY AND/OR UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: *WT1* is a leukemia associated antigen whose expression may be used as an alternative marker for minimal residual disease (MRD) monitoring in acute myeloid leukemia (AML) mainly in patients lacking a specific molecular target.

Aims: To evaluate the prognostic impact of *WT1* expression at diagnosis and during therapy.

Methods: The cohort comprised 84 subjects (median age 52 yrs, range 21-66 yrs; median follow up 1.9 yrs, range 0.6-7.1 yrs) treated from 2008 to 2014 who had overexpressed *WT1* gene at diagnosis and attained hematological remission after up to 2 induction cycles of chemotherapy. Consolidation chemotherapy consisted of 3-4 cycles of chemotherapy or allogeneic hematopoetic stem cell transplantation (HSCT), depending on cytogenetic and molecular characteristics and response to the first induction cycle. *WT1* expression was quantified using a standardized method according to the recommendations of European LeukemiaNet. Upper normal limit of *WT1* expression was defined as ≤50 copies *WT1*/10^4 copies of reference gene *ABL* in peripheral blood. Only patients with *WT1* overexpression >500 copies/10^4 *ABL* at diagnosis were suitable for MRD monitoring. Molecular response (MR) was defined as a decrease of WT1 expression<50 copies/10^4 *ABL*.

Results: Patients with *WT1* expression at diagnosis >500 and ≤5000 copies *WT1*/10^4 *ABL* had 3yr OS 70% and EFS 40%, whereas those with >5000 copies had 3yr OS 50% and EFS 25%, respectively (p=0.036 for OS and 0.030 for EFS). Failure to reach MR was a strong prognostic factor for survival in an overal cohort (3yr OS 63% *vs* 27%, p<0.001; 3yr EFS 38% *vs* 0%, p<0.001), in patients treated with chemotherapy only (3yr OS 66% *vs* 34%, p=0.002; 3yr EFS 31% *vs* 0%, p=0.009) as well as in patients undergoing HSCT in first complete remission (3yr OS 70% *vs* 30%, p=0.009; 3yr EFS 62% *vs* 12%, p<0.001). Median time to MR was 3.0 months (95%CI 2.8-3.1) and was not dependent on the level of pre-treatment *WT1* expression. Reaching MR before start of consolidation therapy was not signicant (3yr OS 67% *vs* 51%; p=0.089). Of 43 *WT1* positive patients at that timepoint, 33 (77%) reached MR by further therapy (chemotherapy or HSCT). Of 27 patients who were *WT1* positive before consolidation I and received another consolidation cycle, 22 (81%) reached MR after consolidation II. There was no significant difference in survival between patients never reaching MR and those with molecular relapse. In a multivariate analysis, the level of *WT1* expression at diagnosis and ability to reach MR were significant prognostic factors for OS and EFS. In addition, performing HSCT as part of first line therapy was another positive prognostic factor for EFS only. *WT1* expression before HSCT was the only significant marker for OS and EFS in transplanted patients.

Summary/Conclusions: The level of *WT1* expression at diagnosis correlates with survival. Monitoring of *WT1* expression is a sensitive tool for MRD monitoring in patiens with *WT1* overexpression at diagnosis. Failure to reach MR is a potent predictor of relapse. These patients could profit from further intensification of the therapy.

E930

CHARACTERIZATION OF PATIENTS WITH RELAPSED OR REFRACTORY AML IN CONTINUED FOLLOW-UP AFTER TREATMENT WITH VOSAROXIN/CYTARABINE *VS* PLACEBO/CYTARABINE IN THE VALOR TRIAL

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Background: Patients with relapsed/refractory (R/R) AML have a median overall survival (OS) less than 1 year. In the phase 3 VALOR trial, vosaroxin/cytarabine prolonged median OS in patients with R/R AML by 1.4 months *vs* placebo/cytarabine (7.5 *vs* 6.1 months; HR=0.87 [95% CI 0.73-1.02]; *P*=0.061). Of 711 enrolled patients, 134 (19%) were alive in follow-up at the primary analysis. After the primary analysis, ongoing patients were followed for survival.

Aims: To characterize patients who continue to be followed for survival in the VALOR trial.

Methods: In VALOR, patients with R/R AML were randomized 1:1 to receive cytarabine (1 g/m^2 IV over 2 h, d 1-5) plus either vosaroxin (90 mg/m^2 IV over 10 min d 1, 4; 70 mg/m^2 in subsequent cycles) or placebo.

Results: As of Jan 22, 2016, 83 patients (12%) were alive in follow-up: 46/356 (13%) in the vosaroxin/cytarabine arm and 37/355 (10%) in the placebo/cytarabine arm. Median follow-up in these patients was 40 months (range 28-60). Patient characteristics are presented (Table); a higher proportion of patients were ≥60 years in the vosaroxin/cytarabine arm (50% *vs* 27% with placebo/cytarabine). Most achieved complete remission (CR) on study (70% with vosaroxin/cytarabine; 51% with placebo/cytarabine); over half maintained CR at database lock (59% with vosaroxin/cytarabine; 49% with placebo/cytarabine). Nearly all received subsequent therapy (93% with vosaroxin/cytarabine; 100% with placebo/cytarabine). Most patients on vosaroxin/cytarabine (85%) and all patients on placebo/cytarabine received posttreatment stem cell transplantation (SCT). Seven patients in the vosaroxin/cytarabine arm did not undergo SCT; all were ≥60 years of age. Median follow-up in these 7 patients was 33 months (range 31-48).

Table 1. Baseline characteristics of ongoing patients.

	Vos/Cyt (n=46)	Pla/Cyt (n=37)
Median (range) age, y	59.5 (23-74)	54 (23-69)
< 60 y, n (%)	23 (50)	27 (73)
≥ 60 y, n (%)	23 (50)	10 (27)
Disease status, n (%)		
Refractory	10 (22)	11 (30)
Early relapsed	18 (39)	10 (27)
Late relapsed	18 (39)	16 (43)
Geographic location, n (%)		
US	15 (33)	16 (43)
Ex-US	31 (67)	21 (57)
ECOG performance status, n (%)		
0	32 (70)	27 (73)
1	11 (24)	9 (24)
2	3 (7)	1 (3)
Cytogenetics by NCCN Guidelines, n (%)		
Favorable	3 (10)	3 (11)
Intermediate	23 (79)	19 (68)
Unfavorable	3 (10)	6 (21)
Missing	17	9

Summary/Conclusions: A small proportion of patients with R/R AML continue to be followed for survival in VALOR. Typically, these patients achieved CR followed by SCT; however, some patients ≥60 years treated with vosaroxin/cytarabine achieved long-term survival without SCT.

E931

DETECTION OF BASOPHIL-ASSOCIATED IMMUNOPHENOTYPIC FEATURES OF ACUTE PROMYELOCYTIC LEUKEMIA BLAST CELLS AT DIAGNOSIS IS ASSOCIATED TO A HIGHER AND MORE SEVERE BLEEDING DIATHESIS

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Background: The introduction of induction therapy with *all-trans* retinoic acid and anthracycline-based chemotherapy in acute promyelocytic leukemia (APL) have translated into complete remission in most patients. However, severe hemorrhage manifestations at diagnosis and/or during induction therapy lead to early death in 10-15% of patients, which is currently a major focus of research in APL.
Aims: Here we performed an extensive immunophenotypic characterization of blast cells from APL and investigated the potential association between their differential phenotypic profiles and the bleeding diathesis depicted at baseline.
Methods: A total of 133 bone marrow samples from newly-diagnosed APL patients were immunophenotypically characterized. In all cases, 8-color flow cytometry was performed for in depth characterization of APL blast cells and residual hematopoietic cell compartments using the EuroFlow AML/MDS panel. The relatioship between the bleeding diathesis and the immunophenotypic features of blast cells at diagnosis could be studied in 86 APL cases.
Results: The multiparameter immunophenotypic characterization of marrow APL blast cells revealed that these patientes systematically depict (among 100% of their leukemic cells) CyMPO$^{+/++}$, CD33$^{+/hi}$, and CD123$^{+/hi}$ expression, together with CD15$^{-/low}$ CD117^{+}, CD71^{+} and CD9^{+}, while lacking the myelomonocytic maturing-associated markers CD16, CD10 and CD300e, as well as the expression of CD105, NG2 (7.1), CD25 and the megakaryocytic lineage-associated markers CD41, CD61, CD42a, CD42b. Other (aberrant) phenotypes were typically found in APL although at lower frequency, including CD64^{low} (55/59 cases; 93%), HLA-DR^{-} (72/78; 92%), CD13$^{+/++}$ (56/61; 92%) and CD38^{+} (47/55; 86%), followed by CD4^{low} (30/58 cases; 52%), CD203c^{+} (40/92; 44%), CD34^{+} (23/78; 30%), the cross-lineage expression of CD7 (14/47; 30%), asynchronous reactivity for CD35 (14/52; 27%) and CD22 (8/52; 15%) and ectopic CD56 (9/60 cases; 15%). The expression of several other markers was rather exceptional in APL blast cells, including, CD19 (7/58 cases; 12%), CD79a (6/57; 10%), CD14, NuTdT (3/54; 5%), CD11b (2/59; 3%) and CD36 (2/61; 1%). Of note, from all analyzed markers the expression *vs* lack of CD203c showed the strongest association with bleeding manifestations at diagnosis (90% *vs* 10% of APL cases; p<0.001), followed by CD7 (89% *vs* 11%; p=0.03), CyCD79a (0% *vs* 100%; p=0.05) and a similar trend for the expression of CD34 by blast cells (74% *vs* 26%; p=0.06). Furthermore, APL cases with severe bleeding at diagnosis depicted a significantly higher frequency of CD203 expression by blast cells, as compared to those with mild and absent bleeding symptoms at baseline (80% *vs* 46% and 10%, respectively; p<0.001). A similar association was respectively found for the expression of CD7 by blast cells (75% *vs* 31% and 7%; p=0.02) and cytoplasmic CD79a (33% *vs* 18% and 0%; p=0.05).
Summary/Conclusions: The presence of basophil differentiation features in marrow APL blast cells, as depicted by their expression of CD203c and/or CD22, and to a lower extent also of certain cross-lineage antigen expression markers (CD7 and/or CD79a) is associated to a higher frequency and severity of bleeding symptoms at diagnosis, which might point to the need of adapted therapy protocols particularly in these cases.

E932

ASSESSMENT OF BAALC AND MN1 GENE EXPRESSION LEVEL COULD CONTRIBUTE TO IMPROVED PROGNOSTIC STRATIFICATION OF THE AML-NK PATIENTS

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Background: There is a constant need for the introduction of new molecular markers in AML-NK group of patients in order to ensure more precise risk stratification. The latest attempt was the inclusion of expression level changes of some genes, like *BAALC* (brain and acute leukemia, cytoplasmic) and *MN1* (meningioma 1). MN1 is an oncoprotein found to function as transcription coactivator, while the BAALC function is not yet clearly defined. Both of the genes are highly expressed in hematopoietic progenitor cells and are down-regulated during differentiation.
Aims: Aim of this study was to evaluate the *BAALC* and *MN1* gene expression levels in *de novo* AML-NK patients, and to investigate association with other molecular and clinical data.
Methods: Fresh bone marrow (BM) samples were collected from 111 AML-NK patients at diagnosis (62 male, 49 female, median age 54 years, range 19-78). Relative quantification analysis of *BAALC* and *MN1* expression level was performed by RQ-PCR methodology, using comparative *ddCt* method with healthy controls as calibrator.
Results: The median expression level of *BAALC* and *MN1* among patients was 1.55 (0.01-4246.00) and 1.59 (0.00-67.74), while the levels detected among healthy controls was 1.00 (0.65-3.63) and 1.00 (0.40-2.53), respectively. The existence of a positive correlation between *BAALC* and *MN1* expression was detected (r=0.693). Using median value of gene expression levels among healthy individuals plus 3xSD as a cut-off value, we found that 42 patients (38%) expressed *BAALC* gene, while 43 patients (39%) expressed *MN1*. Neither *BAALC*$^{+}$ nor *MN1*$^{+}$ status, had any association with usual clinical characteristics. The *BAALC*$^{+}$/ *MN1*$^{+}$ status was not associated with the presence of *FLT3* and *IDH1*/*IDH2* mutations, but exhibited strong correlation with *NPM1*^{wt} status (p<0.001). Only 2/42 *BAALC*$^{+}$ and 2/43 *MN1*$^{+}$ patients were found to have *NPM1* mutations. When among *BAALC*$^{+}$ and *MN1*$^{+}$ patients median expression level of adequate gene was introduced as a cut-off value, patients were divided into *BAALC*^{high}/*BAALC*^{low} (21/21) and *MN1*^{high}/*MN1*^{low} (21/22) patients. *NPM1* mutations could not be found in none of the high expressing group of patients (*BAALC*^{high} or *MN1*^{high}), FLT3-ITD could be detected in only four patients, and therefore the most frequent were the patients with so called *FLT3-ITD*/*NPM1* double negative status. Compared to *MN1*^{low} group, *MN1*^{high} patients tend to have lover CR rate (41% *vs* 59%; p=0.227), and it was confirmed in the survival analyses where *MN1*^{high} patients had lower DFS (12 months *vs* 30 months; p=0.319) and also shorter OS (5 months *vs* 20 months; p=0.069). Similar findings were detected for *BAALC*^{high} patients who, in comparison with *BAALC*^{low} patients, had a tendency to lower CR (42% *vs* 58%) and resistant disease (67% *vs* 33%). In survival analyses *BAALC*^{high} patients had lower DFS (12 months *vs* 18 months; p=0.261) and OS (5 months *vs* 8 months; p=0.196).
Summary/Conclusions: In our cohort of AML-NK patients, overexpression of either *BAALC* or *MN1* is a factor of poor prognosis. This finding could especially contribute to an improved risk stratification among *FLT3-ITD*/*NPM1* double negative patients lacking a reliable prognostic marker.

E933

ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AND NORMAL KARYOTYPE HAVE A FAVOURABLE OUTCOME AFTER INTENSIVE THERAPEUTIC PROGRAMS

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Background: Outcome of AML patients (pts) older than 60 years is poor because of unfavourable disease characteristics; comorbidities frequently tailor under-powered treatment. When standard induction is given to elderly pts the complete remission (CR) rate is around 55%, median overall survival (OS) after intensive post-remission treatments, such as high dose cytarabine (HDAC) or haematopoietic stem cells transplantation, autologous (AUTO) or allogeneic (ALLO), is about 6 months. Cytogenetic is the principal prognostic factor influencing CR and survival.
Aims: Retrospective evaluation of data from AML pts ≥60 yrs of age who received an intensive treatment program at our Institute.
Methods: Period 2/2001 to 11/2015, 111 pts. Criteria for pts selection: PS (ECOG) ≤2, renal and hepatic parameters within normal ranges or<2 times normal values, no active infections and cardiac ejection fraction >50%.
Results: Median pts age was 68 (60-80). Diagnosis: secondary AML or AML with multilineage dysplasia in 64 cases (6 therapy-related), de novo AML in 47 cases. Cytogenetics: favourable (FAV) 4 cases, adverse (ADV) 15 (complex 11), intermediate (INT) 81, 57 of them were normal karytotype (NK), not evaluable (NE) 11 cases. Molecular analysis (56 pts): 10 NPM1 mut, 9 CEBPA mut, 7 FLT3ITD, 1 FLT3TKD, 1 RUNX1-RUNX1T1, 1 JAK2 mut, 27 negative. Overall, 79 pts (71%) obtained the CR after standard induction chemotherapy (CHT), cumulative incidence (CI) of post-induction treatment related mortality (TRM) was 7.5±4%, median OS was 386 days (12-4725), and 3y OS from diagnosis 29.6±9%, median DFS was 297 days (12-3704), and 3y DFS from diagnosis 20.2±8%. We compared the outcomes of 57 NK pts with 50 INT, UNFAV and

NE pts analysed together (noNK). CR rate after induction: 84% (48 pts) and 54% (27 pts) for NK and noNK pts, respectively, p=0.0008. Post-remission treatments (74 pts): 47 NK pts received HDAC in 16 cases, AUTO in 16, ALLO in 9 and CHT in 6, 27 noNK pts received HDAC in 14 cases, AUTO in 4, ALLO in 8 and CHT in 1. CI of post-remission treatment TRM was 13±7% (10 pts, 6 NK and 4 noNK), mostly after ALLO (6 pts). 3y relapse incidence: 55.8±15% for NK, 68.7±15% for noNK, p=0.282. Survival probability of NK pts was significatively higher than no-NK pts: 3y OS from diagnosis was 36.6±12% for NK (median 614 days), 19.4±10% for noNK (median 284 days), p=0.0006, 3y DFS from diagnosis was 27.5±11% for NK (median 453 days), 10.9±7% for noNK (median 219 days), p=0.002. Notably, age did not influence the outcomes: in particular, 3y OS from diagnosis was 46.5±15% if age<70 (median 795 days), and 22.3±13% if age ≥70 (median 467 days), p=0.246. Survival of NK pts remained significatively better than no-NK excluding from the analysis cases with FAV and ADV molecular abnormalities (p=0.0037 for OS, p=0.0013 for DFS).
Summary/Conclusions: Intensive approaches proved feasible in our pts and prolonged survival was observed for several of them. In particular, NK pts showed a survival advantage due to better CR rate after induction and a longer survival free from events, suggesting a higher chemosensitivity of leukemic blasts in absence of cytogenetic abnormalities. Molecular abnormalities and age did not significatively influence the outcome of NK pts. We conclude that absence of cytogenetic abnormalities *per se* probably defines in the elderly a AML subtype with favourable prognosis. Anyway, strategies to prevent disease relapse also in these pts should be explored.

E934

THE HOMOZYGOUS TP53 P72R SNP IS ASSOCIATED WITH NON-FAVORABLE CYTOGENETIC RISK GROUPS IN ACUTE MYELOID LEUKEMIA

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Background: Previous results about a possible predisposition for acute myeloid leukemia (AML) in individuals with the *TP53* P72R SNP are conflicting.
Aims: We tested the hypothesis that the risk for AML development is increased in P72R carriers and that this SNP also influences survival of these patients.
Methods: We performed a case control study of 215 AML patients from Graz, Austria, and Dresden, Germany, and of 3759 controls from Graz and Kiel, Germany. Genotyping was accomplished with constitutional DNA using the TaqMan assay and direct sequencing. The presence of an Hardy-Weinberg equilibrium and the homogeneity of case and control genotypes as well as cytogenetic risk groups according to European LeukemiaNet were tested by the chi-squared test. Survival analyses according to different genotypes stratified for treatment centers were performed by the Kaplan-Meier method and the Wald-test of cox regression. The likelihood ratio test was used to assess the additive model of single alleles.
Results: Genotypes of cases and controls were in Hardy-Weinberg equilibrium (AMLs P=0.192, controls P=0.325; df=1). There was no difference in the genotype distributions between AML patients (PP n=20, PR n=78, RR n=117) and controls (PP n=249, PR n=1482, RR n=2028; P=0.262; df=2). This was also true for the recessive model PP/PR *versus* RR (P=0.949; df=1). Survival analyses of patients who received at least 3+7 induction therapy showed a trend for better overall survival (OS) in the combined group of patients with the PP/PR genotypes (HR=0.70, 95% CI, 0.46-1.1, P=0.091) whereas no difference was present between all three genotype groups. The additive model of Arginine alleles showed a trend for decreased survival (PP>PR>RR; 72R, HR=1.40, 95% CI, 0.99-1.90, P=0.061). There was no association with relapse free survival. Analyses of clinical parameters of all patients showed a significantly different distribution of cytogenetic risk groups with decrease of favorable cytogenetics in patients with the RR genotype (PP/PR, 15/90 [16.7%]; RR 6/112 [5.4%]; P=0.0171; df=1).
Summary/Conclusions: There is no association of the P72R genotype with AML development in this study. The RR genotype shows a trend for lower OS and a statistically significant association with non-favorable cytogenetic risk groups.

E935

FLAG-IDA REGIMEN AS SALVAGE THERAPY IN REFRACTORY/RELAPSED AML PATIENTS: A SINGLE-CENTER EXPERIENCE

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Background: Although treatment outcome in acute myeloid leukemia (AML) adult patient has improved over the past decade, relapse still occurs in up to 50-70% of cases. Furthermore, 15-30% of patients fail to achieve complete remission (CR) because of treatment-resistance. The management of primary refractory and/or relapsed disease remains challenging for clinicians. Although several different chemotherapy combinations are currently administered in refractory/relapsed AML patients, the prognosis is still poor, with a complete remission rate ranging from 30% to 40%.
Aims: In our study, we reviewed the outcome of 116 refractory and/or relapsed AML patients who underwent salvage therapy with the FLAG-Ida regimen between 2005 and 2015 at our institution. The study aim was to determine the efficacy of the FLAG-Ida regimen in order to clarify which variables (WHO PS, LDH, bone marrow, peripheral blood blasts and platelets counts, white blood cells (WBC), PMN, molecular-cytogentic risk, duration of response and relapsed or refractory disease), present before starting FLAG-Ida treatment, might have an impact both on CR and on overall survival (OS).
Methods: We analyzed 116 consecutive adult patients (56 males, 60 females; median age 48 years, range 17-72) with newly diagnosed acute myeloid leukemia refractory to standard induction regimens or relapsed after CR, who received the FLAG-Ida protocol as salvage therapy between January 2005 and December 2015. Sixty-eight of the 116 patients (58%) were in first relapse, forty-seven patients (42%) were refractory to conventional chemotherapy. Median WBC count before salvage therapy was 10.1 $x10^9$/l (range 0.56-88). Median bone marrow and peripheral blasts counts were 52 and 20%, respectively; median platelets count was 91x10e3/uL. According to the FAB classification, 14 patients had M0, 5 M1, 53 M2, 16 M4, 22 M5, 4 M6, 2 had Biphenotype Acute Leukemia. According to molecular-cytogenetic risk stratification 51(44%), 44 (38%) and 21 (18%) patients belonged to poor, intermediate and good risk group, respectively.
Results: Sixty-nine of 116 patients (59%) achieved complete remission (CR); forty-seven 41%) patients were refractory to the salvage therapy. In multivariable analysis, variables with positive impact on response rate were lower WBC counts (<10e3/uL, p=0.0047), higher platlets counts (>50x10e3/ul, p=0.046), molecular-cytogenetic risk (p=0.032), duration of response in relapsed AML (p=0.006) and relapsed rather than primary refractory disease (p=0.042), respectively. Median OS was 17 months (m). Cox regression analysis confirmed that both higher platlets counts, p=0.002 (17 (>50x10e3/ul) *vs* 11 m (<50x10e37uL), log Rank, p=0.05) and relapsed disease, p=0.041 (23 (relapsed) *vs* 17 m (refractory), Gehan-Breslow, p=0.021) correlated with better survival. Of note, molecular-cytogenetic risk evaluated before starting treatment was associated with CR, while no correlation was found with OS in multivariate model.
Summary/Conclusions: Our data seem to confirm the value of FLAG-Ida in relapsed AML rather than refractory disease, and may suggest its best usage as bridge-therapy in patients awaiting allotransplantation.

E936

DECITABINE COMBINED WITH HAAG AS INDUCTION CHEMOTHERAPY FOR NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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Background: Treatment of newly diagnosed acute myeloid leukemia (AML) remains challenging. Several host- and disease-related factors contribute to poor outcome in elderly patients or high risk patients with AML, including medical comorbidities, physical frailty, and increased incidence of poor-risk biologic features. Indicating an urgent need for alternative treatment strategies improving clinical outcome for these patients. It is confirmed that Decitabine has the synergistic reaction with daunomycin or cytarabine. Therefore, we investigated whether Decitabine combined with HAAG regimen might also be effective for the newly diagnosed of AML patients.
Aims: To evaluate the efficacy and toxicity of DAC combined with HAAG regimen as an induction therapy for newly diagnosed AML patients as well as compared with standard IA(IDA 12mg/m^2/d*3d) regimen.
Methods: 130 patients with AML were newly diagnosed in our center bewteen January 2014 and December 2015.The median age was 42□11-82□years. Among them,49 cases received DAC+HAAG regimen(DAC 20mg/m^2/d*5d combined with low dose of homoharringtonine 1mg/m^2/d*7-14d, cytarabine subcutaneously 10mg/m^2/q12h*7-14d, aclarubicin 10mg/d*4-8d and G-CSF priming). The other 81 patients received standard IA regimen (idarubicin 12mg/m^2/d*3d and cytarabine 100mg/m^2/d*7d).The outcomes of all the patients after one cycle of chemotherapy were evaluated.
Results: 1.The complete remission(CR) rate of DAC+HAAG group was superior than that of IA group(81.6% *vs* 74.5%, P=0.517), but with no statistically significant. While the overall response rate (ORR) of DAC+HAAG group was similar wiht IA group(87.8% *vs* 88.7%, P=0.786). 2. Median overall survival (OS) from the starting of DAC+HAAG chemotherapy and IA were 6.5(2-20) months and 8.5(0.5-25) months (P=0.384). Meanwhile, the estimated one-year survival of DAC+HAAG and IA group were 55.8%±8.9 and 77.9%±5.4% respectively (P=0.089).3. The survival of DAC+HAAG were not more prior than IA, but we found that the ages of DAC+HAAG were statistically older than the IA group(P=0.00). However, in DAC+HAAG group, there were 14 elderly (≥60 years old) that not any one in IA group(P=0.000). The CR rate of the old cases was 64.3% and the estimated 1-year survival was 73.4%±13.4%. 4. Treatment related mortality (TRM) was found in 1 case in DAC+HAAG group compared

with 2 cases in IA group. All patients presented cytopenias of grade 4. There were no differences on the recovery time of ANC ≥0.5*10^9/L,HB≥70g/L and PLT≥20*10^9/L after induction chemotherapy (P=0.969,0.392,0.225). 5. The incidence of adverse events including fever, nausea and vomiting, diarrhea were lower in DAC+HAAG group(P=0.024,0.024 and 0.028). While the happened of infection,bleeding,cardiovascular impact, liver dysfunction and renal insufficiency were similar. 6.For the two group, lower blast percentage as diagnosed were significantly associated with a higher OS rate (P=0.000 and P=0.000).
Summary/Conclusions: DAC+HAAG regimen as an induction chemotherapy for newly diagnosed AML patients could better effectively reduce tumor burden than IA regimen(no statistical significance) with mild toxicity. For naïve AML patients, DAC combined with HAAG regimen may be an optimal choice, especially for patients who cannot tolerate high dose of chemotherapy.

E937

AGE FACTOR ON REMISSION INDUCTION THERAPY FOR ADULT ACUTE MYELOID LEUKEMIA

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Background: Therapeutic goal for acute myeloid leukemia (AML) is the cure and usually it can be achieved by intensive chemotherapy. One of the most important factors to choose remission induction therapy is the patient's age.
Aims: We evaluated the impact of age on remission induction therapy for AML.
Methods: The Korean Society of Hematology AML/MDS Working Party built the adult AML registry. The registry collected 14 year or older patients; however this analysis included only 18 years or older patients as adult AML.

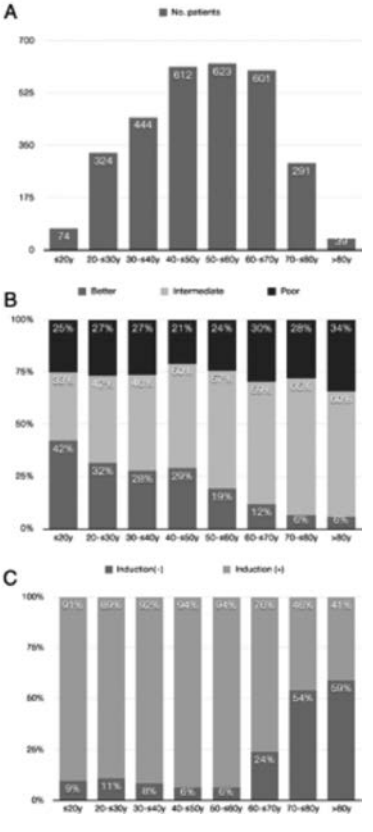

Figure 1.

Results: Total 3041 cases were collected in the registry and 3008 adult AML patients were included in this analysis. Enrolled number of patients were presented in Figure 1A. Risk groups based on karyotype showed strikingly different tendency among age groups. Poor risk group had a tendency of slight increase as patients were getting older (21-34%). However more dramatic change was decreasing better risk group instead of increasing intermediate risk group as ages increased (Figure 1B). Performing induction chemotherapy was similar below 60 years ago (89-94%), however it droped to 76% in age 60-70y, 46% in age 70-80y and only 41% in age older than 80y (Figure 1C). We selected 1935 patients who were older than 17y and not acute promyelocytic leukemia and received induction chemotherapy and known induction responses. The complete remission (CR) rates were decreasing as ages increased: 89.5% in age under 20y; 79% in 20-30y; 75% in 30-40y; 76% in 40-50y; 73% in 50-60y; 63% in 60-70y; 58% in 70-80y; 29% in 80y or older patients. When the analysis was focused on patients who had received cytababine-idarubicin (AI) or cytarabine-daunorubicin (AD) induction chemotherapy (n=1377), the CR rates were following: 86% in age under 20y; 77% in 20-30y; 75% in 30-40y; 76% in 40-50y; 73% in 50-60y; 68% in 60-70y; 62% in 70-80y; 50% in 80y or older patients. When analyzing the population according to risk group, the CR rates were similar in better risk group; 92.9% in age under 20y; 100% in 20-30y; 88.6% in 30-40y; 89.2% in 40-50y; 81.6% in 50-60y; 72.2% in 60-70y; 100% in 70-80y patients. In intermediate risk group, the CR rates were 84.6% in age under 20y; 78.9% in 20-30y; 74.1% in 30-40y; 79.4% in 40-50y; 77.6% in 50-60y; 74% in 60-70y; 59.5% in 70-80y; 66.7% in 80y or older patients. CR rates were dramatically dropped in elderly age with poor risk karyotypes; 77.8% in age under 20y; 66.7% in 20-30y; 70.9% in 30-40y; 59.7% in 40-50y; 61.6% in 50-60y; 54.5% in 60-70y; 44.4% in 70-80y; 0% in 80y or older patients. There was no difference of CR rate between AI and AD (p=0.542): p=0.685 in 30-40y; p=0.713 in 40-50y; p=0.452 in 50-60y; p=0.813 in 60-70y; p=1.000 in 70-80y; p=1.000 in age older than 80y. However there was significant higher CR rate among AI regimen in under 30y (p=0.033).
Summary/Conclusions: Age clearly impacts on outcome of induction chemotherapy. CR rates were similar in favorable risk group in all age groups. However CR rates of unfavorable risk group dramatically droped in elderly patients. There was no clear difference between AI and AD induction in terms of CR rates. Poor outcome in elderly AML can be resulted mainly from poor risk characteristics.

E938

DEFINING FRAILTY OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DIAGNOSIS

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Background: Acute myeloid leukemia (AML) is a disease of older adults, with a median age at diagnosis over 65 years. Evaluation of disease-related and patients specific factors in the context of clinic decision making has therefore been largely subjective. Several studies demonstrated improved survival for older patients receiving intensive induction chemotherapy compared to those receiving supportive care alone. Defining this subset of patients who are not eligible or "not fit" for intensive chemotherapy involves a great deal of subjectivity. Criteria yet have to be standardized across or within institutions.
Aims: Aim of this study was to investigate the validity of three validation scores for distinction of patient fitness at diagnosis in parallel to physician evaluation. Further patient outcome according the respective evaluation was compared.
Methods: A total of 69 clinically and molecularly well characterized consecutive elderly (>60 years) patients with newly diagnosed AML were treated from 2012 to 2015 according to age, performance status and co-morbidities in a single hematology center. Therapy response was defined according to ELN criteria. Therapy intensity decision was based on an initial haematologist evaluation followed by discussion in an interdisciplinary board. In parallel, the local geriatric G8 screening tool, the HCT-CI comorbidity score and the AML score proposed by the German Acute Myeloid Leukemia Cooperative Group, predicting probability of complete remission (CR) and early death (ED) were performed. Overall survival from diagnosis was compared between groups using the Cox model.
Results: Thirty-three patients (47,8%) were evaluated "fit" by the medical board and treated by intensive chemotherapy, whereas 26 patients (37,7%) underwent semi-intensive/experimental therapy and 10 patients (14,4%) received best supportive care. A total of 21 patients (31,4%) achieved a complete remission after induction chemotherapy, whereas 49% patients (25%) were non responders and 13 (19,4%) died. Overall, the median survival time was 5,2 months (95% CI 3,5-8,3). Primary physician care evaluation was able to define in a statistically significant manner a "fit" from an "unfit" patient. Median survival time from the "fit" patients was 8,3 moths (95%CI 6,1-11,2) compared to the "unfit" evaluated patients with 2,9 months (95%CI 1,3-4,4), p=0,004. Parallel evaluation of patients fitness using the G8, HCT-CI and AML scores discriminated significantly "fit" from "unfit" patients considering median survival time, p=0,001, p=0,032 and 0,021, respectively. The ability of the frailty scores on the prediction of fitness classification compared to the physician evaluation was analyzed by calculating the area under the curve (AUC) using a logistic regression model. In this approach an AUC of 1,0 denotes perfect prediction whereas and AUC of 0,5 is analogous to a coin flip. With this regard the AUC for the G8 Score was 0,73, whereas the HCT-CI was 0,72 and the AML score ED 0,78 and the AML CR 0,79 in the present cohort.
Summary/Conclusions: In conclusion, in the present cohort the applied frailty

scores at diagnosis correlated significantly regarding median overall survival. These results may encourage a following larger multi-centre analysis in order to verify the statistic power of the performed analysis.

E939

GENOTYPIC AND PHENOTYPIC CLONAL EVOLUTION IN RELAPSED FAVORABLE-ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Despite high rates of cure with standard chemotherapy in patients with favourable-risk acute myeloid leukemia (AML) according to European Leukemia Net (ELN) classification, some patients will eventually relapse. It is currently unclear whether leukaemia relapse is due to intrinsic chemo resistance or to clonal evolution with acquisition of new driver mutations and clonal selection
Aims: Characterization of phenotypic and genotypic features at relapse compared to diagnosis in patients with molecular/cytogenetic favourable-risk AML presenting relapse after achieving complete remission (CR).
Methods: Retrospective study of 13 molecular/cytogenetic low risk AML patients (Non-M3) (CBF 3 NPM1 10) according to ELN classification criteria from Tor Vergata Hospital (Rome) and Reina Sofia Hospital (Córdoba). They were treated with intensive chemotherapy achieving CR and relapsed after a median of 12.2 months (4.4-255.9) (Table 1). NRAS, KRAS, DNMT3A, IDH1, IDH2 and TP53 genes were analyzed in marrow samples obtained at relapse by Sanger Sequencing using ABI 3130® Genetic Analyzer (Applied Biosystems Inc., Foster City, CA). When a mutation was found, the diagnosis sample for this mutation was screened using Sanger Sequencing also. A complete phenotypic profile was carried out using multiparametric flow cytometry with 3-laser FACSCanto II in both diagnosis and relapse samples. Additionally, all mutations were tested at diagnosis in 7 patients with favourable-risk AML achieving continuous CR (non-relapsing patients)
Results: In non-relapsing patients, only a case with IDH1 (R132H) was found. When analyzing relapse-diagnosis paired samples in 13 AML patients, three patterns of clonal evolution were found: Group A (8 patients): Genotypic (G) and phenotypic (P) persistence of the main clone. Two patients presented a mutation in DNMT3A gene: R882H mutation and c.2705_2706delTC (p.F902fs) mutation (not described so far) respectively. Both mutations were found in diagnosis and relapse samples and phenotypic profile remained unchanged. Group B (2 patients): Phenotypic profile evolution but no changes in genotype. One patient harboured a mutation in DNMT3A gene (R882H) in both diagnosis and relapse samples. Many importants phenotypic changes were observed: In diagnosis sample, CD65+, CD19-, CD15+, CD117+, DR+, CD56LOW, CD7-, CD13+, CD2+, CD11b+, CD14-, CD38+, CD34+, CD33+ were expressed in the main leukemic clone. In the relapse sample, CD15, DR, CD56, CD2 and CD11b not expressed. Group C: 3 patients presented phenotypic and genotypic clonal evolution. The mutations were detected in relapse but not in diagnosis samples: i) D876Y mutation in DNMT3A gene (not described so far), accompanied with phenotypic profile changes with acquisition of CD34 and CD117 and loss of CD15. ii) R140Q mutation in IDH2 gene and G105 mutation in IDH1 gene with a phenotypic evolution with acquisition of CD34 and loss of a suclone CD56 +. iii) G105 in IDH1 gene with a phenotypic evolution with increased of CD33 and acquisition of CD15. These mutations were studied by NGS in the diagnosis samples. IDH mutations were present in both patients, while DNMT3A gene remained wild type.

Table 1.

Summary/Conclusions: i). Driver mutations are found more frequently in patients with favourable-risk AML who relapse compared to those with sustained CR. ii). 3 patterns of relapse were detected: A). No P changes/G (N=8); B). P Changes/No G (N=2). C). P changes/G (N=3). These results highlight the importance of clonal evolution at relapse in favourable-risk AML patients.

E940

DNMT3A MUTATIONS IN ACUTE MYELOID LEUKAEMIA WITH INTERMEDIATE-RISK KARYOTYPE - INCIDENCE AND CORRLEATION WITH OTHER GENE MUTATIONS

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Background: Acute myeloid leukaemia is an aggressive heterogeneous haematopoietic disease. It is characterized by an increased proliferation of haematopoietic progenitor cells that have lost the ability to differentiate. In the last 15 years, major advances have been made in the disease with the discovery of many genetic abnormalities affecting cell proliferation, differentiation, apoptosis or cell cycle regulation. Some of these molecular markers confer good prognosis while others have been found to be adverse prognostic factors. Of the molecular markers, DNMT3A (DNA N-methyltransferase 3A) gene mutation is found to be an independent poor prognostic marker in most studies.
Aims: In this study, we evaluated the mutation status of DNMT3A in AML patients with intermediate-risk cytogenetics, in particular, those with normal karyotype. The incidence of DNMT3A mutations was correlated with the mutation status of other genes like FLT3, NPM1, IDH1 and IDH2 which were also determined.
Methods: For the detection of DNMT3A mutations, exon 26 of the gene was PCR amplified and direct sequencing of the amplicons was performed to detect mutation hotspots in this exon in a cohort of 93 patients.

Table 1.

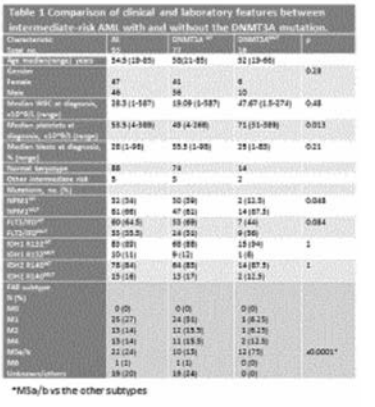
Table 1 Comparison of clinical and laboratory features between intermediate-risk AML with and without the DNMT3A mutation.

Characteristic	N	DNMT3A mut	DNMT3A wt	p
Total no.	93	17	76	
Age, median (range), years	54.5 (18-85)	58(21-85)	52 (19-66)	
Gender				0.28
Female	47	41	6	
Male	46	36	10	
Median WBC at diagnosis, x10^9/L (range)	28.3 (1-587)	19.09 (1-587)	47.67 (1.5-274)	0.48
Median platelets at diagnosis, x10^9/L (range)	53.5 (4-569)	49 (4-268)	71 (11-569)	0.013
Median blasts at diagnosis, % (range)	28 (1-96)	55.5 (1-96)	25 (1-85)	0.21
Normal karyotype	88	74	14	
Other intermediate risk	5	3	2	
Mutations, no. (%)				
NPM1^mut	32 (34)	30 (56)	2 (11.5)	0.048
NPM1^wt	61 (66)	47 (81)	14 (87.5)	
FLT3/ITD^mut	80 (64.5)	33 (69)	7 (44)	0.084
FLT3/ITD^wt	33 (35.5)	24 (31)	9 (56)	
IDH1 R132^mut	83 (89)	68 (88)	15 (94)	1
IDH1 R132^wt	10 (11)	9 (12)	1 (6)	
IDH2 R140^mut	75 (84)	64 (85)	14 (87.5)	1
IDH2 R140^wt	15 (16)	13 (17)	2 (12.5)	
FAB subtype N (%)				
M0	0 (0)	0 (0)	0 (0)	
M1	25 (27)	24 (51)	1 (6.25)	
M2	15 (14)	12 (15.5)	1 (6.25)	
M4	13 (14)	11 (15.5)	2 (12.5)	
M5a/b	22 (24)	10 (13)	12 (75)	<0.0001*
M6	1 (1)	1 (1)	0 (0)	
Unknown/others	18 (20)	18 (24)	0 (0)	

*M5a/b vs the other subtypes

Results: A total of 16(17%) patients were identified with R822 mutations in DNMT3A exon 26. R882H was the predominant mutation in these patients (n=10) while the remaining 6 had R822C. Of these 16 patients, 14 (87.5%) of them were also NPM1-mutated while 9 had FLT3/ITDs as well. The close association of DNMT3A mutations with NPM1 mutations was significant (*p=0.048*) while that with FLT3/ITDs was not quite statistically significant (and *p=0.084*). IDH mutations were detected in only 3 patients with R882 mutations (IDH1 R132H, n=1; IDH2 R140Q, n=2). The relation between DNMT3A mutations and other patient characteristics were determined by the Mann Whitney U test (continuous variables) and the Fisher exact test (categorical variables) (details in Table 1). The platelet counts were significantly higher in the DNMT3A -positive patients than in the patients without DNMT3A mutation (*p=0.0125*). DNMT3A mutations were also significantly enriched in patients with the FAB M5 subtype (12/22, 55%, *p=<0.0001*). Other clinical parameters showed no significant difference between the DNMT3A-positive group and the wild type group. Interestingly, in a patient who was separately investigated, FLT3/ITD^{mut}, NPM1^{mut} and DNMT3A R882C were detected at diagnosis but while FLT3/ITD was not detected on follow-up at relapse, an IDH2 R140Q was observed. The R882C status, however, remained unchanged. Previous larger study series have demonstrated the stability of DNMT3A during the course of disease.
Summary/Conclusions: Within the normal karyotype and other intermediate risk AMLs, we did not observe any significant correlation of DNMT3A mutations with white blood cell counts, FLT3/ITD or IDH mutations, contrary to what was observed in other studies which involved AMLs across all cytogenetic risk groups. We hope the findings from this preliminary study would help improve our understanding of the association of DNMT3A mutations with other gene mutations in intermediate risk AMLs. Further work is needed to investigate the significance of the DNMT3A exon 26 mutations, in particular, the R882 mutations, as a prognostic predictor and the role of screening of these mutations for further risk-stratification of intermediate risk AMLs, particularly in normal karyotype AML.

E941

CLINICAL CHARACTERISTICS OF ACUTE MYELOID LEUKEMIA PATIENTS FROM REGIONS CONTAMINATED BY DEPLETED URANIUM: 20 YEAR ANALYSIS

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Background: During the war in Bosnia (1992-1995), ammunition with depleted uranium was used in several regions, including the town of Hadzici, where the United Nations have measured high concentrations of depleted uranium several years after the war. Depleted uranium (DU), a radioactive heavy metal, is used in military ammunition. Studies have shown the potential health risks of con-

tamination by DU by wounding, ingestion, and inhalation. *In vivo* and *in vitro* experiments showed the carcinogenic potential of DU by neoplastic transformation of human and mouse cells, leading to the development of myeloid neoplasms. It is assumed that the DU exposure induces genomic stability leading to carcinogenesis.
Aims: Increased numbers of patients with blood cancers in DU stricken area was noted by hematologists, so we systematically analyzed hematological patients from this region in the last 20 years, from 01.01.1996.-31.12.2015.
Methods: We systematically analyzed hematological patients from two region in the last 20 years, from 01.01.1996.-31.12.2015. One region had high concentrations of DU (Hadzici) and the other region (ilijas) was used as a control. Both regions have the same population size and same access to tertiary health care. Patient data was collected including age at diagnosis, sex, address, blood parameters, cytogenetics, therapy, and survival.
Results: In the 20 year analyzed period from 01.01.1996–31.12.2015, we found 717 patients with hematological conditions (437 from DU-stricken town *vs* 280 from control town). There were 74 patients with myeloid malignancies (54 *vs* 20), 55 patients with non Hodgkin lymphoma (26 *vs* 29), 21 patients with Hodgkin lymphoma (9 *vs* 12), and 6 patients with ALL (4 *vs* 2). Among the myeloid neoplasms, CML showed 6 fold increase in DU-stricken area and AML showed 3 fold increase. The median age at diagnosis for AML patients was 41 *vs* 73, and for CML patients was 62 *vs* 53. Male to female ratio was for AML 0.5 *vs* 1, and for CML 1 *vs* 7. For AML patients, 22% *vs* 67% of patients achived complete remission after first treatment. The median duration of remission was 10.5 v 17.5 months. OS at 24 months was 22% *vs* 50%.
Summary/Conclusions: Clinical parameters showed more severe course of acute myeloid leukemia patients in DU stricken area compared to the control and international data. Further investigation is needed to elucidate the possible causes of stark increase in myeloid neoplasms in Hadzici area.

E942

COMPARISON OF AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION TO INTENSIVE CONSOLIDATION CHEMOTHERAPY AND MONITORING MINIMAL RESIDUAL DISEASE IN CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA

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Background: One third of patients with core binding factor (CBF) acute myeloid leukemia (AML) relapse and disease relapse is most common cause of treatment failure. For detecting relapse, quantitative RT-PCR of AML1-ETO or CBFB-MYH11 fusion transcript is used to evaluate minimal residual disease (MRD).
Aims: The purpose of this study was to compare the clinical outcome of post-remission therapy between autologous peripheral stem cell transplantation (ASCT) and intensive consolidation chemotherapy in CBF AML patients and to assess MRD in patients who accomplished first CR(CR1) after induction chemotherapy using quantitative RT-PCR of AML1-ETO transcript.
Methods: We retrospectively analyzed adult patients who diagnosed with CBF AML between March 1996 and July 2014, and achieved CR1 after induction chemotherapy at Samsung Medical Center. The patients were divided into two groups who received ASCT or 3 to 4 cycles of intensive consolidation chemotherapy as post-remission therapy. In addition, we also investigated the impact of MRD after induction chemotherapy using quantitative RT-PCR of AML1-ETO for patient with t(8;21). AML1-ETO transripts were normalized to ABL.

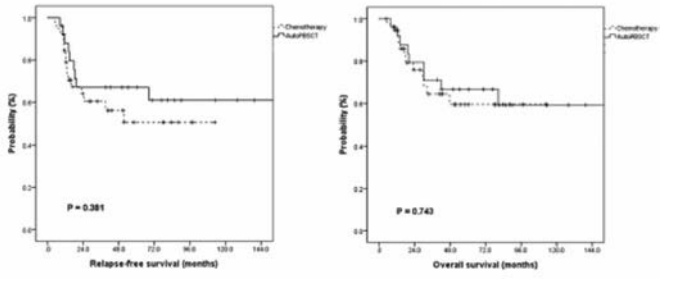

Figure 1.

Results: We identified 65 patients (Male=33, Female=32; t(8;21)=45, inv(16)=20) and the median age at diagnosis was 42 years(range 18-64). 26 patients (40%) received ASCT as postremission therapy and 40 patients were treated with 3 to 4 cycles of intensive chemotherapy. The ASCT group showed a better 5-year relapse free survival (RFS) rate (50.5% *vs* 67.0%, p=0.381) and similar 5-year overall survival (OS) rate (59.5% *vs* 66.6%, p=0.743) during median follow-up 96 months. The median value of AML1-ETO mRNA levels in bone marrow was higher in relapsed patients with t(8;21) than non-relapsed patients (425.79 *vs* 3.29, per ABL x 10^3, p=0.045). We determined cutoff for MRD as the median value of AML1-ETO transcript of all patients with t(8;21), 3.39 per ABL x 10^3. The 5-year RFS rates in the patients without and with MRD were 87.5% and 68.6%, respectively. (p=0.391).
Summary/Conclusions: This study demonstrated that ASCT as postremission therapy showed a better RFS than chemotherapy alone in patients with CBF AML, monitoring minimal residual disease by AML1-ETO mRNA levels as surrogate marker of patients with t(8;21) in CR1 was beneficial.

E943

PROGNOSTIC IMPLICATIONS OF THE IDH1 SINGLE NUCLEOTIDE POLYMORPHISM RS11554137 IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Several studies have shown that synonymous single nucleotide polymorphisms directly impact gene function through various translational or post-translational mechanisms, such as altering mRNA binding, protein folding, the spliceosome, mRNA stability, or expression in general. Mutations in 132 codon of *IDH1* gene are quite frequent event in acute myeloid leukemia (AML). Also, approximately 10% of AML patients were detected to have polymorphism rs11554137 in the 4th exon of *IDH1* gene. Data concerning the impact on prognosis of polymorphism rs11554137 is still controversial.
Aims: To investigate the frequency and prognostic value of polymorphism rs11554137 in *IDH1* gene, its combination with clinical hematological features and karyotype variant in AML pts.
Methods: The study included 112 pts with AML (median age - 55 years). In 103 pts (92.0%) was verified *de novo* AML and in 9 (8.0%) - secondary AML from preceding MDS or lymphoma. Cytogenetic analysis was performed on G-differentially stained chromosomes, so pts were divided into 4 groups: with normal karyotype (NK) - 52 (46.4%), favorable karyotype - 9 (8.0%), unfavorable karyotype - 16 (14.3%), with other chromosomal abnormalities - 35 (31.3%) pts. Screening of *IDH1* gene aberrations was performed by real-time PCR with further analysis of melting curves.
Results: Polymorphism rs11554137 in *IDH1* gene was detected in 7.1% (8 out of 112) of pts with AML. Distribution of pts with AML, depending on the morphological variants showed that polymorphism rs11554137 was more common in pts with M4 variant (5 of 20, p=0.005). All pts with polymorphism rs11554137 had *de novo* AML. The median age of pts with polymorphism rs11554137 (58 years) was significantly higher than in pts without it (55 years) (p=0.005). There was no significant differences when the number of white blood cells and platelets in PB, blasts in BM in pts with and without polymorphism rs11554137 was compared. Slightly more often polymorphism rs11554137 in *IDH1* gene occured in pts with NK (5 of 8 pts; p=0.334), 3 pts with polymorphism had unfavorable karyotype. None of the pts with favorable karyotype was detected to have polymorphism rs11554137. All pts with polymorphism rs11554137 in *IDH1* gene were screened for mutations in *FLT3*, *NPM1, NRAS, CKIT* and *DNMT3A* genes. Only in 2 pts polymorphism rs11554137 in *IDH1* gene was met singly. In the remaining pts (6 of 8) polymorphism rs11554137 was found simultaneously with mutations in other genes: *DNMT3A+, FLT3*-TKD+, *NPM1+, FLT3*-ITD+/*DNMT3A+, FLT3*-ITD+/*FLT3*-TKD+/*DNMT3A+* and in 2 pts polymorphism rs11554137 was detected in combination with *FLT3*-ITD. We also investigated the prognostic value of polymorphism rs11554137 in *IDH1* gene. Comparative analysis of median overall and relapse free survival in pts with and without polymorphism showed significant differences: 5.8 months and 12.8 months (p=0.046) and 4.2 months and 9.5 months (p=0.004) respectively.
Summary/Conclusions: Polymorphism rs11554137 in *IDH1* gene is quite frequent event in pts with AML. It is usually associated with M4 variant of AML and higher median age as compared with pts without polymorphism. Significant prognostic potential of polymorphism rs11554137 in *IDH1* gene allows to consider it as an unfavorable marker correlated with a high risk of relapse and poorer survival. Synonymous polymorphisms represent a special category of molecular aberrations that should be considered in the diagnosis and prognosis of AML pts.

LB2244

FRONT-LINE CENTRAL VASCULAR ACCESS DEVICES IN ACUTE LEUKEMIAS – PERIPHERALLY INSERTED CENTRAL CATHETER (PICC) *VERSUS* TRADITIONAL CENTRAL VENOUS CATHETER(CVC): A PHASE IV RANDOMIZED TRIAL (NCT02405728)

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Background: The use of PICC as an alternative to other CVC devices, particularly for prolonged infusions of cytotoxic agents, blood products and/or other

supportive therapy, is becoming very frequent in cancer patients. PICCs are easier to insert, and associated to a lower rate of complications than traditional CVC. However, in the setting of high-risk patients with acute leukemia there is limited information on the feasibility and safety of PICC as primary vascular access device. Our Hematology Department is conducting a Phase IV randomized trial. We compare PICC *versus* traditional CVC as front-line venous access device in patients with acute leukemias undergoing remission induction chemotherapy. This trial is registered on ClinicalTrials.gov, number NCT02405728 (ongoing).
Aims: Primary endpoint is the occurrence of catheter-related bloodstream infections and/or catheter-related thrombosis. Secondary endpoints are the occurrence of other complications, such as pneumothorax or catheter occlusion, and patient quality of life. Questionnaire covering functional status, sleep and hygiene disturbance had been given to assess patients'quality of life.
Methods: From April 2015 to February 2016, 152 patients with acute leukemia planned for remission induction chemotherapy were randomly assigned (1:1) to PICC (Arm A) or traditional CVC (Arm B). Inclusion Criteria were: age >18 years, suspected survival >4 weeks, and need of central venous access device (long-term; >4 weeks). While exclusion criteria were ongoing uncontrolled systemic infection, presence of significant thrombosis/stenosis in arm or central veins, and inability to communicate and/or to sign informed consent. All insertions were followed by chest X-ray.
Results: 152 patients (130 AML, 22 ALL), median age 47 years (13-82), were randomized in the two arms. In Arm A, 76 PICCs were inserted in 76 hematological patients (median age 51.5 years, r.19-82, 17 females, 59 males) suffering from acute myeloid leukemia (AML: 70) or acute lymphoblastic leukemia (ALL: 6). Double lumen PICCs (5 Fr) were inserted in 70 patients, single lumen PICCs (4 Fr) were inserted in 5 patients, and triple lumen PICC (6Fr) was inserted in 1 patient. 68 PICCs were inserted in the right basilica vein, 5 PICCs were inserted in the left basilica vein and 3 PICCs were inserted in the left brachial vein. In Arm B, 76 traditional CVCs were inserted in other 76 hematological patients (44 males, 32 females) suffering from AML (60) or ALL (16). 45 CVCs were inserted in subclavian vein and 31 CVCs were inserted in jugular vein. Overall, the median duration of in situ catheter placement was 5 months. In the arm A, median duration of device was 6 months (r. 12-8), catheter-related thrombosis occurred in 8 patients (6 basilica vein, 2 brachial vein) and catheter-related bloodstream infections in 4 patients (2 methicillin-resistant, 1 coagulase-negative, 1 *staphylococci*). In the arm B, median duration of device was 3 months (r. 5-10), 20 cases of catheter-related thrombosis (7 subclavian vein, 13 jugular vein) and 15 cases of catheter-related bloodstream infections (3 methicillin-resistant, 2 coagulase-negative, 4 staphylococci, 6 *enterobacteriaceae*) were observed. Thus, PICCs were significantly associated with fewer major complications compared with traditional CVC (catheter-related thrombosis: 10.5% in the arm A *vs.* 26% in the arm B, P=0.01 by $\chi 2$ test; catheter-related bloodstream infections: 5% in the arm A *vs.* 19% in the arm B, P=0.007 by $\chi 2$ test).

Table 1.

	Arm A : PICCs	Arm B : CVCs
N° patients	76	76
Median age	51.5 (19-82)	42.5 (13-74)
M/F	59/17	44/32
AML	70	60
ALL	6	16
Median duration of device	6 months (r. 8-12)	3 months (r. 5-10)
Catheter-related thrombosis	10.5%	26%
Bloodstream infections	5%	19%

Summary/Conclusion: The preliminary observations of this ongoing Phase IV randomized study, focusing on front-line use of central venous access device, suggest that the use of PICC represents an advance in terms of decrease of complication rate and improve of quality of life for patients with acute leukemia.

LB2245

EFFICACY AND SAFETY OF ETOPOSIDE IN COMBINATION WITH G-CSF, LOW-DOSE CYTARABINE AND ACLARUBICIN IN NEWLY DIAGNOSED ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Chemotherapy for elderly patients with acute myeloid leukemia (AML) is still a great challenge. Although 50% AML patients could achieve complete remission (CR) after intensive 3+7 regimen, treatment-related toxicities (TRT) appeared particularly prominent in elderly patients. In 2013, a multicenter randomized controlled trial in southwestern China confirmed that 71.1% refractory or relapsed AML achieved CR after received etoposide combine with low-dose CAG (E-CAG) regimen, and TRT was low, with no mortality.
Aims: This prospective phase II, open label, randomized controlled study was designed to assess the efficacy and safety of E-CAG induction treatment for elderly patients with newly diagnosed AML.
Methods: The effect of E-CAG on the rate of CR was the main study endpoint. The median survival time and the toxicity of the E-CAG regimen were also evaluated.
Results: After induction chemotherapy, patients with E-CAG regimen had a similar CR rate than did patients who received DA regimen (55.1% *vs* 48.9%, P=0.158). The tolerability profiles of E-CAG regimen appeared better than DA regimen. Especially, gastrointestinal reaction and III-IV bone marrow suppression. The median survival time was extended for 4 months in E-CAG group (14.3 months *vs* 10.3 months, P=0.042). The two-year OS probability in E-CAG group and DA group was 24.2% and 11.3%, perspectively.
Summary/Conclusion: The E-CAG regimen seems promising and offers lower toxicity for the treatment of elderly patients with AML, and expected to become a bridge for non-myeloablative stem cell transplantation.

Aggressive Non-Hodgkin lymphoma - Clinical

E944

OUTCOMES WITH RITUXIMAB MONOTHERAPY *VERSUS* R-CHOP IN POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE ARISING AFTER SOLID ORGAN TRANSPLANT: A UNITED KINGDOM MULTICENTRE STUDY

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Background: Post-transplant lymphoproliferative disease (PTLD) remains an important complication of solid organ transplantation (SOT). There is currently a lack of data to guide selection between Rituximab monotherapy (R-mono) and R-CHOP as options for frontline therapy of B-cell PTLD.
Aims: To report outcomes and prognostic factors for patients with B-cell PTLD arising after SOT, comparing R-mono *vs* R-CHOP as initial therapy.
Methods: This retrospective study included adult patients from 6 centres in the United Kingdom diagnosed with biopsy-proven PTLD between 2000-2013. Median follow-up was 4 years 2 months.
Results: Of 116 cases of PTLD identified, 100 were categorised as B-cell PTLD and were included in this analysis. Histological subtypes were 67 DLBCL, 23 polymorphic and 10 B-cell PTLD otherwise unspecified. There were 62 renal, 30 liver, 7 cardiothoracic and 1 pancreatic transplant recipients. Median age at diagnosis was 48 years (range 16-84 years) and 65% of patients were male. Early onset disease, presenting ≤1 year after transplant, comprised 15% of cases. EBV-association was noted in 55/78 (71%) of evaluated cases and this was related to early onset disease (X^2 p=0.004). Initial therapy was R-mono in 26/100 (26%) and R-CHOP in 45/100 (45%). Use of R-CHOP was associated with monomorphic histology (OR 18.3, p=0.001), ≥2 extranodal sites (OR 4.5, p=0.03), late onset disease (OR 6.2, p=0.01) and lack of EBV association (OR 0.08, p=0.002). Of patients treated with R-mono, 25/26 (96%) completed at least 4 infusions of Rituximab 375 mg/m^2 weekly, with no treatment-related deaths. Of those treated with R-CHOP, 33/45 (73%) completed at least 6 cycles, although there were 4 treatment-related deaths. The overall response rate for R-mono was 20/26 (77%; 14 CR, 6 PR, 5 NR and 1 undetermined) compared to 36/45 (80%; 30 CR, 6 PR, 5 NR and 4 undetermined) for R-CHOP. Amongst patients treated with R-mono, 17/26 (65%) had no further therapy and remained disease free with median follow-up of 44 months; 6 other patients received R-CHOP for consolidation of response or relapsed/refractory disease. Overall survival (OS) was 71% and 61% at 3 years for R-mono and R-CHOP respectively (p=0.40; Figure 1A). Amongst all patients with B-cell PTLD, significant baseline predictors of inferior OS were age ≥50 years (HR 3.8, p<0.001), ECOG PS ≥2 (HR 1.9, p=0.04), elevated LDH (HR 2.0, p=0.08 borderline), stage ≥2 disease (HR 2.5, p=0.01) and ≥2 extranodal sites (HR 1.8, p=0.08 borderline). Overall response to R-mono or R-CHOP was also highly predictive of OS (HR 0.16, p=0.0001). B symptoms, onset time, extranodal disease, histology and EBV-association were not predictive. In multivariate testing, age ≥50 years (HR 5.3, p=0.0001) and advanced stage (HR 2.2, p=0.06 borderline) remained significant. Applying a 4 point modified prognostic index (comprising age ≥50 years, stage ≥2 disease, ECOG PS ≥2 and elevated LDH) to patients treated with R-mono or R-CHOP, those with low risk disease (0-1 points) had significantly improved survival compared to those with high risk disease (≥2 points), with 3 year OS of 87% *vs* 51% (p=0.001; Figure 1B). Amongst patients with high risk disease, 13 treated with R-mono had an inferior complete response rate compared to 21 treated with R-CHOP (23% *vs* 67%; OR 0.15, p=0.02), although a survival difference was not detected.

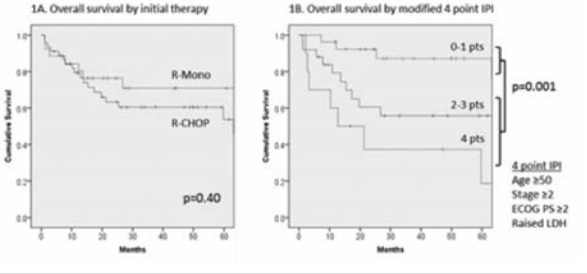

Figure 1.

Summary/Conclusions: We report outcomes for R-mono *vs* R-CHOP as initial therapy for PTLD arising after SOT. R-mono may deliver inferior response rates for patients with high risk disease. Using a modified prognostic index we identify a subset of patients with poor outcome for whom novel therapeutic strategies are required.

E945

EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE OF ADVANCED STAGE: NKEA (NEXT-GENERATION THERAPY FOR NK/T-CELL LYMPHOMA IN EAST ASIA) PROJECT

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Background: Treatment of extranodal NK/T-cell lymphoma, nasal type (ENKL) has proceeded and evolved. Concurrent chemoradiotherapy with platinum-containing regimen and L-asparaginase based chemotherapy such as SMILE (steroid, methotrexate, ifosfamide, L-asparaginase, and etoposide) are the current standard for limited- and advanced-stage ENKL, respectively. However, there are no large-scale study which analyzed the data of patients in general practice.
Aims: To understand the recent conditions of ENKL, we conducted a survey for new-generation therapy for NK/T-cell lymphoma in East Asia (NKEA). Patients with advanced-stage were analyzed in this study.
Methods: This cooperative study by hemato-oncologists and radiation oncologists in Japan (UMIN000015491) retrospectively analyzed data from patients with ENKL who were diagnosed between 2000 and 2013.
Results: Data of 358 patients with ENKL of all clinical stage were collected from 31 institutes. A total of 102 patients were in advanced stage (4 with stage III and 98 with stage IV). There were 64 males and 38 females. The median age was 59 years, ranging from 18 to 86 years old. A total of 27 patients were initially treated with CHOP-type regimen, 33 patients with DeVIC-type, and 30 patients with SMILE-type regimen, but 12 patients did not receive any chemotherapy. In total, 23 patients received additional radiotherapy (RT). The median dose of RT was 48 Gy. Thirty patients received hematopoietic stem cell transplantation (SCT, 10 autologous and 20 allogeneic). Patients who were initially treated with SMILE-type treatment more received SCT (50% *vs* 26% with CHOP-type or 24% with DeVIC-type). The overall response rate was 67% for SMILE-type, 45% for DeVIC-type, and 33% for CHOP-type regimens. The 5-year overall survival (OS) rate was 25% with a median follow-up of 5.8 yrs, and the 5-year progression free survival (PFS) rate of 17%. OS of patients who received SMILE-type, CHOP-type, and DeVIC-type regimen was 39%, 28%, and 19%, respectively (P=0.04). addition of RT did not affect the prognosis of advanced ENKL (5-year OS: 24% for patients without RT and 26% for patients with RT). Prognosis of patients who received SCT was better than those who did not (5-year OS: 55% *vs* 12%). The type of SCT (autologous *vs* allogeneic) did not affect the prognosis (5-year OS: 40% for autologous *vs* 62% for allogeneic). The international Korean Prognostic Indexes were not prognostic (P=0.54 and 0.30, respectively), but the PINK (Prognostic Index for NK) score was prognostic (P=0.03).

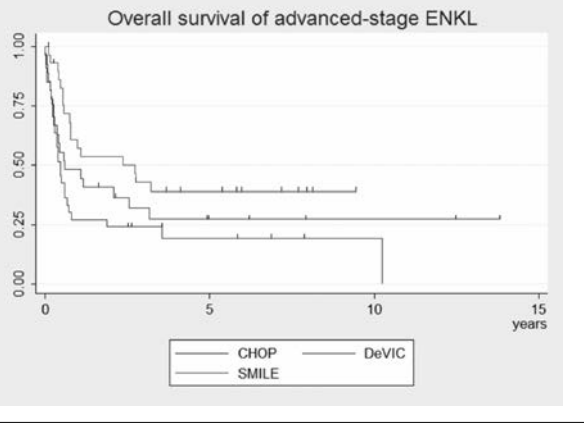

Figure 1.

Summary/Conclusions: The efficacy of SMILE-type chemotherapy for advanced ENKL was shown in this study, although there remains several limitations of retrospective nature. A novel prognostic index of PINK successfully

predicted the prognosis. The prognosis of advanced-stage ENKL has improved as compared with that in the previous-era, but further betterments are required.

E946

VALIDATION OF NCCN INTERNATIONAL PROGNOSTIC INDEX (NCCN-IPI) FOR DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL). THE ADDITION OF B2MICROGLOBULIN RESULTED IN GELTAMO-IPI THAT IS MORE ACCURATE

C Montalban[1,*], A Diaz-Lopez[2], A Lopez-Guillermo[3], I Dlouhy[3], J Rovira[3], S Alonso[4], A Martín[4], JM Sancho[5], O Garcia[5], JM Sanchez[6], M Rodriguez[6], S Novelli[7], A Salar[8], A Gutierrez[9], MJ Rodriguez-Salazar[10], M Bastos[11], JF Dominguez[12], R Fernandez[13], S Gonzalez de Villambrosia[14], JA Queizan[15], R Cordoba[16], R De Oña[1], JM Freue[2], H Garrote[2], L Lopez[2], AM Martin-Moreno[17], J Rodriguez[1], V Abraira[18], JF Garcia[2,19]

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Background: The NCCN International Prognostic Index (NCCN-IPI) for patients with DLBCL treated in the rituximab era showed enhanced discrimination in the original study, when compared to IPI. However, in our previous study (ASH 2015 #3955) it failed to identify patients with a real poor outcome.
Aims: In the present study we explore the prognostic effect of adding new factors to the variables of NCCN-IPI in a large series of patients.
Methods: This nation-wide retrospective study includes a final database of 1848 patients with de *novo* DLBCL diagnosed in 20 Spanish centers within the Grupo Español de Linfomas/ Transplante de Médula Osea (GELTAMO) network treated with standard chemotherapy and rituximab. To study the improvement of the prognostic effect of adding three new variables, normalized high serum β_2 microglobulin ($\beta_2$mcg), primary extranodal involvement and treatments more intense than R-CHOP, to the NCCN-IPI variables, the series was split in two cohorts. The training cohort (1230 patients) was used to develop a prognostic model using Cox regression models for Overall Survival and the final model was validated in an independent validation cohort (618 patients). The Kaplan Meyer method and log-rank test were used for OS and comparison of curves. The prognostic effect of this score was compared with NCCN-IPI and IPI with the reclassification calibration statistics (a modification of Hosmer–Lemeshow goodness of fit).
Results: The COX regression model in the training cohort, showed that distinct extranodal involvement (as included in NCCN-IPI), primary extranodal lymphoma and intense treatment achieved no significance. The final model included the rest of the variables, with points according with the hazard ratios: age (<65/0 pts; ≥65-79/1 pt; ≥80/2 pts), PS(0-1/0 pts ; 2/1 pt ;≥2/2 pts), high LDH, Stage III-IV and high $\beta_2$mcg, with 1 point each, resulting in a score (GELTAMO-IPI) with a maximum of 7 points that separated significantly four risk groups: LR (0 points), LIR (1-3 pts), HIR (4 pts), HR (≥5) with significantly different 5-yOS that was subsequently confirmed in the validation cohort. Using all the patients in the series with available data (n=1672), GELTAMO-IPI discriminates better (5-yOS rates of LR: 93%, LIR 79%, HIR 66%, HR 39%) than NCCN-IPI (93%, 83%, 67%, 49%, respectively) and IPI (88%, 77%, 68%, 51%, respectively); both NCCN-IPI and IPI failed to identify a population with a 5-yOS substantially lower that 50%. Similar results were obtained for 5-yFFS. In the reclassification calibration statistics GELTAMO-IPI was more accurate than NCCN-IPI and IPI.

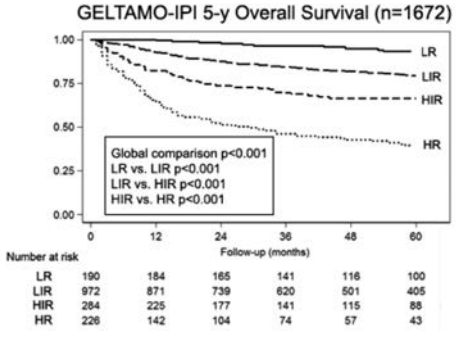

Figure 1.

Summary/Conclusions: GELTAMO-IPI including $\beta_2$mcg along all the variables of NCCN-IPI (excluding selective extranodal involvement) is more accurate than NCCN-IPI and IPI for the prognosis of DLBCL. Additionally, it can be used in primary nodal and extranodal lymphoma and in patients with intense treatment and, most important, identifies a poor prognostic group with 5y-OS of 39%.

E947

EVALUATION OF A PROGNOSTIC MODEL FOR CNS RELAPSE WITHIN THE UK NCRI R-CHOP-14 *VS* 21 TRIAL

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Background: Central Nervous System (CNS) relapse of Diffuse Large B-cell Lymphoma (DLBCL) is associated with a poor prognosis. CNS prophylaxis is administered to patients deemed to be at high risk of CNS relapse but the indications for prophylaxis are not standardized. The German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) recently proposed a new 6-factor prognostic model incorporating the 5 International Prognostic Index (IPI) factors in addition to kidney/adrenal gland involvement to determine the risk of CNS relapse in patients with aggressive B-cell lymphoma. This model stratified patients into 3 risk groups: low [0-1 factors, 2 yr CNS relapse risk=0.6% (95% CI 0.0-1.2)]; intermediate [2-3 factors, 2 yr CNS relapse risk=3.4% (95% CI 2.2-4.6)] and high risk [4-6 factors, 2 yr CNS relapse risk=10.2% (95% CI 6.3-14.1)] (Schmitz et al, Lugano 2013); which was subsequently validated in an independent cohort of R-CHOP-treated patients with DLBCL at the British Columbia Cancer Agency (BCCA) (Savage et al, ASH 2014).
Aims: In this analysis we applied the DSHNHL prognostic model to the UK NCRI prospective R-CHOP-14 v 21 trial cohort to determine if similar risk groups could be identified.
Methods: The randomised phase III UK R-CHOP-14 *vs* 21 trial assessed rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP) given 2 weekly *versus* 3 weekly in 1080 DLBCL patients accrued between 2005-2008 (Cunningham et al, 2013). Administration of CNS prophylaxis was at the discretion of investigators but recommended for patients with involvement of bone marrow, peripheral blood, nasal/paranasal sinuses, orbit and testis (12.5mg intrathecal methotrexate (IT MTX) for the first 3 cycles of treatment or according to local guidelines). Details of CNS prophylaxis were retrospectively collected from participating sites using case report forms.

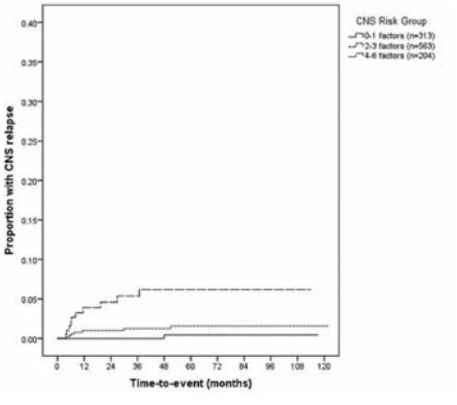

Figure 1.

Results: With a median follow-up of 6.5 years the incidence of CNS relapse in our cohort was 1.7% (18/1,080). 17.3% (170/982) of patients in the R-CHOP-14 *vs* 21 trial received CNS prophylaxis: IT MTX=94.0%, high-dose intravenous MTX=1.3%, prophylaxis type unknown=4.5%. We have previously reported that the incidence of CNS relapse for patients receiving prophylaxis was 3.5% which may suggest some benefit of this therapy (Gleeson et al, ASH 2014). The 6-factor DSHNHL model stratified patients into 3 risk groups for CNS relapse at 2 years: low (0-1 factors)=0%; intermediate (2-3 factors)=1.0% (95% CI 0.2-1.8) and high risk (4-6 factors)=4.6% (95% CI 1.5-7.7) [Figure 1]. Patients developing CNS relapse (n=18) were divided into the following DSHNHL risk groups: low n=1/313, intermediate n=7/563 and high-risk n=10/204. The proportion of patients receiving CNS prophylaxis was 15.3%, 14.2% and 31.4% for low, intermediate and high-risk groups respectively.

Summary/Conclusions: The overall incidence of CNS relapse within the R-CHOP-14 *vs* 21 trial cohort was low (1.7%). The DSHNHL model identified 3 risk groups for CNS relapse at 2 years in the R-CHOP-14 *vs* 21 cohort, with patients in the high-risk group demonstrating a 2-year incidence of CNS relapse of 4.6%. We observed a lower rate of CNS relapse than that reported by both the DSHNHL and BCCA groups. This may have been influenced by population differences, as the DSHNHL cohort included patients with aggressive B-cell non-Hodgkin lymphoma and the BCCA cohort is a population-based dataset. In addition the proportion of patients receiving CNS prophylaxis may have differed between patient cohorts.

E948

SAFETY AND CLINICAL ACTIVITY OF TEMSIROLIMUS IN COMBINATION WITH RITUXIMAB AND DHAP IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA-PRELIMINARY RESULTS OF THE STORM TRIAL

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Background: Prognosis of diffuse large B-cell lymphoma (DLBCL) has improved with the advent of Rituximab. However, there is increasing evidence that treatment of patients with relapsed and refractory disease is challenging.
Aims: The purpose of this trial is to evaluate the safety, tolerability and efficacy of the combination of the mTOR inhibitor Temsirolimus and a standard salvage regimen (R-DHAP) in patients with relapsed or refractory DLBCL.
Methods: This is a prospective, multicenter, phase II, open-label study. Patients with relapsed or refractory DLBCL with a maximum of two prior treatment lines were eligible. The STORM regimen consisted of Rituximab 375mg/m^2 (day 2) and DHAP (Dexamethasone 40mg day 3-6, Cisplatine 100mg/m^2 day 3, Cytarabine 2x2g/m^2 day 4) with Temsirolimus added on day 1 and 8 of a 21 d cycle, with 2-4 cycles planned. In part I, dose levels for the mTOR inhibitor Temsirolimus from 25, 50, 75 and 100 mg were predefined. Based on the observed toxicity profile, the independent data safety committee recommended a Temsirolimus dose of 25mg given on day 1 and 8 for the part II extension cohort of the trial.
Results: In part I of this clinical trial 15 patients were included - 8 patients in the 25 mg cohort and 7 patients in the 50 mg cohort. Median age was 70 (range 49-76) years and median number of prior regimen was 1. Two DLTs (one venous thrombosis in the 25 mg cohort, one esophagus infection in the 50 mg cohort) were observed. The most frequent non-hematologic side effects were nausea (9 pts, 60%), epistaxis (7 pts, 47%), fatigue (6 pts, 40%), increased ALT (6 pts, 40%) and increased creatinine (6 pts, 40%). Frequent grade 3/4 events (n>2) in both cohorts (25mg|50mg) included leukopenia (11 pts, 73% - with a mean duration of 4.4 days | 6.7 days), thrombocytopenia (11 pts, 73% - with a mean duration of 4.6 days | 11.9 days), lymphopenia (6pts, 40%), anemia (5 pts, 33%), neutropenia (3 pts, 20%), renal failure (3 pts, 20%) and infections (4 pts, 27%, bladder infection, esophagus infection, central venous access infection, soft tissue infection, mucositis). All but one evaluable patient responded (10/11 pts, 91%), with two CRs and one CRu (27%). Four patients could not be evaluated for response at the time of the first report. After a median follow up of 12 (range 5-22) months, no relapse had been documented (1 pt lost to follow up); since then, progression of disease has occurred in three patients and led to these patients' death. However, only for one of these patients participation in the study ended prematurely because of progressive disease under therapy, while the other two had already dropped out due to adverse events. As far as the extension cohort of part II is concerned, preliminary data of 17 patients are reported below. Median age was 61 (range 42-74) and median number of prior regimen was one. Out of these 17 patient data sets, two could not be evaluated for response. The response rate of the remaining 15 patients was 87% (13/15 pts). Two therapy-related deaths occurred (one patient died from sepsis during neutropenia, another from cerebral bleeding, both events occurring after cycle 3). After a median follow up of 7 months for the total study population, median PFS and OS have not been reached.
Summary/Conclusions: Temsirolimus can be safely added to DHAP and Rituximab with promising activity. This conclusion drawn from part I of the study has been confirmed by the preliminary data of part II. Recruitment of the part II of the trial is continuing.

E949

Abstract withdrawn.

E950

PROGNOSTIC FACTORS IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL) AFTER RITUXIMAB-CHOP (R-CHOP) WITH OR WITHOUT RADIOTHERAPY (RT): MATURE RESULTS OF A COOPERATIVE RETROSPECTIVE STUDY

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Background: Prognostic factors (PFs) have not been extensively studied in PMLBCL and prognostic models specifically applicable to this entity have not been developed, mainly due to its rarity. R-CHOP provides very good results in PMLBCL, minimizing failure rates. High IPI and serous effusions emerged as adverse prognostic factors in 181 Japanese patients treated with RCHOP±RT (selected among broader population with heterogenous treatment), while serous effusions, B-symptoms and age were identified in 96 RCHOP±RT-treated patients in a 2012 abstract from Vancouver. Given that more intensive chemotherapy (R-da-EPOCH) might be better than R-CHOP, the applicability of various PFs needs to be urgently evaluated in the Rituximab era in order to define subgroups of patients at high risk for treatment failure and death.
Aims: The identification of PFs for the outcome of patients with PMLBCL treated with RCHOP± RT.
Methods: 213 patients with PMLBCL were treated in a multicenter setting with RCHOP±RT (usually 6-8 cycles). The following potential prognostic factors were evaluated: Age (median 31; range 17-82; >60 years only 4%), gender (female 64%), B-symptoms, stage III/IV, infradiaphragmatic disease, extranodal involvement (either stage IV or stage E), pleuritis, pericarditis, performance status (PS) ≥2, LDH levels, anemia, leukocytosis ≥10x10^9/L, ESR ≥30mm/h, albumin <4g/dL, bulky disease (≥10cm), age-adjusted IPI (aaIPI; ≥2 in 21%). A modified version of aaIPI was also analyzed, attributing 1 point to either stage III/IV or E-disease instead of stage III/IV only (aaIPI-mod).
Results: With 52 failures recorded (51 within 17 months from diagnosis), the 3-year freedom from progression (FFP) was 75%. With 24 deaths recorded (excluding 2 unrelated deaths), the 5-year overall survival (OS) was 87%. aaIPI≥2 identified a minority of patients (21%) with a 5-year FFP of 64% *vs* 79% for those with aaIPI 0-1 (p=0.04) and 5-year OS of 76% *vs* 91% (p=0.0095). The aaIPI-mod was more effective in predicting the outcome and indentified a larger poor prognostic group (41% of total): The 5-year FFP was 61% *vs* 86% for those with aaIPI-mod 0-1 (p<0.0001), while 5-year OS was 75% *vs* 97% (p<0.0001). Many of the examined variables had a significant (p<0.05) or borderline (p<0.15) association with both FFP and OS (extranodal disease, LDH, PS, abdominal disease, bulky disease, serous effusions, anemia). In multivariate analysis of FFP, extranodal involvement and bulky disease were independent PFs (p=0.006 and p=0.04). None, 1 or 2 of these factors were present in 29%, 42% and 30% of the patients. FFP at 5 years was effectively predicted being 88%, 79% and 59% for patients with 0, 1 or 2 PFs respectively, while 5-year disease specific survival was 100%, 91% and 72%.
Summary/Conclusions: In the largest patient series reported so far, RCHOP±RT provided satisfactory results in PMLBCL with long-term FFS of 75% and excellent OS of 87%. The aaIPI was moderately predictive of the outcome but a modified version performed better. Either the aaIPI-mod or the combination of extranodal involvement and bulky disease defined a subgroup comprising ~40% and ~30% of patients respectively with a ~40% risk of failure and ≥25% risk of death, who might be suitable for trials of treatment intensification.

E951

A POOLED DATA ANALYSIS OF TWELVE CLINICAL TRIALS OF FONDAZIONE ITALIANA LINFOMI (FIL): KHORANA SCORE AND HISTOTYPE PREDICT THE INCIDENCE OF EARLY VENOUS THROMBOEMBOLISM (VTE) IN NON HODGKIN LYMPHOMA

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Background: Recent studies show that the risk of VTE in Non Hodgkin Lymphoma (NHL) patients (pts) is similar to that observed in high risk solid tumors (i.e. pancreatic, ovarian cancer). VTE in NHL occurs in most cases within 3 months from diagnosis and can have substantial impact on treatment delivery and outcome as well as on quality of life. However few data are available on potential predictors.
Aims: To better clarify the epidemiology of early (within 6 months from treatment start) VTE in NHL pts we conducted a pooled data analysis of 12 clinical trials from FIL. Our analysis included basic demographic features, lymphoma-related characteristics as well as the Khorana score KS (based on histology, body mass index BMI, platelets count PLTs, white blood cells count WBC and hemoglobin values Hb) which is extensively used in solid tumors to predict VTE risk.
Methods: From Jan. 2010 to Dec. 2014 all pts with B-cell NHL enrolled in prospective clinical trials from FIL for frontline treatment were included. For 9 studies the study period included the entire trial population. The analysis was conducted based on CRFs as well as pharmacovigilance reports. VTE definition and grading was stated according to standard criteria of toxicity (CTCAE V4.0). Cumulative incidence of VTE from study enrollment was estimated using the method described by Gooley et al. accounting for death from any causes as a competing risk. The Fine & Gray survival model was used to identify predictors of VTE among NHL pts. Factors predicting the grade of VTE were investigated using an ordinal logistic regression model. This pooled data analysis was approved by local IRB.
Results: Overall, 1717 pts belonging to 12 studies were evaluated. 8 studies were phase I/II or II (25% of pts) and 4 phase III (75% of pts). M/F ratio was 1.41, median age was 57 (IQ range IQR 49-66). Histologies were: diffuse large B-cell lymphoma DLCL-B 34%, follicular lymphoma FL 41%, mantle cell lymphoma MCL 18%, other 6%. Median BMI was 25 (IQR 22-28). Median Hb, WBC and PLTs counts were 13g/dl (IQR 11.5-14.2), 7,1x10^9/l (IQR 5.6-10.3) and 224x10^9/l (IQR 169-298) respectively. In 1189 pts was assessed KS: 58% low risk, 30% intermediate risk, 12% high risk. Human erytropoetin support was given to 9% of pts. All pts received Rituximab. Planned therapeutic programs included autologous stem cells transplantation in 27% of pts, conventional chemotherapy in 67% and conventional chemotherapy plus lenalidomide in 6%. Overall 59 any grade VTE episodes occurred in 51 pts (2.9%), including 21 grade III-IV VTEs (18 pts). None was fatal. Median time from study enrollment to VTE was 63 days (IQR: 35-110). Considering death as a competitive event the 6 months cumulative incidence of VTE was 2,2% (95%IC: 1.1-3.3) in low risk, 4.5% (95%IC: 2.3-6.7) in intermediate and 6.6% (95%IC: 2.4-10.8) in high risk KS (p=0.012). KS was predictive also for grade III-IV VTEs as they were 0,7% (95%IC:0.1-1.4) in low risk and 2% (95%IC:0.8-3.3) in intermediate-high risk (p=0.048). Results were similar also after excluding lenalidomide containing studies. Fine and Gray multivariate analysis, adjusted for age and stage, showed that KS (intermediate risk adjHR=1.96; 95%IC: 0.84-4.56 and high risk adjHR=3.81; 95%IC: 1.51-9.58) and DLCL-B histotype (adjHR=2.58; 95%IC: 1.01-6.55) were independently associated to an increased risk of VTE. Moreover an ordinal logistical regression model indicated that the increase of 1 point in the KS resulted in an increased risk of VTE (OR=1.85; 95%CI: 1.23-2.79).

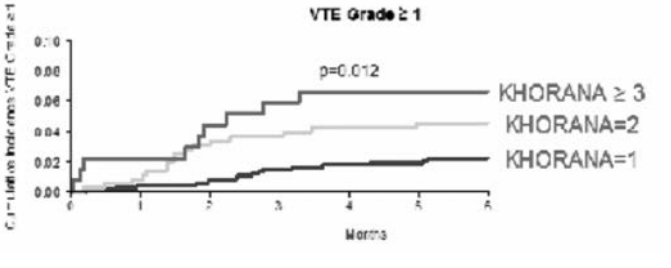

Figure 1.

Summary/Conclusions: Our results suggest that DLCL-B hystotype and KS are predictors of VTE in NHL. The latter might become a simple and effective tool to assess the risk of VTE in NHL. Prospective validation including also pts not eligible for clinical trials is needed.

E952

HEMOGLOBIN LEVEL IMPROVES THE ABILITY OF THE NATIONAL COMPREHENSIVE CANCER NETWORK INTERNATIONAL PROGNOSTIC INDEX TO PREDICT OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA A POPULATION BASED COHORT STUDY

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Background: Prognosis and treatment options for patients with diffuse large B-cell lymphoma (DLBCL) has for the last 20 years been conveyed on the basis of The International Prognostic Index (IPI). Improving IPI by adding biomarkers is, however, an ongoing discipline. The National Comprehensive Cancer Network IPI (NCCN-IPI) adds to the classical IPI factors more refined age and lactate dehydrogenase (LDH) strata and weighs extranodal disease according to the involvement or not of high risk organs such as bone marrow, CNS, liver/GI tract or lung. The NCCN-IPI was derived and validated on two cohorts with a mean age of 57- and 62-years, respectively, *i.e.* slightly younger than one would expect in a population-based DLBCL cohort. In Denmark, the universal healthcare system is tax funded and free of charge for all inhabitants ensuring a broad coverage of high quality healthcare. For lymphoma, a national group elaborate management guidelines and a prospective collection of patient-related data stored in the group's database (LYFO).
Aims: i) To validate the NCCN-IPI in a population-based cohort of de-novo DLBCL patients treated with anthracycline-based chemotherapy; (ii) to analyze, in a multivariate model, whether adding information on pre-therapeutic hemoglobin level improves on the ability of the NCCN-IPI to predict outcome.
Methods: All patients were diagnosed with de novo DLBCL between 2000 and 2012 and treated with anthracycline-based chemotherapy with or without rituximab (R). Patients with indolent- or composite lymphoma were excluded, as were patients with primary CNS-lymphoma. Clinical data were obtained from LYFO and biochemical data were retrieved partly from LYFO and from hospital laboratories. Data on vital status were obtained from the Danish Civil Registration System. Patients were followed from the date of biopsy until death or the end of study (December 10, 2015). Overall survival was described using Kaplan-Meier curves according to NCCN-IPI, treatment and hemoglobin values. Univariate and multivariate analysis were performed using Cox proportional hazard model including NCCN-IPI group, anemia and treatment as covariates with mutual adjustment. Anemia was defined as hemoglobin<7.3 mmol/l l for women and<8.3 mmol/l for men. The proportional hazard assumption was evaluated graphically using log-log plots. The study was approved by the Danish Data Protection Agency (no. 1-16-02-562-13).
Results: A total of 3654 patients fulfilled the inclusion criteria and had a median follow-up of 1888 days. Median age was 65 years (range 15-98), 2033 (56%) were men, 2081 (57%) had Ann Arbor stage III-IV, and 712 (19%) had a performance score ≥2. The pre-therapeutic LDH ratio (measured *vs* max reference value) was >1 in 2225 (61%). Anemia was present in 1784 (49%) patients. The NCCN-IPI separated our cohort in low (8%), low intermediate (35%), high intermediate (39%), and high risk (18%) patients. For R-chemo treated patients, the four NCCN-IPI risk groups (low, low-intermediate, high-intermediate, high) revealed 5-year OS values of 0.96 (CI95%, 0.92-0.98), 0.81 (CI95% 0.77-0.83), 0.60 (CI95% 0.56-0.63), and 0.30 (CI95% 0.26-0.37), respectively (Figure 1A). When hemoglobin level was added to the model it showed a significant impact on OS for both intermediate risk groups. Estimated 5 year overall survival in anemic *vs* non anemic patients with high-intermediate risk NCCN-IPI was 0.53 (95%CI 0.48-0.58) and 0.69 (95%CI 0.63-0.73) (Figure 1 B) and for low-intermediate 0.74 (95%CI 0.68-0.79) and 0.84 (95%CI 0.80-0.87). Patients with anemia as defined had a HR of 2.46 (95%CI 2.08-2.90).). After adjusting for NCCN-IPI factors, the HR remained significant (1.51; 95%CI 1.29-1.83).

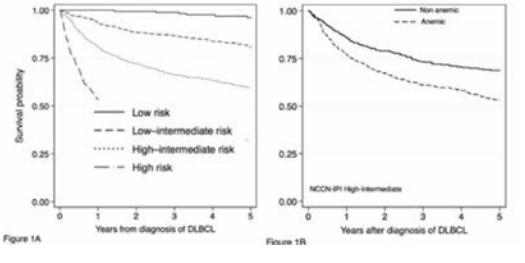

Figure 1.

Summary/Conclusions: In a population-based cohort of de novo DLBCL patients, we found that pre-therapeutic anemia was present in 49% of the patients and had independent prognostic impact also when adjusted for NCCN-IPI risk factors, and stratified for rituximab treatment. The adverse prognostic value of pretherapeutic anemia was independent of gender and age strata. Our patient cohort was older than the ones of the original NCCN-IPI publications (median age 65 yrs *vs* 54 yrs), which probably explains the difference in the proportion of high-risk patients (18% in our cohort *vs* 8% and 14% in the two original NCCN-IPI cohorts).

E953

ROUTINE IMAGING FOR PERIPHERAL T-CELL LYMPHOMA IN FIRST COMPLETE REMISSION DOES NOT IMPROVE SURVIVAL: A DANISH SWEDISH POPULATION-BASED STUDY

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Background: Routine surveillance imaging plays a limited role in detecting lymphoma relapse and a recent Danish-Swedish study showed that imaging-based follow-up practice was not associated with better outcome for diffuse large B-cell lymphoma (DLBCL) patients.
Aims: Using a similar approach, we evaluated the outcome of Danish and Swedish patients with nodal peripheral T-cell lymphoma (PTCL) in first CR for whom traditions for routine imaging have been different.
Methods: Patients from the Danish and Swedish lymphoma registries were selected by the following criteria: a) newly diagnosed nodal PTCL from 2007 to 2012, b) age ≥18 years, and c) CR after CHOP or CHOEP therapy with or without consolidating high-dose therapy (HDT). Follow-up for Swedish patients included symptom assessment, clinical examinations and blood tests at 3- to 4-month intervals for 2 years, with longer intervals later in follow-up. The national Swedish guidelines only recommended imaging when relapse was clinically suspected. Follow-up for Danish patients was similar but included routine imaging, usually computed tomography (CT) every 6 months for 2 years and at some centers annual CT from the 3rd till the 5th year of follow-up as well.
Results: In total, 109 Danish patients and 123 Swedish patients with nodal PTCLs were included. PTCL not otherwise specified (NOS) was diagnosed in 95 patients, anaplastic large cell lymphoma (ALCL) in 88 patients, and angioimmunoblastic T-cell lymphoma (AITL) in 49 patients and with similar subtype frequencies for Danish and Swedish patients. The baseline demographic characteristics were also fully comparable between Danish and Swedish patients (Table 1). The OS estimates for Danish and Swedish patients in CR following frontline therapy were similar for all patients (P=0.6, Fig 1) and in PTCL subtype specific analyses. The most important adverse predictor of OS following CR was age >60 (Hazard Ratio [HR], 3.73; 95% CI, 2.08 to 6.66, P<0.01). The 2-year probability of relapse was similar for Danish and Swedish patients (25%; 95% CI 16-33% *vs* 29%; 95% CI 19-38%, P=0.53). The post-relapse OS was similar for Danish and Swedish patients overall (P=0.8) and in PTCL subtype specific analyses.

Table 1. Demographic and clinicopathological information on Danish and Swedish patients with nodal PTCL.

	DK (n=109)	SWE (n=123)	Missing (DK/SWE)	P-value
Median age (years)	61 (21-89)	64 (20-87)	0/0	0.29
Male-female ratio	1.27	1.51	0/0	0.59
IPI > 2, n (%)	31 (29.8)	36 (30.0)	5/3	1.00
ECOG >= 2	14 (13.0)	16 (13.2)	1/2	1.00
Induction chemo CHOP	77 (70.6)	72 (58.5)	0/0	0.07
Induction chemo CHOEP	32 (29.4)	51 (41.5)	0/0	0.07
ALCL	42 (39)	46 (37)	0/0	0.33
AITL	27 (25)	22 (18)	0/0	0.33
PTCL NOS	40 (37)	55 (45)	0/0	0.33

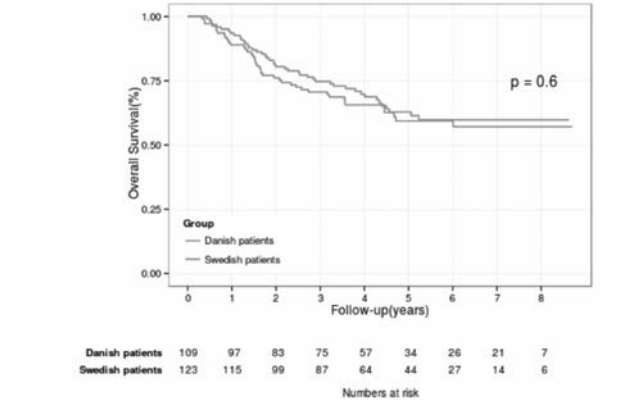

Figure 1.

Summary/Conclusions: Relapse following frontline treatment for PTCL is typically associated with a poor prognosis and an imaging based follow-up practice did not translate into better survival for Danish patients. Thus, a follow-up strategy without routine surveillance imaging appears safe and equally effective to a FU strategy with imaging for patients with nodal PTCL in first CR.

E954

REAL-TIME CELL-OF-ORIGIN SUBTYPE IDENTIFICATION BY GENE EXPRESSION PROFILE IN THE PHASE 3 ROBUST TRIAL OF LENALIDOMIDE+R-CHOP *VS* PLACEBO+R-CHOP IN PREVIOUSLY UNTREATED ABC-TYPE DLBCL

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Background: Gene expression profiling (GEP) represents a gold standard in identifying patients with activated B-cell–like (ABC) DLBCL, a subtype associated with inferior outcomes. Until recently, real-time identification of patients with ABC DLBCL as an integrated biomarker and inclusion criterion was not feasible due to technology limitations. The randomized, double-blind, global phase 3 ROBUST study (NCT02285062) compares lenalidomide+R-CHOP (R^2-CHOP) with placebo+R-CHOP in patients with previously untreated ABC-type DLBCL with participating sites in Asia, Australia, Europe, New Zealand, and North America.
Aims: Explore the real-world feasibility of GEP screening to determine DLBCL cell of origin (COO) in a global phase 3 study and quantify turnaround time and the percentage of screened patients having ABC-subtype DLBCL.
Methods: ROBUST methods have been described (Nowakowski, ASCO 2015). Patients must provide written informed consent. A key entry criterion is previously untreated, histologically confirmed ABC-type CD20+ DLBCL. Formalin-fixed paraffin-embedded excisional/surgical or core needle biopsy samples (Storhoff, Blood 2015) are analyzed by central pathology using the NanoString Lymphoma Subtyping Test (LST; Wallden, JCO 2015), based on the Lymph2Cx GEP assay (Scott, Blood 2014). Turnaround time is defined as number of days between central pathology sample receipt and results being provided to the study site.
Results: As of 11 Jan 2016, 357 patients were screened for ROBUST using the LST. Samples were analyzed in 2 central pathology labs in the US and UK. COO was ABC and non-ABC in 118 (33%) and 212 (59%), respectively. 27 patients (8%) had samples that could not be processed for technical reasons (incorrect/insufficient slides or blocks, or low tissue RNA concentration and/or purity). Mean turnaround time was 2.25 days (range, 0-9). 86 patients have met all inclusion criteria and were enrolled in ROBUST.
Summary/Conclusions: Real-time COO assessment is feasible in a multicenter, global phase 3 DLBCL study with short turnaround time. The percentage of patients with ABC-type DLBCL was similar to that reported in the literature. Our findings have important implications for the design and size estimation of both current and future studies in newly diagnosed DLBCL utilizing COO as a biomarker, promising a significant step forward in precision medicine. Updated results will be presented.

E955

MYD88 (L265P) MUTATION IS ASSOCIATED WITH AN UNFAVORABLE OUTCOME OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: Primary central nervous system lymphomas (PCNSL) typically show an immunophenotype resembling that of activated B-cell-like diffuse large B-cell lymphoma (ABC-DLBCL). Somatic mutations in *MyD88, CD79B*, and *CARD11* were shown to activate the NFκB signaling pathway in ABC-DLBCL. These mutations are reported to be strongly over-represented in PCNSL. Nonetheless, clinical relevance of these mutations remains unclear. On the other hand, the several specific clinical parameters, such as MSKCC and IELSG prognostic score systems, were reported to predict the treatment outcome of PCNSL patients. Recently advanced genetic knowledge, however, is not incorporated in any of these proposals.
Aims: We conducted this study to elucidate the impact of gene mutations on clinical outcome of PCNSL.
Methods: We analyzed 42 immuno-competent patients suffering from newly diagnosed PCNSL who were aged over 60 years, or aged 55-60 years if they had poor performance status, and in whom enough material for DNA extraction was available. They were treated with modified version of the EORTC protocol, consisted of systemic administration of intermediate-dose methotrexate (MTX), lomustine, procarbazine, and methylprednisolone, and intrathecal chemother-

apy with MTX and cytarabine (Taoka K., et al., Int J Hematol 2010; Lee SY., et al., Oncol Res Treat 2014), at the University of Tsukuba Hospital between March, 2005 and May, 2015. Targeted deep sequencing was performed by using Ion Ampliseq technology for 12 genes, including *MyD88*, *CD79B*, *PIM1*, *TBL1XR1*, *BTG2*, *PRDM1*, *TNFAIP3*, *CARD11*, *B2M*, *TOX*, *TMEM30A*, and *PRKCD*. All candidate mutations were validated by genomic PCR followed by Sanger sequencing. Overall survival (OS) and progression-free survival (PFS) were estimated using the Kaplan-Meier method, and compared by the peto-peto generalized Wilcoxon test.

Results: At least one mutation was detected in 38/42 cases (90.4%). Frequencies of mutations of 12 genes were similar to those of the previous reports (*MyD88*, 79%; *CD79B*, 52%; *PIM1*, 69%; *TBL1XR1*, 24%; *BTG2*, 29%; *PRDM1*, 24%; *TNFAIP3*, 12%; *CARD11*, 19%; *B2M*, 11.9%; *TOX*, 11.9%; *TMEM30A*, 4.8%; *PRKCD*, 4.8%). The median follow-up time was 26 months (range, 1-105 months), with OS at 3 years of 46% and PFS at 3 years of 26%. By univariate analysis, age >75 (P=0.0105) and altered mentation (P=0.0202) were significantly associated with inferior OS. Regarding PFS, CrCl >90 (P=0.0286) and altered mentation (P=0.0473) were significant factors. In addition, *MyD88* L265P mutation showed a tendency to be associated with inferior OS and PFS, although statistically non-significant (OS: P=0.127, PFS: P=0.0872, Figure1). When adjusted to a multivariate Cox regression analysis, *MyD88* L265P mutation remained as a significant risk factor for death (hazard ratio (HR), 2.9; 95% confidence interval (CI), 1.0–8.3, P=0.0470), together with altered mentation (HR, 4.7; 95% CI, 1.5-14.7, P=0.0072). Regarding PFS, only *MYD88* L265P mutation (P=0.0303) was significantly associated with a higher risk of progression.

Summary/Conclusions: This is the first study that provides evidence that elderly PCNSL patients with *MyD88* L265P mutation show an unfavorable prognosis. Given that *MyD88* L265P mutation was reported to be also associated with an unfavorable outcome of DLBCL, not otherwise specified and cutaneous DLBCL of leg type, dysregulated cellular program by this mutation may be an important determinant for the prognosis of DLBCL in general. These findings open new perspectives in the utility of *MyD88* L265P mutation in the clinical sequencing settings of PCNSL.

E956

SUBCUTANEOUS *VERSUS* INTRAVENOUS RITUXIMAB ADMINISTRATION IN FIRST-LINE DIFFUSE LARGE B-CELL LYMPHOMA AND FOLLICULAR LYMPHOMA: PREFMAB STUDY OF PATIENT PREFERENCE AND SATISFACTION IN 19 COUNTRIES

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Background: A subcutaneous (SC) formulation of rituximab has been developed with comparable clinical activity to rituximab intravenous (IV), but a shorter administration time (~5 min *vs* 1.5-6 hrs). The SC administration route has been shown to reduce healthcare resource burden and has potential to reduce patient treatment burden compared with rituximab IV. In PrefMab, patients preferred rituximab SC over IV when given with chemotherapy, and reported higher levels of convenience and satisfaction with the rituximab SC administration route. However, in a large, international study, regional differences in patient preference due to local variations in clinical practice or culture may not be clear in the overall data.

Aims: To evaluate country-specific patient preference and satisfaction with rituximab SC *vs* IV.

Methods: PrefMab (NCT01724021) is a randomized, open-label, crossover phase 3b study. Patients were aged 18-80 years and had: previously untreated CD20+ diffuse large B-cell lymphoma (DLBCL; international prognostic index [IPI] 1-4 or 0 with bulky disease) or follicular lymphoma (FL; FLIPI grade 1-3a); at least 1 bi-dimensionally measurable lesion ≥1.5cm at its largest dimension; and ECOG performance status ≤3. All patients gave informed consent. Patients received 8 cycles of rituximab according to 2 schedules: 1 cycle rituximab IV (375mg/m^2) and 3 cycles rituximab SC (1400mg) then 4 cycles rituximab IV; or 4 cycles rituximab IV (375mg/m^2) then 4 cycles rituximab SC (1400mg). Patients also received 6-8 cycles of chemotherapy (CHOP [cyclophosphamide, doxorubicin, vincristine, prednisone], CVP [cyclophosphamide, vincristine, prednisone], or bendamustine as per standard local practice). A Patient Preference Questionnaire (PPQ) was completed at cycles 6 and 8. A Rituximab Administration Satisfaction Questionnaire (RASQ) was completed at cycles 4 and 8; domains were scored 0 (least)-100 (best). The current analysis included countries with data for ≥10 patients.

Results: At the primary data cut-off (January 19, 2015), 743 patients had been randomized to treatment in 32 countries. The median age was 60 years, most patients had DLBCL (63% *vs* 37% FL) and baseline characteristics were balanced between arms. Nineteen countries (listed in Figure) with data available for ≥10 patients were eligible for and included in the analysis. At cycle 8, most patients preferred rituximab SC to IV, with the median score ranging from 60-100% (Figure); similar results were seen at cycle 6. Preference was not substantially impacted by underlying disease. Patient satisfaction was higher for the SC administration route. Compared with the IV route, median RASQ scores were ≥10 points higher for SC in: 0/19 countries in the physical impact domain; 8/19 countries in the psychological impact domain; 16/19 countries in the impact on activities of daily living domain; 15/19 countries in the convenience domain; and 14/19 countries in the satisfaction domain. In 18/19 countries most patients (>70%) thought the time taken to administer rituximab SC was "just right", although 53% of patients in Brazil felt it was "too short". In all countries, most patients (>50%) felt they had "more than enough time" to discuss their illness with their nurse and/or doctor, regardless of administration route, although in Germany, Brazil and Canada >50% patients felt the SC route impacted on this time.

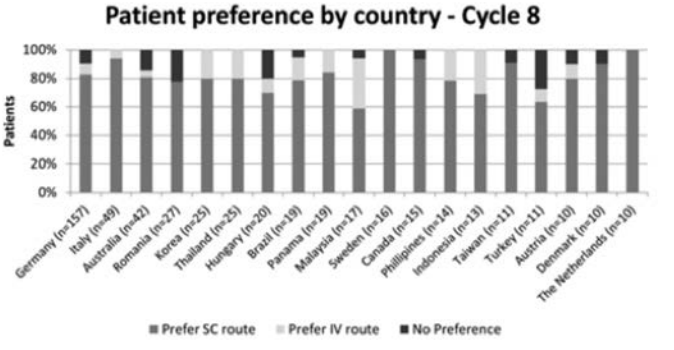

Figure 1.

Summary/Conclusions: Patient preference and satisfaction was higher with rituximab SC *vs* IV, and did not vary substantially by the country in which treatment was received.

E957

EXTRACORPOREAL PHOTOPHERESIS: SAFE AND EFFECTIVE FOR THE TREATMENT OF MYCOSIS FUNGOIDES

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Background: Primary cutaneous T-cell lymphomas (CTCL) are second most common extranodal non-Hodgkin lymphoma with an yearly incidence of 0.45 per 100,000 person. The treatment of mycosis fungoides (MF), the most frequent CTCL, is determined by disease extent, prognostic factors and patient characteristics. Extracorporeal Photopheresis (ECP) was approved by the US Food and Drug Administiration for the palliative treatment of MF since 1988.

Aims: The aim of this study is to analyze the clinical activity, toxicities, response and outcome rates of ECP; compare with combination therapies in the treatment of patients with MF.

Methods: We retrospectively included 50 MF patients who have diagnosed at our center. ECP was given empirically in cycles of 2 consecutive days in every 2 to 4 weeks at any time during their follow-up.

Table 1.

Patients (n=50)	
Median age, years (range)	55 (25-79)
Sex,n (M/F)	43/7
Stage at diagnosis, n (%) Stage 1-2 Stage 3-4	 17 (34%) 33 (66%)
Treatments prior to ECP, n (%) < 3 lines ≥ 3 lines	 25 (50%) 25 (50%)
Number of average ECP cycles, n (range)	39 (2-161)
Combination therapy with ECP, n (%)	36 (72%)

Results: The patient characteristics is shown in table. Treatments prior to ECP were; topical retinoids (bexarotene), topical corticosteroids, phototherapy (PUVA), Narrowband ultraviolet B light (NBUVB), Interferons, Metotrexate (MTX), CHOP (cyclophosphamide, daunorubicin, vincristine, prednisolone). ECP is combined with gemcitabine, PUVA, MTX, Bexaroten, IFN or Vorinostat up to patient characteristics. Overall response rate (ORR) was 42% (21/50)

with median time to response 11 months (range, 3-48 months). 15 of the responded patients (71%) were in stage III and IV at diagnosis. 10 of the responders (48%) had 3 or over treatment lines prior to ECP. 29 patient had not responded (58%) and 3 patients underwent (6%) allogeneic hematopoietic stem cell transplantation during follow-up. 8 patients (16%) had adverse events related with ECP; catheter related infection, headache, fever, shivering, nausea. Overall survival (OS) rate for 50 patients were 72 months (range, 3-211). The number of cycles in ECP responder patients were 32, and 44 for non-responders. Stage 3 and 4 patients had received average of 31 cycles compared to 55 cycles in stage 1 and 2 patients (P=0.006). The increased number of ECP is not correlated with the response (P=0.203). There were no statistically difference in OS in early stage *vs* late stage patients (77 *vs* 69 months, P=0.077). Time to response in early stage disease were 14 months and 8 months with late stage disease (P=0.267). Combined treatment with ECP statistically had improved OS (84 months *vs* 62 months, P=0.005).

Summary/Conclusions: The overall survival has improved in combined treatment with ECP patients. ECP may be a preferred treatment alternative due to low risk of adverse events. There is a conflicting data on treatment schedule and continuation. Prospective controlled clinical trials which are conducted on same ECP protocol and duration will better document the efficacy of this therapeutic modality.

E958

R-DA-EPOCH WITHOUT RADIOTHERAPY IN PRIMARY MEDIASTINAL LYMPHOMA PATIENTS RESULTS IN SIMILAR OUTCOME IN COMPARISON WITH A HISTORICAL COHORT TREATED WITH RADIOTHERAPY BASED REGIMENS

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Background: Historically, patients with Primary Mediastinal B Cell Lymphoma (PMBCL) have been treated with chemotherapy according to R-CHOP or R-VACOP-B regimen followed by radiotherapy with favorable outcome. Omission of radiotherapy in treatment of PMBCL represents a challenge due to the long-term toxicity observed in young people affected by this disease. Recently, Rituximab Dose Adjusted EPOCH (R-DA-EPOCH) regimen based on continuous drugs infusion not followed by radiotherapy demontrated to be an attractive alternative treatment for these patients.

Aims: We retropsectively compared the outcome of R-DA-EPOCH without radiotherapy with R-CHOP, R-VACOP-B or high dose sequential therapy followed by radiotherapy in newly diagnosed PMBCL patients treated at Istituto Nazionale dei Tumori in Milano from 2002.

Methods: Clinical, laboratory and imaging data of patients with PMBCL at diagnosis have been retrospectively collected and analyzed for clinical characteristics, response rate, progression free survival (PFS) and overall survival (OS).

Results: Overall, 67 newly diagnosed PMBCL patients have been treated between 2002 and 2016, 26 patients treated after September 2009 received 6 cycles of R-DA-EPOCH (R-DA-EPOCH group), whereas from 2002 to 2009, 31 patients received 6 cycles of R-CHOP (n=15), 12 weeks of R-VACOP-B (n=13) or high dose sequential therapy supplemented with rituximab (n=3), followed by radiotherapy (RT group). Ten patients treated without Rituximab were excluded from this analysis. Median age at diagnosis was 30 years in both groups (range, 18-61 and 21-55). With the exception of an higher proportion of stage III/IV patients in RT group (42% *vs* 11%) (p=0.01), other clinical characteristics were similar: extranodal disease in 11% and 35%, elevated LDH in 65% and 48% and presence of bulky disease (diameter >10 cm) in 77% and 68% of patients in R-DA-EPOCH and RT group respectively (p=ns). Eighteen patients (69%) completed R-DA-EPOCH without radiotherapy, 4 patients received radiotherapy because of residual disease at PET (Deauville Score 4), whereas 4 patients progressed. No patients relapsed after a median follow-up of 21 months (range, 3–79). In RT group, 24 patients (77%) completed the treatment with radiotherapy whereas 7 (22%) patients experienced disease progression before radiotherapy. Overall, 6 patients (19%) relapsed after a median of 7 months (range, 4-19). Eight out of 13 relapsed and refractory patients underwent autologous transplant and 5 of them are in complete remission. Median follow-up for RT patients was 73 months (range, 5-155). One patient developed a secondary malignancy 3 years after R-VACOP-B, whereas until today no secondary tumors have been observed among R-DA-EPOCH patients. 3-yrs OS and PFS for the whole population were 80% and 66%. In R-DA-EPOCH group 3-yrs PFS was 82% and in RT group was 57% (p=0.07); 3-yrs OS was 88% and 76% in R-DA-EPOCH and RT group, respectively (p=ns). 3-yrs PFS was 46%, 76% and 82% in R-CHOP, R-VACOP-B and R-DA-EPOCH (p=0.02), whereas 3-yrs OS was 62%, 92% and 88% in the three subgroups respectively (p=ns).

Summary/Conclusions: Omitting radiotherapy is feasible in patients treated with R-DA-EPOCH regimen. At the present, no significative differences in PFS and OS have been observed when R-DA-EPOCH patients are compared with a retrospective cohort treated with chemotherapy and radiotherapy. A longer follow-up of R-DA-EPOCH patients could assess significativity of the slighty advantage observed in term of PFS and will allow a proper assessment of long-term toxicity.

E959

COMPARISON OF BONE MARROW ASPIRATE FLOW CYTOMETRY AND TREPHINE IMMUNOHISTOCHEMISTRY IN STAGING OF PATIENTS WITH LYMPHOMA

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Background: Accurate staging of lymphoma is essential for guiding management and predicting outcome. Bone marrow biopsies are performed as part of routine staging for patients with lymphoma and immunohistochemistry on the trephine biopsy is considered the gold standard. Multiparameter Flow Cytometry (MFC) is increasingly sensitive at detecting small clonal B-cell populations and abnormal T-cell phenotypes on bone marrow aspirate samples but it is not clear that this adds additional clinically relevant information.

Aims: To compare results of MFC on bone marrow aspirates with immunohistochemistry on trephine biopsies for patients with T- and B-cell lymphomas to assess whether MFC is of additional clinical utility.

Methods: Results of lymphoma staging marrows received by the King's College Hospital Haematological Malignancy Diagnostic Centre between June 2014 and June 2015 were retrospectively reviewed. All aspirate samples had MFC performed using the following antibodies; CD19, CD5, CD4, CD8, CD2, CD20, CD23, CD10, CD79b, CD49d, CD38, FMC7, kappa and lambda.

Results: 117 patient samples were received. The lymphoma diagnoses included diffuse large B-cell lymphoma (DLBCL; n=63), follicular lymphoma (FL; n=29), primary central nervous system lymphoma (n=9), marginal zone lymphoma (n=6), high grade transformation of low grade lymphoma (n=4), mantle cell lymphoma (n=2), post-transplant lymphoproliferative disorder (n=2) and T-cell lymphoma (n=2). The concordance rate between trephine histology and MFC was 86.3% (n=101). Both were negative in 89 cases (76.1%) and positive in 12 cases (10.3%). Three cases (2.6%) were detected by MFC alone but not apparent on the trephine histology. The diagnoses in these cases included primary central nervous system lymphoma (3% CD5-/CD10- clonal B-cells; n=1), mantle cell lymphoma (2% clonal B-cells; n=1) and DLBCL (4% CD5-/CD10- clonal B-cells; n=1). In these three cases the clinical management was not altered by knowledge of the MFC results. Ten cases (8.5%) were histologically positive but negative by MFC. The diagnoses in these cases were FL (n=4), DLBCL (n=2), T-cell lymphoma (n=1) and FL transformed to DLBCL (n=3). There were three cases (2.6%) with suboptimal trephines. One of which was a case of DLBCL in which MFC was positive (5% CD5-/CD10- clonal B cells).

Summary/Conclusions: As reported in other series, the concordance between multiparameter flow cytometry and the trephine biopsy is high. Rarely, MFC can detect low-level bone marrow involvement not detected by immunohistochemistry. However, these results are unlikely to alter clinical management. In resource stretched health care systems, MFC on lymphoma staging marrows may not be justified.

E960

EVALUATION OF THE NEW PROGNOSTIC SCORE IN HIV RELATED NON HODGKIN LYMPHOMA. EXPERIENCE IN UNIVERSITY HOSPITAL SON ESPASES

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Background: The use of the International Prognostic Index (IPI) in aggressive lymphoma is widespread in the context of HIV infection. However recently it has been reported a new index tailored to patients infected with human immunodeficiency virus (HIV) which takes into account the characteristics of immunosuppression, viral load and evolutionary status of HIV infection: the AIDS-related lymphoma International Prognostic Index (ARL- IPI).

Aims: To evaluate this new index inside the Balearic Lymphoma Group in comparison with other prognostic scores used in aggressive lymphomas.

Methods: We identified all aggressive lymphomas diagnosed between 2002 and 2015 based on the records of the Service of Pathology contrasted with their HIV status. After that, the characteristics of their HIV infection (CD4 counts, viral load and AIDS-defining illness) with the main prognostic factors, treatment and evolution of the lymphoma were subsequently collected. Survival analysis was performed using Kaplan - Meier curves with the log -rank test.

Results: Table 1 shows main characteristics of the patients (n=50). Median age was 41 years, most with advanced disease (84% Ann Arbor (AA) stage III-IV) and poor prognosis factors: 70% a-IPI >1 and 71% TS>2. Seventy-two per-

cent of cases had a prior history of AIDS; while 46% had CD4 counts lower than 200 / microL and 54% VIH viral loads higher than 10000 copies/mL. ARL-IPI showed 26%, 42% and 26% of patients respectively in the low, intermediate and high risk subgroups. Overall response (p=0.002) and CR rates (p=0.012) were significantly different in these three risk subgroups: respectively low risk (92% and 77%), intermediate risk (74% and 65%) and high risk (31% and 23%). Median follow-up was 53 (0-167) months. PFS and OS at 4 years was 53% and 51%, respectively. Univaried survival analysis showed several factors significantly associated to worse PFS (ECOG PS>1, p=0.001; III-IV AA stage, p=0.018; a-IPI 2-3, p=0.01; R-IPI 1-2 or 3-5, p=0.029; TS>2, p=0.006; intermediate and high ARL, p=0.002 and lower CD4 count, p=0.019) and OS (ECOG PS>1, p<0.001; bulky disease, p=0.043; a-IPI 2-3, p=0.041; intermediate and high ARL-IPI, p=0.001 and lower CD4 count, p=0.001) (Image 1).

Table 1.

Sex (M/F)	45 (90%) / 5 (10%)
ECOG PS > 1	16 (32%)
B symptoms	29 (58%)
High LDH	32 (64%)
High Beta-2-microglobuline	40 (91%)
> 1 extranodal site	12 (24%)
Bulky disease	5 (10%)
Diagnosis:	
- DLBCL	31 (62%)
- Plasmablastic lymphoma	10 (20%)
- Burkitt lymphoma	7 (14%)
- Peripheral T-cell lymphoma	2 (4%)
a-IPI:	
- 0-1	15 (30%)
- 2-3	35 (70%)
R-IPI:	
- Low risk (0)	4 (8%)
- Intemediate risk (1-2)	32 (64%)
- High risk (3-5)	14 (28%)
LRS-IPI:	
- Low risk	13 (26%)
- Intermediate risk	24 (48%)
- High risk	13 (26%)
CD4 count:	
- <50	5 (10%)
- 50-199	18 (36%)
- 200-499	12 (24%)
- >500	15 (30%)

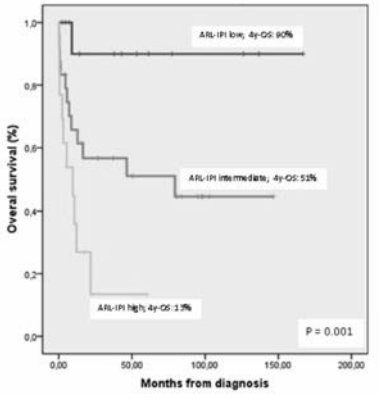

Figure 1.

Summary/Conclusions: In our study ARL-IPI showed a very good definition of prognostic groups in terms of response and survival. Interestingly, ARL-IPI identifies a high risk subgroup with a very poor prognosis. Our results validate the usefulness of this new prognostic index in the HIV population with aggressive lymphoma.

E961

SERUM IL-10 LEVEL PREDICTS POOR PROGNOSIS IN PATIENTS WITH ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA: RESULTS OF A SINGLE CENTER COHORT STUDY

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is derived from T-follicular helper cell, and tumor cells are often outnumbered by admixed reactive inflammatory cells. Accordingly, the secretion of various cytokines and their interaction with tumor microenvironment has been more studied compared to other subtypes of PTCL. Interleukin-10 (IL-10) is one of T-helper cell-associated cytokine and promotes M2 macrophage inhibiting the anti-tumor action of non-neoplastic T-cells. Thus, serum IL-10 level might be associated with treatment outcome of AITL patients.

Aims: The primary objective is to evaluate whether serum IL-10 level at diagnosis could predict survival outcome of AITL patients. The secondary objectives are the comparison of serum cytokine profiles according to subtypes of PTCL and their interaction with clinical and laboratory features.

Methods: Patients were from two prospective cohort studies of our institution: the first study (NCT#00822731) and the second study (NCT#01877109). Patients were diagnosed with AITL, peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), ALK-negative and positive anaplastic large cell lymphoma (ALCL) between September 2008 and December 2014 according to the pathology criteria of the World Health Organization. Serum samples at diagnosis after we obtained written informed consent were collected and stored at -80°C until analysis. Procarta cytokine profiling kit (Panomics, CA, USA) with Bio-Plex Cytokine Assay System (Bio-Rad Laboratories, Herucules, CA, USA) was used to measure different cytokines, including IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, and interferon-γ. Survival status and disease status were updated in February, 2016.

Results: A total of 97 patients were analyzed: AITL (n=37), PTCL-NOS (n=40), ALK-negative ALCL (n=11), and ALK-positive ALCL (n=9). All patients were treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) or CHOP-like regimens after diagnosis. The response to the first-line treatment was as follows: complete response (n=64), partial response (n=11), and progressive disease (n=22). With the median follow-up of 42 months, 58 patients experienced relapse, progression, or any kinds of death, and 44 patients died. The 3-year overall survival of AITL patients (66%) was superior to that of PTCL-NOS (43%) and inferior to ALK-positive ALCL (75%) in consistent with previous reports. Among measured cytokines, serum levels of IL-10 and IL-12 were significantly higher in AITL patients than other subtypes (P<0.05), and other cytokines did not show any significant association with subtypes. The cutoff for serum IL-10 and IL-12 was determined by the ROC curve, and high serum IL-10 (>1.845 pg/mL) was significantly associated with poor overall survival (P=0.012, figure). All patients with high serum IL-10 showed disease progression after CHOP chemotherapy. This association was only found in AITL patients not other subtypes. In addition, other cytokines including IL-4,5, and 12 did not show a significant association with survival outcomes in AITL patients as well as other subtypes.

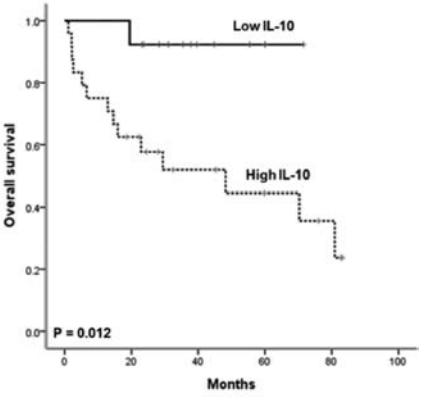

Figure 1.

Summary/Conclusions: Serum IL-10 level at diagnosis might be a useful biomarker for predicting survival outcome in patients with AITL. Our findings should be confirmed by a future study with larger population.

E962

REDUCED RCHOP FOR ELDERLY PATIENTS WITH DLBCL

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Background: RCHOP regimen is considered the standard of care for patients with diffuse large B-cell lymphoma (DLBCL). Elderly patients often have comorbidities, worse performance status, less hematologic reserve and are more prone to changes in pharmacokinetics, which lead to higher rates of toxicity. Therefore it was suggested that elderly patients should be treated with reduced intensity RCHOP regimen. Very few studies examined regimens of reduced intensity RCHOP.

Aims: To evaluate the effect of dose reduction of RCHOP on survival, disease control and toxicity in patients 70 years or older with DLBCL.

Methods: We performed a retrospective cohort study including patients with DLBCL treated with RCHOP at the Rabin Medical Center between the years 2004-2014. We excluded patients with transformation or CNS involvement. We collected age, sex, performance status (PS), B symptoms, bone marrow involvement, number of extranodal involvement sites, Ann Arbor stage, international prognostic index (IPI), bulky disease, Charlson comorbidity index (Charlson), hemoglobin (Hb), LDH, neutrophil, lymphocyte, monocyte, and

platelet count, creatinine, albumin, CRP, cardiac ejection function, RCHOP dose (for each drug separately) and treatment date, response, and survival. We defined full, standard dose (SD) as 90% and above of standard adriamycin dose, and reduced dose (RD) as less than 90% in the first cycle. OS was compared using Kaplan-Meier survival analysis. Variables potentially associated with mortality were entered into a Cox regression multivariate analysis.
Results: 140 patients were eligible. Median dose reduction was by 88%. Median age was 80 years in the RD group, and 76 years in SD group (p<0.001). Patients in the RD group were older, had a worse PS, and a higher IPI and Ki67, and lower albumin, Hb, and lymphocyte count. There were no differences between the groups regarding sex, B symptoms, bone marrow involvement, number of extranodal involvement sites, stage, bulky disease, Charlson, LDH, neutrophil, monocyte, and platelet count, creatinine, and CRP. 3% of patients had cardiac ejection function<45%. Patients treated with RD RCHOP had statistically significant lower risk of achieving complete response (CR) (HR for in a univariate model of OS RD group (HR 2.53, 95% CI 1.57-4.07, p=0.0001), older age, advanced stage, more than 1 extranodal site involvement, performance status more than 1, higher IPI, lower albumin and Hb levels had negative prognostic effect on survival. In multivariate model 1 including age, sex, Charlson, and Hb the negative effect of RD treatment remained statistically significant compared to SD (HR 1.93, 95% CI 1.18-3.15, p=0.01) as well as age and Hb. In model 2 including treatment group, age, sex, PS, IPI, LDH, and albumin there was a trend towards worse survival with RD RCHOP compared to SD (HR 1.64, 95% CI 0.96-2.82, p=0.069). Age, sex, IPI, and albumin remained prognostic in this model. There was no statistically difference in the rate of any infection between the groups (p=0.623). Patients treated with RD RCHOP were hospitalized more than patients treated with SD RCHOP (median 4 *vs* 1 day, respectively).
Summary/Conclusions: Based on retrospective data, dose reduction of adriamycin in the first cycle of R-CHOP may reduce the chance to achieve CR and impair OS. This should be further evaluated in randomized trials. We will further explore the effect of dose intensity and density in all cycles of RCHOP on PFS and OS.

E963

A PREDICTIVE SCORE FOR CNS DISSEMINATION AND ITS EFFECT ON CNS PROPHYLAXIS IN A MONO-INSTITUTIONAL SERIES OF 242 PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED IN THE RITUXIMAB ERA

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Background: CNS dissemination is a lethal event in DLBCL. Early detection and effective CNS prophylaxis may reduce related mortality. However, risk predictors reported in the rituximab era exhibits a low diagnostic sensitivity and the most effective prophylaxis remains to be defined.
Aims: We analysed these two unmet clinical needs in a retrospective series of 242 pts with DLBCL treated in the rituximab era.
Methods: Consecutive HIV-neg adults with DLBCL without CNS involvement treated with R-CHOP or similar were considered. CNS, mediastinal and leg-type DLBCL, high-grade transformed lymphomas and pts registered in prospective trials were excluded. Following institutional guidelines, no DLBCL pt diagnosed before 2007 received CNS prophylaxis; after 2007, CNS prophylaxis, consisting of 3-4 cycles of methotrexate 3 g/m^2±intrathecal liposomal cytarabine (IT) was indicated in pts with high CNS recurrence risk. IT was delivered one dose per R-CHOP course, and methotrexate was delivered after R-CHOP treatment. CNS dissemination risk was defined by involvement of the testis, kidney/adrenal, spine, skull, paranasal sinuses, orbit, and/or breast or by the simultaneous presence of advanced stage and high serum LDH. In the present study, CNS dissemination risk was defined as low in pts without involvement of high-risk extranodal organs and with an International Prognostic Index (IPI) of 0-3; intermediate in pts without involvement of high-risk extranodal organs and with an IPI of 4-5; and high in pts with involvement of above-mentioned high-risk extranodal organs and with any IPI.
Results: 242 pts were analysed (median age 66, range 18-89). According to the proposed model, CNS dissemination risk was low in 148 (61%) pts, intermediate in 29 (12%) and high in 65 (27%). Pts with low and intermediate risk were managed without prophylaxis, whereas this strategy was indicated in 45 high-risk pts: 35 pts received intravenous±IT chemotherapy, 10 pts receive only IT chemotherapy due to MTHFR polymorphisms, comorbidity or old age. CNS prophylaxis was well tolerated; unexpected toxicity and interruptions due to toxicity were not recorded. At a median follow-up of 51 months (12-171), 11 (4.5%) pts experienced CNS relapse: in the brain parenchyma in 6 cases, in the meninges in the others. CNS relapse rate was<1% (1/148) in low-risk pts, 10% (3/29) in intermediate-risk, and 11% (7/65) in high-risk pts. Eight of these pts died of CNS progressive lymphoma after 7-37 months (median 12). In the high-risk subgroup, CNS relapse rate was 25% (5/20) in pts who did not receive CNS prophylaxis, 20% (2/10) in pts treated IT chemotherapy alone and 0% (0/35) in pts who received intravenous±IT chemotherapy (p=0.004). Overall, 18 high-risk pts experienced relapse, and CNS was the most commonly involved site (7/18). Moreover, the addition of intravenous CNS prophylaxis was associated with a significantly improved PFS (3-yr: 80% *vs* 42%; p=0.001) and OS (3-yr: 86% *vs* 43%; p=0.00007).
Summary/Conclusions: With all the limitations of a retrospective series, this study suggest that pts with DLBCL and involvement of certain extranodal organs and/or high IPI should receive CNS prophylaxis with intravenous high-dose methotrexate and IT liposomal cytarabine. This strategy shows a relevant effect on survival of high-risk pts as CNS is a common relapse site, associated with a high mortality.

E964

A PHASE I STUDY IN T CELL LYMPHOMA PATIENTS TREATED WITH ANTI-CD70 SIMPLE ANTIBODY™ ARGX-110

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Background: CD70 is a cell surface marker transiently expressed on activated B, T cells and dendritic cells. The interaction via its receptor CD27 results in survival, proliferation and lymphocyte differentiation. In a large number of solid tumours types, CD70 is overexpressed but without receptor co-expression. As overexpression of CD70 is paralleled by high expression of its receptor CD27 in TCL, the CD70/CD27 axis appears critical for proliferation and survival of malignant cells in T-cell lymphoma.
Aims: ARGX-110 is a novel glycoengineered monoclonal antibody targeting CD70 and blocking CD27 signaling, leading to a direct mode of action lysing CD70$^+$ tumor cells (via CDC, ADCP and enhanced ADCC) and indirect anti-tumor restoring immune surveillance.
Methods: (NCT01813539) A phase I trial was initiated with ARGX-110, dose escalated to investigate safety, clinical pharmacology and determine the RP2D, in refractory or relapsed solid tumors and hematological malignancies on patients with CD70+tumors (defined by IHC >10%+). ARGX-110 was given to 65 patients at doses from 0.1 to 10 mg/kg intravenously every 3W.
Results: *Preclinical data:* Immunohistochemistry on TCL patient samples confirmed overexpression of CD70 (>10% CD70+ tumor cells) in 40/73 PTCL (55%) and 15/21 CTCL (71%) samples. Serum soluble CD27 levels were elevated in TCL patients compared to healthy subjects (670±1096 *vs* 192±44 IU/ml, respectively, p= 0.0062). *Phase 1 heme results:* Nine patients received ARGX-110 for a TCL. Histological TCL types were 3 Cutaneous TCL (CTCL) and 6 Peripheral TCL (PTCL). ARGX-110 was well tolerated with no grade >3 adverse events related to the study drug. A clinical and/or biological response was observed in 4/9 of T-cell Lymphoma patients; CTCL (n=3) and PTCL-AITL (n=1) patients. Response consisted in 2 patients with CTCL Sezary syndrome (treated at 0.1 and 10 mg/kg, respectively) in a >90% reduction in the circulating malignant clone, with a clinical partial response in the skin in one patient. The other patient with CTCL T_{FH} lymphoma (treated at 5 mg/kg) achieved a PR. One patient with PTCL with Angioimmunoblastic T-cell Lymphoma (AITL) reached a PR (5 mg/kg dose) and had a resolution of lymphoma associated auto-immune hemolytic anemia.
Summary/Conclusions: In the TCL patients treated with ARGX-110, a signal of clinical and/or biological anti-tumor activity was observed. These preliminary results support further investigation of ARGX-110 in TCL.

E965

DOUBLE PROTEIN LYMPHOMA PATIENTS OF THE NON-GCB SUBTYPE HAVE AN INFERIOR PROGNOSIS WHEN TREATED WITH CHEMOIMMUNOTHERAPY

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Background: Diffuse large B-cell lymphoma (DLBCL) can be stratified by cell of origin (COO) derivation into the prognostically favorable germinal center B-cell (GCB)–like and unfavorable activated B-cell (ABC) or non-GCB-like subtype based on gene expression signatures or immunohistochemical determination. Double-hit lymphoma (DHL) were identified as a subgroup of aggressive lymphomas with both MYC and BCL2 gene rearrangements, characterised by a rapidly progressing clinical course and short survival. Recently, DLBCL patients with an immunohistochemical coexpression of MYC and BCL2 also demonstrated inferior prognosis suggesting that concurrent expression of MYC/BCL2, termed double-protein-expression lymphoma (DPL), rather than cell-of-origin classification, is a better predictor of prognosis in patients treated with R-CHOP.
Aims: We determined the frequency of DPL in a large single center cohort of DLBCL uniformely treated with R-CHOP and evaluated patients' outcome in contrast to "standard" DLBCL not exhibiting concurrent expression of MYC/BCL2.

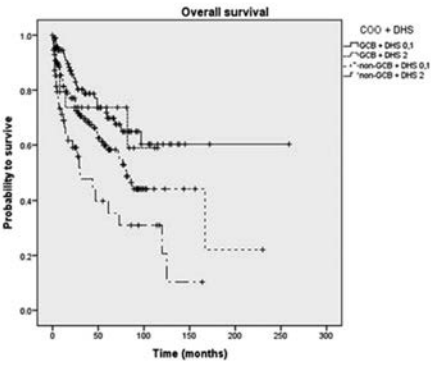

Figure 1.

Methods: We evaluated retrospectively the appearance of DPL in 440 patients with DLBCL, consecutively diagnosed and treated between 2004 and 2014 at our institution. To determine the double-hit score, immunohistochemistry for c-MYC and BCL2 was performed on diagnostic samples. Furthermore, the Choi algorithm was used to subclassify samples by cell-of-origin. On the basis of immunohistochemical MYC and BCL2 expression, a double-hit score (DHS) was assigned to all patients with DLBCL. The DHS-2 group was defined by high expression of both MYC and BCL2 protein.
Results: A GCB subtype was found in 162 patients, whereas 223 samples were classified as non-GCB subtype. For 55 patients, no classification according to cell-of-origin could be performed. Of 440 DLBCL, 289 patients were evaluable, 58 of them showed a double-hit score of 0, 168 patients had a DHS of 1, and 63 a DHS of 2, respectively. Twenty-one of 76 patients with a DHS of 2 could be classified as GCB, whereas 42 patients exhibited a non-GCB subtype. Using Kaplan-Meier curves and log-rank tests, we could demonstrate a significant inferior outcome for DLBCL patients with the non-GCB subtype as compared to the GCB subtype (, p<0.001). Further, DPL patients had also a shorter overall survival (OS) (p=0.05). Combining the cell-of-origin classification with the DHS, we were able to further discriminate a subgroup of patients with an even worse clinical outcome to standard treatment (median OS 81 *vs* 44 months, p<0.001) (Figure 1).
Summary/Conclusions: In this large single center cohort, we could demonstrate a significant negative effect on OS for DLBCL patients coexpressing MYC and BCL2. We could further define a particular subgroup of patients within the non-GCB cohort demonstrating double hit positivity (DHS of 2) that is associated with a particular short survival. These non-GCB-, DHS positive DLBCL patients are candidates for novel therapeutic strategies.

E966

A CLINICO-PATHOLOGIC AND IMMUNOHISTOCHEMICAL ANALYSIS OF ORAL AND EXTRA-ORAL PLASMABLASTIC LYMPHOMA IN SOUTH AFRICA

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Background: Plasmablastic lymphomas (PBLs) are a group of aggressive non-Hodgkin lymphomas originally described to be exclusive to the oral cavity in patients with immunodeficiency. This group of uncommon lymphomas, which shows morphologic, phenotypic and molecular features of terminally differentiated B-cells is increasingly being described in many extra-oral sites as well, in both HIV and non-HIV infected patients. Data relating to age, gender, HIV status and immunophenotypic profile have been published in case reports, case series and systemic reviews. To date there are no data on extra-oral PBLs in South Africa where HIV is higly endemic. We present a series of 101 oral (55) and extra-oral (45) PBLs which forms the largest global series described to date in a region of Africa where HIV is endemic.
Aims: To characterize the clinical, morphological and immunological features of oral and extra-oral plasmablastic lymphomas in a defined South African population.
Methods: This is a retrospective study on archival cases of extra-oral and oral PBLs diagnosed over the period 2006 to 2015 in the Department of Anatomical Pathology, University of the Witwatersrand, Johannesburg, South Africa. Clinical parameters analyzed included patients age, gender, HIV status and site of tumor presentation. Each case was reviewed histologically to confirm the diagnosis and assessed immunohistochemically with CD45 (LCA), CD20, CD79a, PAX5, CD138, MUM1, BLIMP1, VS38c, Ki-67, BCL6, CD10, and HHV8 using standard immunohistochemistry protocols. The presence of EBV-encoded early nuclear RNAs (EBER1 and EBER2) was assessed by chromogenic *in-situ* hybridization. Ethical clearance was obtained from the University of the Witwatersrand human research ethics committee.
Results: Forty-six patients were diagnosed with extra-oral PBLs and 55 patients with oral PBLs. These included all adult patients, except for one case of a nine-year old child. The age ranged from 9 to 59 years (mean:35.5; median:35) for extra-oral cases and 22 to 78 years for oral PBLs (mean:41.1; median:41). Of the 18 and 34 patients with extra-oral and oral PBLS with known HIV status, 17 (95%) and 33 (97%) patients respectively were infected with HIV. None of the patients had systemic disease at presentation. The overall male:female ratio was 1.9:1 (67 males and 34 females), with 66% of PBLs occurring in males. The male:female ratio for extra-oral and oral PBLs was 1.4:1 (27 males (59%) and 19 females) and 2.7:1 (40 (73%) males and 15 females) respectively. The anus was the favoured extra-oral site of presentation (13 of 46 cases, 28%), followed by soft tissue (11 of 46 cases, 24%). The favoured oral site for PBL was the maxilla (16 of 55 cases, 29%) followed by the palate (11 of 55 cases; 20%). The histomorphology in both oral and extra-oral PBLs was similar showing both plasmablastic and plasmacytic features. The immunohistochemical profile of all PBLs in the study recapitulated that found for both oral and extra-oral PBL in the literature, except for CD45 (leucocyte common antigen), which signalled positively in a higher percentage of cases. Overall 73 of 80 cases (91%) were positive for CD45; 36 of 42 extra-oral cases (85.7%) and 37 of 38 oral cases (97%). The positive membrane signal for CD45 was of variable intensity, between 5 and 100% of tumor cells. EBV was positive by *in-situ* hybridization in 71 of 74 cases (96%), *i.e.* in 37 of 40 (93%) extra-oral cases and all 34 oral PBLs (100%).
Summary/Conclusions: Extra-oral PBL was identical to its oral counterpart in gender and age distribution, HIV status, morphological appearances, immunophenotypic profile and association with EBV. The high association with EBV as assessed by *in-situ* hybridization studies mirrors that of extra-oral and oral PBLs reported in the literature. A peculiarity observed within this case cohort was the high level of expression of CD45. This has been reported to be of low or near absent in most cases of PBL, as defined by the WHO 2008 and recorded in the literature. Although the HIV status was only known for approximately half of these cases, HIV associated PBL manifested as a single disease in both oral and extra-oral forms, and should be regarded as the same tumor.

E967

A NGS IGK ASSAY FOR THE DETECTION OF CLONALITY AND THE MONITORING OF MINIMAL RESIDUAL DISEASE

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Background: During B-cell development, functional immunoglobulin genes are assembled from individual V, D, and J gene segments to generate V-D-J combinations of length and sequence that are unique for each cell. Rearrangements within *IGH* occur first, followed by *IGK*. In addition to V-D-J rearrangement, the *IGK* Kde locus can rearrange to Vk gene and to an isolated RSS in the Jk-Ck intron (INTR). Leukemia and lymphomas originating from the malignant transformation of individual lymphoid cells generally share one or more of these cell specific or “clonal” gene rearrangements. Assays that identify clonal lymphocyte populations in clinical specimens are used on a routine basis to assist in the diagnosis of lymphoproliferative disease. The clinical relevance of minimal residual disease (MRD) detection in lymphoproliferative disease has been demonstrated by many studies. However, allele-specific primers are required for the qPCR-based MRD monitoring. The emergence of cost-effective, next-generation sequencing (NGS) platforms and development with associated bioinformatics tools have resulted in powerful new approaches for clonality detection and MRD monitoring without the requirement of allele-specific designs.
Aims: To establish an *IGK* NGS assay that, combined with an *IGH* NGS assay, will increase the chance of identifying clonal populations and provide easy tracking of identified clonal population for MRD in lymphoid malignancies.
Methods: Genomic DNA from cell line, peripheral blood, bone marrow aspirates, and FFPE were tested for *IGK* rearrangements using the LymphoTrack® *IGK* MiSeq® Assay (Invivoscribe Inc., San Diego, USA). The *IGK* libraries were run individually or in combination with *IGH* and or *TRG* libraries. The sequencing data was analyzed using LymphoTrack® bioinformatics software, which performs the separation of *IGK*, *IGH*, and *TRG* data as necessary, and generates frequency distributions and identifies the rearranged DNA sequences for each target. Total of 198 peripheral blood samples were also tested by the capillary electrophoresis (CE) based IdentiClone® *IGK* assay.
Results: Data generated with the *IGK* NGS assay and bioinformatics software identified clonality and corresponding DNA sequences of *IGK* Vk-Jk, Vk-Kde, and INTR-Kde gene rearrangements. Excellent linearity of the *IGK* NGS assay was obtained from contrived cell line DNA in the range of 2.5-20% when running individually (R^2=0.9897) or in combination with *IGH* and *TRG* assays (R^2=0.9905). The concordance between the 198 samples tested by *IGK* NGS and CE assays was 77%. There were 43 samples indentified to be clonal out of 198 samples tested by both *IGK* and *IGH* NGS assays. Among those samples, 31 and 25 samples were clonal for *IGK* and *IGH*, respectively, and 18 samples were clonal for both *IGK* and *IGH*. It was demonstrated that the NGS

assay allows for easy tracking of clonal populations at the sensitivity of 10^{-4} or at even lower limits of dectection, provided sufficient amount of DNA is tested.
Summary/Conclusions: A comprehensive assay has been developed for the MiSeq® platform that identifies clonal *IGK* rearrangements and the associated specific DNA sequences. The *IGK* assay, in combination with the *IGH* assay, increased the detection of clonality by 72% comparing to *IGH* alone. The assay can be used both to detect clonality and monitor the identified clonal populations in MRD.

E968

DE NOVO TRANSFORMED FOLICULAR LYMPHOMA. OUTCOMES AND ROLE OF AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: The role of histologic transformation (HT) on survival of follicular lymphoma (FL) in the rituximab era has been recently analyzed in several studies. However, there is no information about the outcome of patients with simultaneous presence of FL and diffuse large B cell lymphoma (DLBCL) in the same initial diagnostic biopsy (*de novo* transformed FL; dn-tFL).
Aims: We examined clinical characteristics, survival following transformation (SFT) and impact of intensive regimens (including ASCT) in a group of patients diagnosed of dn-tFL, and compared them with a group of FL patients who developed HT (tFL).
Methods: We recruited 1809 patient candidates from a retrospective series in 18 centres from the Spanish Group of Lymphoma and Autologous Stem Cell Transplantation (GELTAMO). Of them, 75 patients were diagnosed of dn-tFL, and the other 1734 of FL grade 1-3a, from which 106 developed HT. Treatment at HT consisted in rituximab containing regimens.
Results: Clinical variables were not significantly different between tFL and dn-tFL. 5-year SFT was significantly higher in dn-tFL as compared to tFL patients (75% *vs.* 25%, p<0.0001; Figure 1A). Variables influencing 5-year SFT in dn-tFL were complete response (CR) to front line therapy (90% *vs.* 41%, p<0.001; Figure 1B), and low-risk FLIPI score (100% *vs* 58%, p=0.018). In the multivariate analysis, only CR to front line therapy (HR 6.5, 95% CI: 2.0-20.7) had an independent influence on 5-year SFT. In the conventional tFL comparison cohort, achievement of CR (47% *vs* 5%, p<0.001; Figure 1B), but not low-risk FLIPI (40% *vs.* 27%, p=0.2) influenced 5-year SFT. A total of 19 dn-tFL patients did not achieve CR to first-line therapy, and received salvage therapy, which was followed by ASCT as consolidation therapy only in 4 cases. 5-year SFT in the ASCT group was higher (67% *vs.* 42%, p=0.07). However, these groups were not comparable since patients receiving ASCT were significantly younger than those not receiving ASCT (median 54 years *vs.* 71 years, p=0.031).

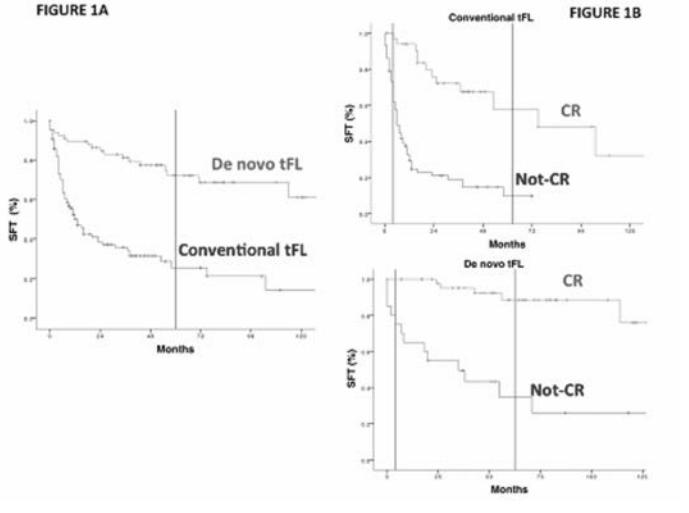

Figure 1.

Summary/Conclusions: De novo tFL patients have better SFT than patients that develop HT after FL diagnosis. Although differences are evident in terms of general SFT, both groups are characterized by a decisive influence of therapy response in SFT. Interestingly, although FLIPI score is not the standard index to evaluate aggressive lymphoma, it accurately stratified composite FL+DLBCL and not the conventional tFL. In our series, ASCT shows a potential benefit as a rescue therapy in those dn-tFL patients not responding to first-line treatment.

E969

LOW EXPRESSION OF MIR-155 IN VINCRISTINE RESISTANT DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Vincristine is a key chemotherapeutic drug of first line therapy R-CHOP in Diffuse Large B-cell Lymphoma (DLBCL). The clinical course for DLBCL patients has great variability and an unfavorable outcome is observed in more than 40% of cases due to intrinsic or acquired treatment resistance. Accordingly, identification of molecular resistance mechanisms and early prediction of drug specific resistance is an urgent clinical need.
Aims: This study investigates the potential of miRNAs as predictive biomarkers of vincristine resistance and the involvement of miRNAs in the resistance mechanisms.
Methods: Systematic vincristine dose-response screen was performed on 12 DLBCL cell lines to identify 50% growth inhibition dose (GI_{50}) for the individual cell lines. Ranking of GI_{50} allowed trichotomisation into groups of drug specific sensitive (n=3), intermediate (n=6), or resistant (n=3) cell lines. Based on global miRNA expression profiles of each cell line in untreated condition, class comparison was performed and 13 differentially expressed miRNAs were identified between sensitive and resistant cell lines (fold-change>|2|, p-value<0.05). Especially, miR-155 showed significant association between low expression and increased vincristine resistance having 33.5 fold decreased expression in resistant cell lines (p=0.0005). Based on gene expression profiles, 66 DLBCL patients biopsied at time of diagnosis were classified into the prognostic molecular subclasses ABC/GCB and B-cell associated gene signatures (BAGS), reflecting naturally occurring B-cell subsets of naïve, centroblast, centrocyte, memory, and plasmablast cells as cell of origin. Additionally, resistance gene signature (REGS) for vincristine was assigned to the clinical samples giving each case a likelihood of predicted response to vincristine. Expression levels of miR-155 were determined in the DLBCL samples by RT-qPCR.
Results: A significantly higher expression of miR-155 was observed in the prognostic adverse ABC subclass compared to GCB (n=66, p-value=0.0373) indicating miR-155 as an unfavorable marker. However, using BAGS classification clinical samples assigned into the prognostic favorable centrocyte subclass had higher miR-155 expression compared to the prognostic adverse centroblast subclass (n=59, p-value=0.003). Observations further supported when BAGS classification was stratified into ABC and GCB subclasses. Within the GCB subclass centrocyte DLBCL cases had significantly higher miR-155 expression compared to centroblast cases (n=27, p=0.0223). Within the ABC subclass no difference between the BAGS subclasses was detectable. Thus, using the prognostic BAGS classification system within the GCB subclass low miR-155 expression is an unfavorable marker. REGS stratification of clinical samples did not show significant miR-155 expression differences between REGS classes. However, assigning REGS to ABC and GCB subclasses, separately, a significant difference between low miR-155 expression and vincristine resistance was observed within the GCB subclass (n=27, p-value=0.051) but not within the ABC subclass (n=26, p-value=0.99). This supports the *in vitro* observations of low miR-155 expression and vincristine resistance within the GCB subclass.
Summary/Conclusions: In conclusion, we observed different miR-155 expression patterns between ABC and GCB subclasses. Moreover, a significant association between low miR-155 expression and vincristine resistance was demonstrated within GCB assigned cases. To unravel the biological mechanism determining miR-155 impact on vincristine resistance functional studies including expression manipulation in DLBCL cells with lentiviral vector system are ongoing.

E970

RITUXIMAB AND HIGH DOSE CYTARABINE WITH AUTOLOGOUS TRANSPLANTATION FOR MANTLE CELL LYMPHOMA

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Background: Mantle cell lymphoma (MCL) is an aggressive and incurable form of non-Hodgkin lymphoma (NHL), typically presenting at a late stage, often with extra nodal involvement, and with a preponderance for older males. Front-line therapy incorporating rituximab and high-dose cytarabine (R-HDAC), often in combination with other drugs or alternating with another regimen, followed by autograft in first response is standard of care for younger, fitter patients. Reported overall response rates (ORR) and progression free survival (PFS) are typically 70-90% and 50-60% respectively.

Aims: To explore the tolerability and efficacy of rituximab and single-agent R-HDAC prior to autologous stem-cell transplant in patients with untreated MCL.
Methods: We studied 45 unselected, transplant eligible patients with MCL treated upfront with R-HDAC alone across four UK centres between February 2010 and August 2015. The median age was 60, the majority were male (84%), and the majority had advanced disease (91% Stage III/IV). None had central nervous system (CNS) involvement at presentation. All patients received at least one cycle of R-HDAC. The median number of cycles of R-HDAC given was 5, and 89% received at least three cycles. Only two patients stopped R-HDAC therapy early due to toxicity; one due to PJP pneumonia after the first cycle who was stepped down to Bendamustine based treatment, and one due to severe sepsis after the second cycle who stopped therapy at this point having already achieved a partial response. One further patient completed five cycles of R-HDAC but was felt unfit for autograft at that point.
Results: Nine patients (20%) progressed on R-HDAC, of whom only three (33%) achieved a remission with second line therapy, two of whom subsequently died of progressive disease. In total 31 patients (69%) have now undergone transplantation; 28 (62%) received an autograft (the majority BEAM conditioned), two had an allogeneic transplant and one a syngeneic transplant. Two patients are currently completing R-HDAC treatment and will undergo BEAM autograft thereafter. Transplant related mortality was within expected limits, with one patient dying as a result of sepsis due to BEAM autograft toxicity. Median duration of follow up of the entire cohort was 38 months. Overall response rate for R-HDAC with or without transplantation was 35/45 (78%), with most patients achieving a complete remission (CR) (n=30, 67%). Median OS and PFS were not reached. The OS at 4yrs is 78% (18/23); and PFS at 4yrs is 61% (14/23). Eight patients relapsed after achieving CR1 with HDAC +/- transplantation, five (63%) have subsequently achieved a second remission. The most commonly employed strategies amongst patients who required subsequent treatment were Ibrutinib (47%), Bortezomib (35%), CHOP (29%) and Bendamustine (17%) based therapies. CNS involvement was seen at relapse/progression in four cases.

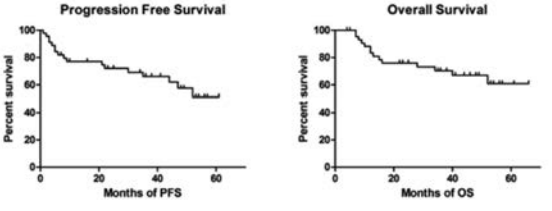

Figure 1.

Summary/Conclusions: This study confirms that induction with R-HDAC, consolidated with stem cell transplantation where appropriate, is a safe and effective upfront treatment strategy in MCL, and compares favourably with other induction regimens in terms of toxicity and duration of response. By avoiding the use of other agents as part of upfront treatment excess toxicity is avoided. Using R-HDAC upfront also leaves a greater range of therapies available for use at subsequent relapse, with good remission rates after second line therapy shown in this study. However, direct comparison with other upfront treatment strategies has not been made, and a prospective randomised controlled trial is needed.

E971

TREATMENT OF INITIAL CENTRAL NERVOUS SYSTEM INVOLVEMENT IN SYSTEMIC AGGRESSIVE LYMPHOMA WITH HIGH DOSE METHOTREXATE AND R-CHOP

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Background: Central nervous system (CNS) involvement in systemic aggressive B-cell non Hodgkin lymphomas (B-NHL) at initial diagnosis is uncommon. Two recent studies suggest a beneficial effect of intensive chemotherapy followed by autologous stem cell transplantation (ASCT) for these patients, but since ASCT was only offered to patients in complete remission optimal treatment remains unclear.
Aims: We investigated the outcome of patients with concomitant systemic and CNS B-NHL (sCNS B-NHL) at diagnosis treated with high dose methotrexate (HD-MTX) and R-CHOP, but no ASCT.
Methods: We performed a retrospective cohort study of patients with sCNS B-NHL at diagnosis identified from the electronical databases of 2 referral centers in the Netherlands. Patients had histological proven aggressive B-NHL and stage 4 disease according to Ann Arbor classification with concomitant paryncymal CNS involvement. Patients were treated according to one of two regimens: either 6 alternating cycles of HD-MTX and R-CHOP, or four cycles of HD-MTX followed by 6 cycles of R-CHOP. In the latter MTX was part of the MBVP regimen which also includes teniposide (VP16) and carmustine (BCNU). Primary endpoints were progression free survival (PFS) and overall survival (OS).

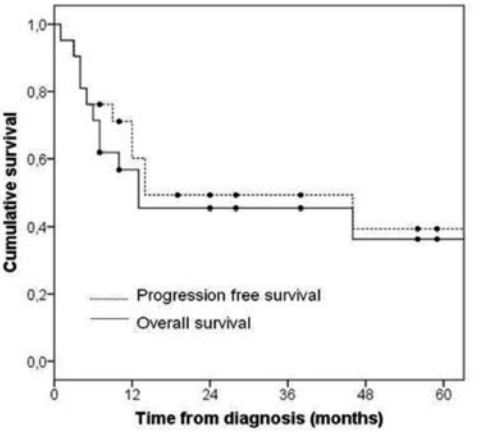

Figure 1. Overall and progression free survival.

Results: From 2001-2015 21 patients with sCNS B-NHL were identified. Median follow-up was 28 months. Characteristics at diagnosis: median age 54 years (range 19-71); WHO performance ≥2: 33%; IPI-score 0-1: 9%, 2-3: 66%, 4-5: 25%. Histological B-NHL types: diffuse large B-cell lymphoma: 66%; iatrogenic immunodeficiency B-NHL: 14%; transformed marginal zone lymphoma: 10%; intravascular B-NHL: 10%. Systemic organ involvement: testicular: 44% of patients at risk; breast: 17% of patients at risk; bone: 38%; kidney: 19%. Concomitant spinal fluid localization occured in 43% of patients. Fifteen out of 21 patients completed therapy as planned. The median number of MTX cycles at a dose of 3 g/m^2 was 4 (range 1-8). Sixty-two percent of patients also received intrathecal MTX. The median number of R-CHOP cycles was 6 (range 0-8). Whole brain radiotherapy was given to 33% of patients in partial remission. Besides the additional VP16 and BCNU in patients treated with MBVP, there was no difference between the two regimens. 3-year PFS and OS for the entire cohort were 45% (95%CI 34-56%) and 49% (95%CI 38-60%) respectively. The treatment related mortality (TRM) was 24% (5/21). No significant difference in OS was found between the two regimens (HR OS 1.25, 95%CI 0.38-4.2 p 0.72). The 3 patients with IPI-score 0-1 were alive at the last control, whereas all 4 patients with IPI-score 4-5 died within 2 years after diagnosis. There were 6 relapses (28%): 2 systemic, 2 in CNS, and 2 both systemic and in CNS.
Summary/Conclusions: In this second largest cohort described to date, the outcome of patients with sCNS B-NHL treated with HD-MTX and R-CHOP was comparable to that of more intensive regimens. Prospective studies are needed to define the optimal treatment for these patients.

E972

FEASIBILITY AND EFFICACY OF DHAOX REGIMEN AS SALVAGE AND MOBILIZING THERAPY IN RELAPSED /REFRACTORY LYMPHOMAS

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Background: As already known, cisplatinum containing regimens for the management of relapsed-refractory lymphomas are complicated by different adverse events, mainly renal impairment. Since 2004 we used oxaliplatinum instead of cisplatinum, in association with cytarabine and dexamethasone (DHAOX).
Aims: The aim of the present study was to perform a retrospective analysis on feasibility and efficacy of this salvage regimen in patients with R/R lymphomas.
Methods: We retrospectively analyzed the outcome of 83 patients with R/R lymphomas who received DHAOX regimen from October 2004 to January 2016. The median age was 59 (22-79) and the median follow up is 56 months. Our series includes 41 patients with Diffuse Large B cell Lymphoma (DLBCL, 49%), 10 with Hodgkin Lymphoma (HL, 12%), 13 with Mantle Cell Lymphoma (MCL,16%), 15 with Indolent Lymphomas (18%), 4 with Peripheral T cell Lymphoma (5%). DHAOX schedule contains dexamethasone (40 mg/die on days 1-4), oxaliplatinum (130 mg/sqm on day 1) and cytarabine (2 g/sqm bid on day 2). In the majority of patients DHAOX was used as second line therapy. All patients received prophylactic G-CSF to reduce neutropenia. We analyzed overall survival (OS) and relapse free survival (RFS) in the whole cohort and then in DLBCL and HL patients. We evaluated haematological and non haematological adverse events (AEs).
Results: Non haematological AEs of grade >2 were observed in 27 patients (33%). The most frequent were: oral mucositis and other gastrointestinal events (6 patients, 8%), FUO (17 patients, 21%), sepsis (3 patients, 4%), paresthesias

(5 patients, 6%), and other different AEs in 4 patients (gout, atrial fibrillation, hypocalcemia and pleural effusion). 13 patients (16%) needed transfusional support. No patients experienced renal impairment. In 39 patients DHAOX regimen was utilized as mobilizing therapy to collect CD34+ cells for autologous stem cell transplantation (ASCT). In 32/40 patients (83%) more than $4x10^6$ CD34+ cells/kg were collected following G-CSF stimulation (5 mcg/Kg/die from day 5), in 1 patient associated with plerixafor. Median OS was 84 months. The projected OS at 12, 24, 36 months was 74.5%, 64.3% and 54.5%, respectively. Forty-one patients with DLBCL have been treated (median age 55 yy, range 22-79), 33 in second line (81%), 8 in third or subsequent line (19%). CR was obtained in 22 cases (60%), PR in 4 cases (11%). Eleven patients did not respond (29%). ORR was 71%. The median OS was 33 months and the projected OS at 12, 24 and 36 months was 66.3%, 57.9%, 45.5% respectively.
Ten patients with HL were treated (median age 44, range 28-74), 7 in second or third line (70%), 3 in fourth or subsequent lines (30%). CR and PR were obtained in 3 (30%) and 1 patients (10%), respectively. ORR was 40%. Considering only patients in second or third line, the ORR was 29%. Stem cell harvest was attempted in 2 patients, reaching the stem cell goal in both cases. The median OS was 84 months. The projected OS at 12, 24 e 36 months was 88.9%, 77.8%, 66.7% respectively.

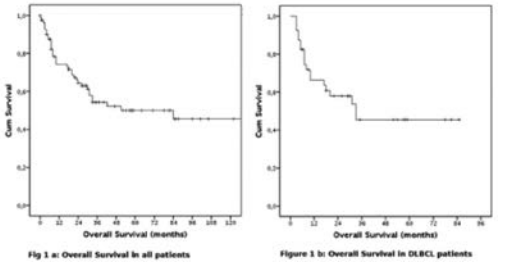

Figure 1.

Summary/Conclusions: DHAOX regimen was generally well tolerated, with acceptable toxicity profile and no documented significant renal impairment. By reducing days of hospitalization and the need of supportive care, we obtained a significant saving of economic resources despite the higher cost of oxaliplatinum. Stem cell mobilization with DHAOX resulted feasible and a successful apheresis was achieved in most patients. In conclusion, DHAOX regimen showed a good efficacy and a safe profile in the setting of relapsed/refractory non Hodgkin Lymphomas, mainly DLBCL; on the contrary, we experienced poor results in relapsed/refractory HL, suggesting that other schedules might be better in these patients.

E973

Abstract withdrawn.

E974

OUTCOMES OF 34 PRIMARY BREAST DIFFUSE LARGE B-CELL LYMPHOMA IN RITUXIMAB ERA AT A SINGLE INSTITUTION

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Background: Primary breast lymphoma (PBL) is a rare subtype of lymphoma accounting for 1 to 2% of extra-nodal lymphomas with the most subtype of diffuse large B cell lymphoma (DLBCL), thus the data of pathology and outcome is limited.
Aims: To elucidate the clinicopathological features and the outcomes including incidence of central nervous system (CNS) relapse in Rituximab-era.
Methods: Data were collected on patients with PBL diagnosed between January 1992 and 2016 at the Cancer Institute Hospital of JFCR, retrospectively. Patients with stage III and IV were excluded. Female DLBCL with stage I / II were also collected as a control in the matched case-control study.
Results: A total of 34 patients; 9 treated with CHOP-like regimens (CHOP group), 10 treated with rituximab-CHOP (R-CHOP group), and 15 treated with R-CHOP and intrathecal (IT) prophylaxis, were analyzed. Median age was 57 years. All patients were female with 21 patients (70.5%) of presenting with right breast tumor. Non-GC type defined by Hans algorithm had a higher proportion of 14 out of 24 analyzable patients (58.3%) without poor prognostic value. In comparison with early stage DLBCL treated with R-CHOP with or without IT prophylaxis except for breast, younger age (57 years v.s. 66 years) and higher CNS relapse rate (8.8% v.s. 0%) had significant difference between two groups. Then 1: 3 pair-matched case-control study limited in patients with stage I / II, female, and rituximab use, adjusted with age was carried out. Non-GC had higher rate in PBL than early stage DLBCL (66.7% v.s.44.4%), however, with no significant difference. On survival analysis with a median follow-up of 63months, PBL did not have poor prognosis with a 3-y-PFS (89.2% *vs* 90.2%, p=0.55) and 3-y-OS (90.0% *vs* 94.5%, p=0.87). Among patients with PBL, after a median follow-up of 47.5 months (range 1-286 months), the 3-year Progression free survival (PFS) and overall survival (OS) according to three groups; CHOP group, R-CHOP group, and IT+R-CHOP group were 55.6%, 80%, and 100% (p=0.058), and 55.6%, 80%, and 100% (p=0.06), respectively. CNS relapse had occurred in 3 out of 34 patients: all were stage II, one in CHOP group and two in R-CHOP group. Notably IT+ R-CHOP group had no relapse to date.

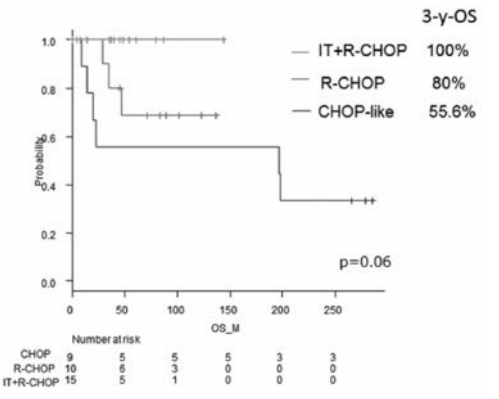

Figure 1.

Summary/Conclusions: PBL patients are younger than early stage DLBCL, and still considered as a high-risk for CNS relapse in Rituximab era. Nevertheless, IT added to R-CHOP potentially improve outcomes including CNS relapse.

E975

PATIENTS WITH PRIMARY CNS LYMPHOMA OR PRIMARY TESTICULAR DIFFUSE LARGE B-CELL LYMPHOMA HAVE AN INFERIOR PROGNOSIS WHEN CO-EXPRESSING C-MYC AND BCL2

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Background: Primary central nervous system lymphomas (PCNSLs) and primary testicular lymphomas (PTLs) are extranodal diffuse large B-cell lymphomas (DLBCLs) exhibiting a similar genetic signature and are characterised by an inferior response to current treatment regimens. DLBCL can be stratified by cell of origin (COO) derivation into the prognostically favorable germinal center B-cell (GCB)-like and the unfavorable activated B-cell (ABC) or non-GCB-like subtype based on gene expression signatures or immunohistochemical determination. The majority of PCNSL or PTL lymphoma derives from the non-GCB subtype. Recently, DLBCL patients with an immunohistochemical co-expression of c-MYC and BCL2 demonstrated inferior prognosis suggesting that concurrent expression of MYC/BCL2, termed double-protein-expression lymphoma (DPL), is a predictor of prognosis. Up to now there are no data, investigating the impact of DHL in patients with PCNSL or PTL.
Aims: We determined the frequency of DPL in a single center cohort of PCNSL or PTL and evaluated patients' outcome in contrast to "standard" DLBCL not exhibiting concurrent expression of MYC/BCL2.

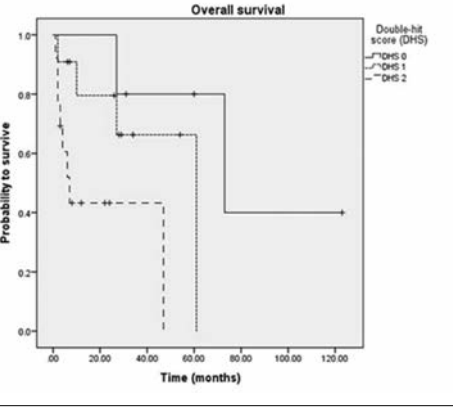

Figure 1.

Methods: We evaluated retrospectively the appearance of DPL in 42 patients with CNS or testicular DLBCL, consecutively diagnosed and treated between

2004 and 2014 at our institution. To determine the double-hit score, immunohistochemistry for c-MYC and BCL2 was performed on diagnostic samples. Furthermore, the Choi algorithm was used to subclassify samples by cell-of-origin. On the basis of immunohistochemical c-MYC and BCL2 expression, a double-hit score (DHS) was assigned to all patients with DLBCL. The DHS-2 group was defined by high expression of both MYC and BCL2 protein.
Results: Except for 3 samples, all patients were classified as non-GCB subtype. For 7 patients, no classification according to cell-of-origin could be performed. Of 42 primary extranodal DLBCL (PCNSL, n=28, PTL, n= 14), 29 patients were evaluable, 5 of them showed a double-hit score of 0, 11 patients had a DHS of 1, and 13 a DHS of 2, respectively. Using Kaplan-Meier curves and log-rank tests, probably due to small sample size, we could not demonstrate a significant different outcome for PCNSL or PTL patients with the non-GCB subtype as compared to the GCB subtype (*p*=0.74). However, we could clearly demonstrate a significant impaired outcome for patients with DHS of 2 (OS 61 *versus* 7 months, *p*=0.01) (Figure 1). Particularly, for patients with non-GCB subtype, we were able to further discriminate a subgroup of patients with an even worse clinical outcome to standard treatment (*p*=0.007).
Summary/Conclusions: In this single center cohort, we could demonstrate a significant negative effect on OS for PCNSL or testicular large cell lymphoma patients co-expressing MYC and BCL2. We could further define a subgroup of patients within the non-GCB cohort demonstrating double hit positivity (DHS of 2) that is associated with a particular short survival. These non-GCB-, DHS positive DLBCL patients are candidates for novel therapeutic strategies.

E976

CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT IN DIFFUSE LARGE B-CELL LYMPHOMA. A SINGLE CENTER ANALYSIS OF THE RISK FACTORS AND THE IMPACT OF CNS PROPHYLAXIS IN THE RITUXIMAB ERA

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Background: Central nervous system (CNS) relapse is a serious and generally fatal complication of diffuse large B-cell lymphoma (DLBCL) occurring in almost 5-7% of patients in the pre-rituximab era. The median time from diagnosis to detection of CNS disease is less than 1 year, suggesting that seeding in the CNS occurs early in the course of disease, with the exception of primary testis lymphoma in which it can occur up to 10 years after diagnosis. Up to date, there is no general consensus on how to define high-risk patients and what is the optimal CNS prophylaxis. The addition of rituximab to CHOP chemotherapy (RCHOP) has improved the clinical outcome of DLBCL. Nevertheless, the impact of rituximab on the incidence of CNS complications is still unclear.
Aims: This retrospective study aimed to analyze the predictive factors of CNS relapse and the impact of CNS prophylaxis in DLBCL patients treated at our Institute over a 12-years period.
Methods: We collected 393 patients with DLBCL diagnosed between 2002 and 2014. The patients with all stage of disease, regardless of the IPI score, were eligible and all received RCHOP every 14 or 21 days for at least 4/6 cycles. Patients with HIV or CNS involvement at diagnosis were excluded. Prophylaxis with intrathecal methotrexate (IT-MTX) was administered in patients with a high risk of CNS relapse according to the Italian Society of Hematology's guidelines. The diagnosis of CNS relapse was based on CT/MRI assessment and/or cerebrospinal fluid cytology.
Results: The median age was 58 years (IQR 47-68), 54% were males; 212/393 patients (54%) had stage III-IV, a bone marrow (BM) involvement was found in 51/393 patients (13%), while 49 (12%) had an involvement of more than 1 extranodal site, testis involvement was in 20/393 patients (5%). B symptoms were experienced by 100/393 patients (25%) and an elevated LDH was present in 115/393 patients (29%). According to the IPI score, 197/393 patients (50%) had a IPI<2 and 196/393 (50%) a IPI>2. Twelve/393 patients (3%) experienced a CNS relapse: 7 of them had a brain mass, 2 had a leptomeningeal involvement and 3 had both.CNS prophylaxis was carried out in 81/393 patients (21%): 27 with a IPI<2 and 54 (67%) with a IPI≥2. In the low risk group, only 1 patient with a primary testis involvement developed a CNS relapse 26 months from diagnosis, despite prophylaxis. In the high risk group according to IPI, 11 patients experienced a CNS relapse; only 4 of them had received IT-MTX[R1]. At univariate analysis: gender (p=0.029), advanced stage (p=0.004), PS>2 (p=0.009), presence of B symptoms (p=0.000), elevated LDH (p=0.006), involvement of >1 extranodal sites (p=0.000), BM involvement plus elevated LDH (p=0.003), testis localization (p=0.019) and IPI>=2 (p=0.000) were associated with an increased risk of CNS relapse. At multivariate analysis, only PS>2 (p=0.031, 95%CI: 0.009-0.189), testis involvement (p=0.001, 95%CI: 0.048-0.2) and BM involvement plus elevated LDH (p=0.016, 95%CI: 0.021-0.2) were predictive factors of high risk of CNS relapse. The 2-year overall survival of the CNS relapsed patients was 9%, with a median follow-up of 8 months from diagnosis (IQR 4-28).
Summary/Conclusions: CNS relapse remains a fatal event for patients with DLBCL even in the rituximab era. Poor PS, testis and BM involvement combined with elevated LDH represent the most important prognostic factors for CNS relapse. In our experience, single IT-MTX prophylaxis did not reduced CNS.

E977

SURVIVAL IMPACT OF SERUM ALBUMIN (SA) IN PATIENTS WITH ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) RELATED NON-HODGKIN'S LYMPHOMAS

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Background: Despite effective combination antiretroviral therapy for HIV, there is still increased incidence of Acquired Immunodeficiency Syndrome (AIDS) related Non-Hodgkin lymphomas (NHLs). AIDS related NHLs represent a challenge for the practicing oncologist. Prognostic factors have been shown to be useful to risk stratify the disease.
Aims: Here we examine potential prognostic indicators that may improve the performance of current prognostic systems in AIDS related NHLs.
Methods: Patients with HIV associated aggressive B-cell NHL were identified between 2001- 2014 at Moffitt Cancer Center. Patients with and primary central nervous system lymphoma were excluded. Clinical data including CD4 count, HIV viral load (VL) and opportunistic infections (OI) were included. Survival curves analyzed using the Kaplan Meier method and statistical significance was assessed using the log-rank test. A p<0.05 was considered statistically significant.
Results: A total of 85 patients were included. The male:female ratio was 6.7. Median age was 44.3 years (range 25-65). The median time from HIV to NHL diagnosis was 29 months (0-284). Seventy-eight percent presented with stage III/IV disease. The Eastern Cooperative Oncology Group performance (ECOG) status was >1 in 62%. The most common histologies were Diffuse Large B Cell Lymphoma (DLBCL) and Burkitt's lymphoma with 49 and 34%, respectively. Bulky disease was present in 27.3%, elevated LDH in 61.5%, and CD4 count<100/mL at diagnosis in 29.5% patients. The mean Hb, SA and CD4 count were 3.6 g/dL, 11.8 g/dL and 205.9 k/uL, respectively. A serum albumin (SA)<3.7 g/dL and Hb<10 g/dl were present in 33% and 23.2% of patients, respectively. Forty-six percent had had cART at time of diagnosis. The cohort's median OS was 5.9 years. KM curves showed poor median OS with SA<3.7 g/dl (OS=3.9 y, p=0.021), Hb<10 g/dl (0.7 y, p= 0.001) and OI (OS= 1.0 y, p=0.018), There was a trend with worse OS with NCCN-IPI score >3 (OS=1.1 y, p= 0.165). MV analysis showed that SA<3.7 retained statistical significance (HR: 2.45, CI: 1.03-5.84, p=0.042).

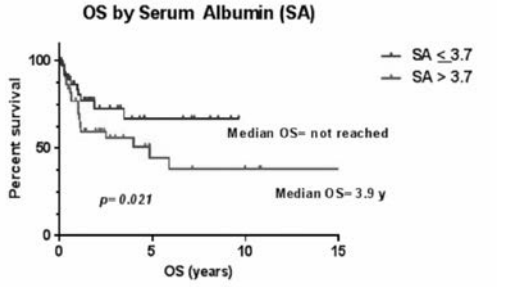

Figure 1.

Summary/Conclusions: New prognostic scoring systems have improved the risk stratification of AIDS-related lymphomas (Barta el al, Haematologica 2014). SA<3.7 is associated with worse OS in this study. SA could improve the stratification of AIDS related lymphomas in the cART era. A validation of the role of SA should be performed in larger cohorts.

E978

OUTCOME FOR PATIENTS WITH RELAPSED/REFRACTORY LYMPHOMA TREATED WITH GEMCITABINE AND OXALIPLATIN WITH OR WITHOUT RITUXIMAB

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Background: Relapsed/refractory aggressive lymphoma remains challenging to treat. There is no standard salvage chemotherapy regimen. Studies suggest that the outpatient combination of Gemcitabine, Oxaliplatin (Gem-Ox) with Rituximab (R) achieves similar response rates (RR) with less toxicity than intensive inpatient regimens[1].

Aims: To review outcomes for patients with refractory/relapsed aggressive lymphoma treated with Gem/Ox+/-R.
Methods: This retrospective study analysed clinical data on patients with relapsed/refractory aggressive lymphoma treated with Gem/Ox with or without R between 2010 and 2015 at 3 teaching hospitals in London, UK. The treatment schedule was Gemcitabine 1000mg/m^2 and Oxaliplatin 100mg/m^2 +/- Rituximab 375 mg/m^2 on day 1 and repeated every 15 days for up to 8 cycles.
Results: 44 patients, 26 male, with a median age of 66 years (range 22-83) were included. 30 (68%) patients had DLBCL; 27 had received prior R. 6 had T-cell lymphoma, 3 Hodgkin lymphoma and 5 had high grade transformation of low grade B-cell lymphoma. The median number of prior treatments was 1 (range 1-6). 7 patients had received an autologous transplant, 1 of whom had also received an allogeneic transplant. Remission duration (RD) to the prior treatment regimen was<12 months in 26 (59%) patients. 26 (75%) patients received R with Gem-Ox. The median number of cycles received was 4 (range 1-6). Response was assessed by CT scan in 19 PET/CT in 19, MRI in 2 and by clinical evaluation in 4. The overall response was 43% (n=19) with a complete response (CR) of 29% (n=13). CR was achieved in 11/18 (61%) patients with a prior RD ≥12 months compared to 2/26 (8%) patients whose prior RD was<12 months (p=0.0002). 27/44 (61%) patients were transplant eligible, 7 of whom had received a prior autologous transplant. The overall response in transplant eligible patients was 44% (n=12) 8/27 (30%) went on to receive a stem cell transplant; 4 autologous and 4 allogeneic transplants. At a median follow up of 6 months (range 0.5-33), the median overall survival (OS) is 8 months. For the 19 responding patients the median RD has not been reached. Median OS was significantly better in patients who had had a ≥12 month response to their prior treatment regimen; 13 *vs* 4.5 months, p=0.02 (Fig 1). Toxicity data were available for 36 patients. 17(47%) patients experienced grade 3/4 haematological toxicity. 8 (22%) had at least one febrile episode requiring hospitalisation. There were 4 (11%) grade 3/4 non-haematological toxicities, including diarrhoea, vomiting, peripheral neuropathy and bowel perforation. Dose reductions were required in 5 patients. 33 (75%) patients have died, 26 (60%) from lymphoma. Other causes of death include 1 old age, 2 post transplant graft *vs* host disease, 1 treatment-related sepsis 3 unknown.

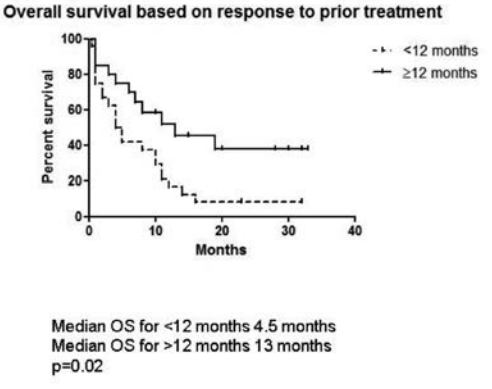

Figure 1.

Summary/Conclusions: In this study of 'real world' patients, Gem-Ox+/-R is a well-tolerated, outpatient regimen achieving good response rates in patients with chemo-responsive disease. Responses are comparable to more toxic, inpatient, platinum-based regimens. It can also successfully bridge patients to stem cell transplantation. Best responses are achieved in patients who have had a prior remission of at least 1 year.

E979

DOSE-ADJUSTED ETOPOSIDE-PREDNISONE-VINCRISTINE-CYCLOPHOSPHAMIDE-DOXORUBICIN-RITUXIMAB (DA-EPOCH-R) FOR PRIMARY MEDIASTINAL B-CELL LYMPHOMA (PMBCL) AND OTHER VERY HIGH-RISK OR ADVANCED LYMPHOMAS

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[1]Hematology, [2]Nuclear Medicine, [3]Pharmacy, Ospedale dell'Angelo and SS. Giovanni e Paolo, Venezia Mestre, [4]Pathology, Ospedale SS. Giovanni e Paolo, Venezia, Italy

Background: Dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab (DA-EPOCH-R) is highly effective in PMBCL (Wilson W et al. NEJM 2013;368:1408) and is adopted in very aggressive lymphomas with encouraging results. While it is commonly used in US, experience in European countries is limited.
Aims: DA-EPOCH-R was used at Venice hospitals to treat adult patients with PMBCL and aggressive lymphoma variants associated with a poor response to R-CHOP.
Methods: DA–EPOCH-R consists of 6-8 cycles q21d with escalating doses of etoposide, cyclophosphamide and doxorubicin. Etoposide, vincristine (fixed dose 0.4mg/m^2/d) and doxorubicin are given by continuous IV infusion over 96 hours (dd 1-4). Drug level 1 is with etoposide 50, doxorubicin 10 and cyclophosphamide (d5) 750mg/m^2/d. A 20% dose escalation after each cycle and up to level 6 is prescribed to patients who do not develop a neutropenia <500/µl. The dose is reduced by 20% if neutropenia lasts >1 week or platelets fall <25.000/µl. Concomitant drugs are prednisone 60mg/m^2/bd (dd 1-5), rituximab 375mg/m^2 d1, and G-CSF from d6. Complete remission (CR, CRu [unconfirmed]) is defined according to standard criteria and PET/CT assessment after a minimum of 4 cycles.
Results: Thirty-three patients were treated between august 2013 and january 2016. Twenty-eight patients were treated frontline for PMBCL (n=14), non-GCB/highly proliferative (Ki67 ≥80%) diffuse large B-cell lymphoma (DLBCL) (n=5), double-hit DLBCL (n=6), plasmablastic lymphoma (n=2, one without R), and EBV+ lymphomatoid granulomatosis (n=1). Five patients were treated at salvage after 1-2 prior lines for DLBCL (n=3), Burkitt lymphoma (n=1) and Hodgkin disease (n=1, without R). Median patient age was 57.5 years (range 17-74 years), 61% were male, 73% had ECOG PS >1, 64% had stage III-IV disease and 58% had intermediate-high/high IPI. Altogether, the 33 patients received 158 courses, without change in dose level in 5 salvage patients. Twenty-six of 28 untreated patients had 2 or more cycles and are evaluable for dose level increments. Dose level could not be increased in 8 patients (31%), reached level 2 in 5 (19%), level 3 in 7 (27%), level 4 in 5 (19%), level 5 in 1 (4%) and level 6 in none. Toxicity consisted of severe neutropenia and thrombocytopenia in 67% and 10% of the cycles, respectively, neutropenic fever with admission to hospital (n=5, 15%), vincristine-related neuropathy (n=3, 9%), and thromboembolism (n=3, 9%). Due to poor clinical conditions, treatment was stopped after cycle 1 in a DLBCL patient (aged 65) with concurrent lung cancer, and switched to R-CHOP after 2 and 4 cycles in 2 patients (aged 74 and 61, respectively) with double-hit DLBCL, both remaining well and alive. Twenty-five patients completing 4 courses with PET/CT-based restaging are evaluable for response (Table). CR and ORR rate was high in patients treated frontline, particularly in PMBCL (ORR and PFS 100%). Response rate was appreciable in double-hit and very aggressive lymphomas as well, but was instead poor in patients treated at salvage.

Table 1.

	CR	CRu	CR/CRu	PR	ORR	NR	Progression-free (m.)
1st line							
PMBCL (n=12)	8 (66.6%)	2 (16.6%)	10 (83.3%)	2 (16.6%)	12 (100%)	0	12 (100%) at 15.5 (2-29+) mos.
Double hit (n=6)	3 (50%)	1 (16.6%)	4 (66.6%)	1 (16.6%)	5 (83.3%)	1 (16.6%)	5 (83.3%) at 9.5 (3-14+) mos.
Others (n=7)	5 (71.4%)	1 (14.3%)	6 (85.7%)	0	6 (85.7%)	1 (14.3%)	6 (85.7%) at 11 (1-21+) mos.
>1st line							
All (n=5)	2 (40%)	0	2 (40%)	0	2 (40%)	3 (60%)	0 (0%) at 4 mos.

PR, partial remission; ORR, overall response rate; NR, no response

Summary/Conclusions: DA-EPOCH-R proved globally feasible and safe in this aged patient population, and highly active in PMBCL as observed in the original study. The small patient number and the limited follow-up period do not allow definite conclusions, although the regimen would be useful in PMBCL (avoiding mediastinal irradiation) and potentially useful in very aggressive B-cell lymphomas at risk of failing standard R-CHOP therapy.

E980

ELDERLY AGE IS NOT EXCLUSION FACTOR FOR INTENSIVE THERAPY BY SCHEME R-DA-EPOCH/R-HMA FOR PATIENTS WITH UNTREATED HIGH-GRADE DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Median of age for patients with diffuse large B-cell lymphoma (DLBCL) is 60. Approximately 50% of DLBCL are defined as high-grade by IPI and these forms are characterized by aggressive course and poor response to standard chemotherapy (CT). High-dose methotrexate courses, as example Hyper-CVAD/HMA or mNHL-BFM-90 protocols are applied for patients with high risk DLBCL. But for elder patients this approach usually is not employed due to the extreme toxicity. Addition of R-HMA to R-DA-EPOCH favourably changes the outcome in patients with untreated high-grade diffuse large B-cell lymphoma [ASH 2015 # 2708].
Aims: To evaluate the efficacy and toxicity of R-DA-EPOCH/R-HMA protocol in patients with untreated high-grade diffuse large B-cell lymphoma depending on age.
Methods: 35 untreated DLBCL patients from 4 centers were enrolled in a prospective study between August 2013 - Jan 2016; stage II-IV; ECOG 0-3; median age 55 years (27-76); age ≥60y/<60y 50%/50%; M/F 60%/40%; IPI: 63% high-intermediate and 37% high risk; 20% with bone marrow involvement. All patients underwent 4-8 courses (2-4 cycles) of chemotherapy: R-DA-EPOCH (standard dose and scheme), R-HMA (R 375 mg/m^2 d1, MTX 1000 mg/m^2 24 hours d 2, AraC 3000 mg/m^2 q 12 hrs d 3-4). For patients older than 60 year dose of R-HMA was reduced (R 375 mg/m^2 d1, MTX 500 mg/m^2 24 hours d 2, AraC 1000 mg/m^2 q 12 hrs d 3-4) and R-EPOCH were applied with-

out dose adjusted. In 4 cases of DLBCL with bone marrow involvement BEAM conditioning and autologous stem cell transplantation were applied.

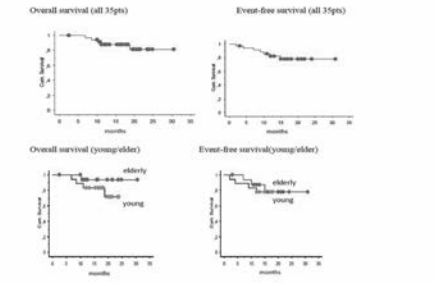

Figure 1.

Results: The median follow-up is 16 months (3-31). There was no mortality associated with toxicity. The main non-hematological toxicities of R-HMA were infections (mucositis, pneumonia, sepsis, enteropathy) grades 1-2 and 3-4 in 90% and 10%, respectively. Hematological toxicity grade 4 for less than 4 days we observed only after courses R-HMA. Complete remission (CR) was achieved in 31 (88,6%) patients. In group of young patients we observed 2 progressions of the disease after first cycle of chemotherapy, 2 cases with partial remission after 2-3 cycles and following progression of disease. There are three failures in patients older than 60 years: two relapses (after 5 and 13 month CR) and one death after 7 month CR by reasons not related with DLBCL. With a median follow 16 months overall and event-free survival of 35 patients constituted 81,6% and 78,2%, respectively. There is no difference in elderly and young groups: OS was 93,3% *vs* 71,4% (p=0,2), EFS was 77,8% in both. So the combination of R-DA-EPOCH/R-HMA may be considered as optimal intensive approach in elder patients.
Summary/Conclusions: The R-DA-EPOCH/R-HMA protocol demonstrated acceptable toxicity and high efficacy in patients with high-grade DLBCL. This protocol has shown optimistic results in the elderly patients and it could be recommended for further investigation in that group.

E981

FREQUENCY AND CLINICAL IMPLICATIONS OF SOX11 EXPRESSION IN BURKITT LYMPHOMA

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Background: The transcription factor SOX11 is normally expressed during embryogenesis in humans. Several studies have shown that SOX11 is aberrantly expressed in various types of haematopoetic and solid malignancies, and appears to affect clinicopathological characteristics. In mantle cell lymphoma, SOX11 serves as a diagnostic antigen and has been shown to be associated with superior outcome (Nordström *et al.* British Journal of Haematology 2014), although its role as a prognostic indicator remains controversial. In chronic lymphocytic leukemia, SOX11 was associated with inferior overall survival (Roisman *et al.* Tumor Biol 2015), whereas SOX11 expression correlated with superior outcome in epithelial ovarian cancer (Sernbo *et al.* BMC Cancer 2011). Various possible target genes and transcriptional programs have been identified, by which SOX11 may exert both repressive, and exaggerative, functions on proliferation (Kuo *et al.* Oncogene 2015, Vegliante *et al.* Blood 2013). In Burkitt lymphoma (BL), two small series have reported expression of SOX11 in 30-50% of cases (Mozos *et al.* Haematologica 2009, Dictor *et al.* Haematologica 2009). As of yet, no study has been performed specifically to evaluate the implication of SOX11 expression in BL.
Aims: The aim of this study was to investigate the frequency of SOX11 positive BL cases in a larger joint cohort from Denmark and Sweden, and correlate its expression to clinical and pathological parameters.
Methods: 45 BL cases were collected from Sweden and Denmark. Samples were analyzed for expression of SOX11 and other immunohistochemical markers. Clinical data were obtained from the Danish Lymphoma Database (LYFO) and the Swedish Lymphoma Registry. Additionally, 9 pediatric BL cases were analyzed for SOX11 expression, but with no clinical data available.
Results: In the adult study population, 14/45 (31%) expressed nuclear staining of SOX11. The SOX11 positive subgroup had a higher median age (53 compared to 41), as well as a larger proportion with elevated LDH. Other prognostic factors were equally distributed between the groups. Although a similar proportion of patients received high-intensive chemotherapy in both subgroups, 3 (21%) received no treatment in the SOX11 positive cohort. There was no significant difference in immunohistochemical characteristics between SOX11 positive and negative cases. Five-year overall survival (OS) in the SOX11 positive group was 57%, compared to 77% in the SOX11 negative cohort. When adjusting for treatment and and prognostic factors in multivariable analysis, no significant difference in outcome was seen between groups (hazard ratio: 1.5, 95% confidence interval: 0.3-6.3, p=0.6). In the pediatric cohort, 5/9 (56%) expressed SOX11.
Summary/Conclusions: SOX11 expression was found in a minority of BL cases, and was associated with higher age in the adult BL cohort. In contrast to SOX11 expression in other malignancies, SOX11 expression showed no significant impact on outcome, in our study, when adjusting for prognostic factors and treatment.

E982

LONG-TERM FOLLOW-UP OF PRIMARY GASTRIC DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH MNHL-BFM-90

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Background: R-CHOP chemotherapy can induce favorable result for localized-stage primary gastric diffuse large B-cell lymphoma (PG-DLBCL) patients. But the presence of adverse factors (AF) and advanced stage decrease the efficacy of this therapy: 3-year progression-free survival (PFS) and overall survival (OS) are 43% and 64% respectively. The optimal treatment strategy for this pts still remains unknown.
Aims: Efficacy and safety assessment of the modified chemotherapy protocol NHL-BFM-90 (m NHL-BFM-90) in the treatment of the PG-DLBCL with AF.
Methods: Forty previously untreated pts with PG-DLBCL underwent m NHL-BFM-90 treatment between January 2004 and December 2015; mean age 47 years (range 37-72); age ≥60 years 9 pts (22,5%); M\F=23\17; stage >I 27 pts (68%); all pts had one or more AF. None of the patients received surgical treatment before chemotherapy and no consolidation radiotherapy. NHL-BFM-90 program (2 courses A and 2 courses B) was modified for PG-DLBCL in the following way: doxorubicin (50mg\m^2) was added on the third day of course A. Twenty one (52%) pts received rituximab on day 0 prior to each course of therapy.
Results: The overall response rate (ORR) was 100%. Complete remission was achieved in 38 (95%) pts. From 2 pts with partial remission one died from progression of disease, one received salvage therapy. With a median follow-up of 74 months (range 3-146) disease-free and overall survival of 40 pts constituted 87,5% and 92,5%, respectively. All but one treatment failure belonged to the group without rituximab. Hematologic toxicity of grade 3 and 4 was observed in 80% of pts. Severe complications became the reason for subsequent switch to CHOP therapy after 2 courses in 6 cases. There was no treatment-related mortality.
Summary/Conclusions: The mNHL-BFM-90 demonstrated acceptable toxicity and high efficacy in patients with PG-DLBCL with AF.

E983

PRIMARY BONE MARROW LYMPHOMA: A HEMATOLOGICAL EMERGENCY IN ADULTS WITH FEVER OF UNKNOWN ORIGIN

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Background: Primary bone marrow lymphoma (PBML) denotes non-Hodgkin lymphoma (NHL) arising primarily in the bone marrow (BM) without any lymphadenopathy. It is often masked by prolonged fever leading to delayed diagnosis and notorious for various definitions adopted by previous studies.
Aims: This study aimed to lighten clinical panorama of PBML and to identify potentially warning parameters of PBML.
Methods: Two cohorts of PBML patients were enrolled. Cohort I consisted of 163 PBML cases from 16 case series and 23 case reports for meta-analysis of clinical characteristics. Cohort II comprised 221 adults with fever of unknown origin (FUO) from Taipei Veterans General Hospital who underwent a BM study, and 52 cases of them were finally diagnosed of NHLs, including 26 PBML cases. The latter cohort was used to identify warning parameters of PBML by comparing data upon admission between all FUO patients with and without a subsequent PBML diagnosis.
Results: In cohort I, PBML had heterogenous histology and could be clinically separated into two distinct entities: lymphoma limited entirely to the BM, liver, and/or spleen (i.e. BLS-type, n=79), and isolated BM lymphoma (i.e. IBM-type, n=84). Two third (67%) of PBML patients initially manifested with fever, and one third (31%) complicated with hemophagocytic lymphohistiocytosis (HLH). The BLS-type cases were mainly reported from the countries in Eastern Asia, more often presented as FUO and HLH at diagnosis, and suffered from a poorer prognosis (median overall survival of BLS-type *vs* IBM type=272 *vs* 580 days,

P<0.001). For 26 PBML patients of the cohort II, 89% were classified as BLS-type; median overall survival revealed dismal 9 days, with 54% having early mortality within 30 days. Compared to non-PBML NHL in the cohort II, PBML is an independent risk factor of both early mortality and overall survival. Among 221 FUO adults in cohort II, 4 warning parameters able to alert underlying PBML were independently identified, including serum alkaline phosphate >2× UNL, serum immunoglobulin G<0.67× UNL, cytopenia ≥2 lineages, and leuco-erythroblastosis in the peripheral blood.

Table 1. Univariate and multivariate analyses of warning parameters for primary bone marrow lymphoma in 221 adults with fever of unknown origin.

Warning parameters for PBML	Univariate		Multivariate	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Bicytopenia or pancytopenia	22.844 (6.572–79.404)	<0.001	20.559 (3.101–136.279)	0.002
Leucoerythroblastosis in PB smear	1.390 (1.193–1.619)	<0.001	1.384 (1.008–1.901)	0.045
LDH >500 IU/L (2x UNL)	15.693 (4.543–54.212)	<0.001	4.956 (0.832–29.511)	0.079
IgG <1000 (0.67x UNL)	4.912 (2.015–11.972)	<0.001	5.086 (1.046–24.728)	0.044
ALP >200 U/L (2x UNL)	6.000 (2.473–14.556)	<0.001	5.857 (1.179–29.085)	0.031
PT >12 sec (1x UNL)	6.416 (2.551–16.133)	<0.001	4.457 (0.939–21.161)	0.060

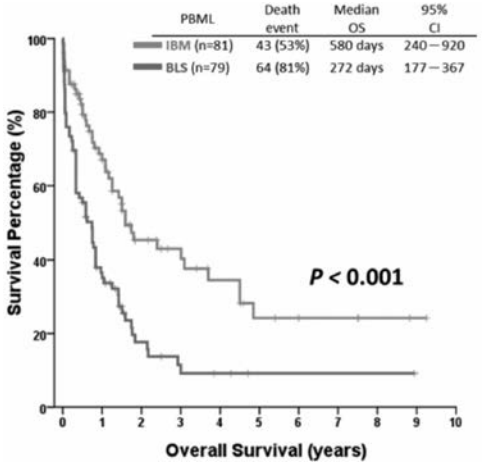

Figure 1.

Summary/Conclusions: As a particular clinical phenotype of NHL, PBML is often masked by prolonged fever and should be regarded as a hematological emergency when approaching patients with FUO. Our study provided additional diagnostic clues to early recognize FUO adults with underlying PBML.

E984

CHEMOTHERAPY OF 40 PRIMARY MEDIASTINAL B-CELL LYMPHOMA PATIENTS

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Background: Primary mediastinal B-cell lymphoma (PMBCL) amounts from 0,9 to 4% of Non-Hodgkin Lymphomas. Predominantly suffer young women. All relapses in PMBCL occur as early events. Taking into account unfavorable prognosis in case of relapse or progression of PMBCL (2-year overall survival (OS) is about 15% [Kurvilla J., 2008]), it's necessary to achieve a complete remission (CR) in first line treatment.
Aims: To evaluate an efficacy of R-DA-EPOCH therapy+R-DHAP№2 and autologous peripheral blood stem cell transplantation (autoSCT) with BEAM conditioning regimen in treatment of PMBCL pts.
Methods: In 40 pts (14 males, 26 females) diagnosis of PMBCL was established according to WHO criteria. All pts had stage II of disease according to Ann Arbor classification. Median age of pts was 27 years old (from 19 to 67 years old). Increased LDH concentration was revealed in 34 from 40 (85%) pts. Bulky mediastinal disease was in 31/40 (77.5%) pts. In case of CR after 6 courses according to computer tomography and PET scanning treatment was completed. In case of PR pts underwent 2 R-DHAP courses with autoSCT (BEAM conditioning regimen). All cases of treatment failure occurred till one year of observation. Kaplan-Meier method was used to calculate OS and OFS.
Results: After 6 R-DA-EPOCH therapy CR was achieved in 32/40 (80%) pts, 8/40 (20%) pts had PR and underwent 2 R-DHAP courses followed by auto-SCT (BEAM). 2- OFS and OS was 96% and 100%. Early relapse occurred in 1/40 (2,5%) patient. Median follow up was 20 months (3-32).
Summary/Conclusions: R-DA-EPOCH allows getting high efficacy of primary mediastinal B-cell lymphoma therapy. Although future analysis will show distant results of R-DA-EPOCH chemotherapy. Taking into account unfavorable prognosis in case of relapse or progression of PMBCL autoSCT in first line therapy might have benefit in patients with partial remission after R-DA-EPOCH.

E985

VITREORETINAL LYMPHOMA: A DESCRIPTIVE STUDY OF A VERY RARE OCCURRENCE FROM A SINGLE INSTITUTION IN ITALY

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Background: The vitreoretinal lymphoma is a rare subset of primary central nervous system lymphoma (PCNSL) with a poor prognosis. Because of its rarity and the difficult of collecting tumor simples, this entity remains poorly understood and the optimal management is still unknow.
Aims: to describe a single insitution experience with Vitreoretinal lymphomas.
Methods: The 2011-2015 archives of the hematology and oftalmology units of the Reggio Emilia Hospital Lymphoma were searched for cases with vitreoretinal lymphoma. Cases were classified as primary vitreoretinal lymphoma (PVRL) of systemic lymphomas with concurrent Vitreoretinal involvement (CVRL). We defined stage IE as mono or bilateral involvement of the vitreum. We provide a complete description of pathological, clinical, and therapeutic findings.
Results: We identified 7 patients with either PVRL (3) or with CVRL (4). Four patient were female, the median age at diagnosis was 62 years (range 45-74), three had a stage IE and four a stage IVA. All patients were HIV negative. Among the 4 CVRL 2 patients showed brain involvement, one patient nodal involvement, one patient gastric involvement. All patients were initially referred with ocular symptoms mimicking uveitis. The median time between the onset of the ocular symptoms and the lymphoma diagnosis was 13 months (range 2-30 months). All patients underwent a diagnostic vitrectomy. The cytopathological analysis was positive in five cases and uncertain in two cases (CVRL). The immunoistochemical analysis showed atypical CD20+ lymphocytes in three cases. Vitreous supernatant was submitted for IL-10 and IL-6 measurement by ELISA using the IL-10 to IL-6 ratio as a diagnostic marker. Elevation of IL-10 levels and IL-10:IL-6 ratio greather than 1 was found in all cases. Molecular analysis of the specimens detected IgH gene rearrangements in 4 cases. Notably the three PCR negative samples displayed a sharp unbalanced IL-10:IL-6 ratio and atypical CD20+ lymphoid large cells. Cerebral Spine Fluid (CSF) analysis was negative in all patients. All patients were treated as CNS lymphomas and received combined high-dose systemic chemotherapy with Rituximab, High dose ARA-C and High dose Methotrexate, and intravitreal methotrexate iniection (Intravitreal methotrexate 400 microg/0,1 ml twice weekly for 4 weeks, once weekly for 8 weeks, and then once montly for 9 months for a total of 25 injection). All 7 patients achieved a complete remission (CR) at the end of therapy. Four patients underwent autologous stem cell transplant (ASCT) in first remission.m The median observation period was 16 months (range, 2-48 months). All patients were alive at this time point except for one patient who relapsed four months after ASCT and died one month later.
Summary/Conclusions: This single center retrospective series highlights the insidious onset of both PVRL and CVRL and the difficulties in diagnosing this particularly rare lymphoma occurrence.

E986

RCHOP *VS* -CHOP CHOP IN DIFFUSE LARGE CELL FOLLICULAR EPIDEMIOLOGICAL OF PATIENTS WITH LYMPHOMA LYMPHOMA TREATED IN CARACAS, VENZUELA FOR 14 YEARS

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Background: Few epidemiological Venezuelan Lymphoma patients data are found in the literature to compare survival and the current standard treatment with chemoinmunotheraphy with Lymphoma patients from developed countries.
Aims: The purpose of this paper is to present epidemiological data of patients with lymphoma treated by the Commission of lymphoma IOH and some private clinics in Caracas during the past 14 years and compare the efficacy of treatment of RCHOP *vs* CHOP
Methods: Materials and Methods A total of 1812 patients with lymphoma, of which 1214 were classified as non-Hodgkin lymphoma and 598 as Hodgkin's lymphoma were treated, and of these 1117 patients with lymphoma were studied from the pto epidemiologically between 1996- 2010 Lymphoma diagnosis was made based on clinical history, X-rays, CT, lymph and tissue biopsy (depending on the case), immunohistochemistry studies when required.
Results: Results Patients with lymphoma were classified into Hodgkin and non-Hodgkin 390 cases 827 cases. Hodgkin's lymphoma (HL) is subclassified in Nodular Sclerosis 62.6%, 27.7% mixed cellularity, lymphocyte-predominant 4.3% 5.4% Depletion Lymphocytic The age and sex of the patients were Nodular Sclerosis: 32.45 years (18-65), 129 female and 115 male, Mixed cellularity: 22.3

years (18-56), 37 female and 72 male, predominantly lymphocytic 22.6 years (18-67), 6 female and 11 male, depletion Lymphocytic 22 years (20-42) female and 15 maleThe stages were stage I: 5,5%, stage II: 44%, stage III: 31.1% stage IV: 19.4%.HL patients were treated with different chemotherapy protocols: MOPP, ABVD, STANDFORD V, HYBRID, COPP / ABVD, COPP / EBVD, BEA-COPP and in some cases radiotherapyNon Hodgkin Lymphomas were subclassified by 36.4% Diffuse Large Cell, Follicular 24.1%, 10% Maltoma, 5.5% of peripheral blood cells, 3% mantle cell lymphoma, 20.8% other types of lymphoma such as mycosis Mycosis, immunoblastic Anaplastic, Lymphoblastic, Burkitt, Cutaneous B cell 45% of patients with follicular lymphoma had a high score FLIPI and 60% of diffuse large cell lymphoma had a high intermediate score with high-risk prognostic factors and only 15% of these were low-risk DLB-CLNHL were treated with different protocols according to the year of admission and stage of CHOP disease, CHOP Bleo, MACOB B, ATT, Hyper-CVAD, CHOP MTX and from 2005 to 2010 55 patients were treated with R-CHOP and year 1999-2004 29 patients were treated with CHOP resulting overall survival in DLB-CL with RCHOP 92.1% *vs* 67.9% and 89.5% LF *vs* 50%, event-free survival of 90% DLBCL with RCHOP CHOP *vs* 44% (p 0.002) and LF with CHOP *vs* 89.8 RCHOP age influenced patient survival (p 0.56). Other variables such as sex, type of lymphoma, stage had no impact on the EFS.

Summary/Conclusions: Conclusion: 1117 patients with lymphoma were studied retrospectively. Patients were classified in 34.9% and 65.1% non -Hodgkin lymphoma. The most common subtype of LH was nodular sclerosis (62.57%) and was more common in women and young adults of mixed cellularity HL was the second most common and the prevalence in lymphocytes and lymphocyte depletion constituted only 6.8% and 8 7%% the most common was the NHL diffuse large cell and the second Follicular NHL. The Venezuelan population is made up of a mixture of different indigenous races, Caucasian, Negroid, but it seems that this disease follows the same patterns reported in other populations Overall survival, event-free survival of DLBCL and LF was statistically significant in favor of RCHOP, which should be the treatment of choice in these pathologies.

E987

IS THAT MALE GENDER AN ADVERSE RISK FACTOR ONLY IN YOUNG PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA?

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Background: The International Prognostic Index (IPI) is the most important clinical tool for determining prognosis in diffuse large B-cell lymphoma (DLBCL). It incorporates five adverse risk factors: age above 60 years; Ann Arbor stage III or IV; elevated serumnlactate dehydrogenase (S-LDH); Eastern Cooperative Oncology Group (ECOG) performance status of 2 or higher; and involvement of two or mor extranodal sites. GUSTAF HEDSTRÖM was published in Acta Oncologica (in April 2015), one of the largest population-based studies of DLB-CL presented (7166 patients were included), he found male gender to be a significant adverse risk factor compared to fertile women, without msurvival differences between genders in the older sub-population. According Nurith H, the reduced rate of NHL among females may be explained by direct effects of estrogens on lymphoma cell proliferation or by its effect on anti-tumor immune response: the influence of sex hormones on lymphoid malignancies has been the subject of clinical and *in vitro* research. Epidemiological studies highlighting the association between sex hormones and NHL have provided some clues. Lee and colleagues reported that an increasing number of pregnancies and live births is associated with a decreasing trend in the risk of DLBCL.

Aims: The primary objective of this study was to analyze the impact of gender on age in patients with newly diagnosed DLBCL.This retrospective study included 95 patients with diffuse large B cell lymphoma who were treated the Medical Hematology Department, HCA during the time period from 2000 to 2015.

Methods: We conducted a retrospective cohort study of all DLBCL patients diagnosed between 2000 and 2015, to evaluate the impact of gender for survival from DLBCL. Survival curves n were estimated according to the Kaplan-Meier method and associated log rank, a Cox model was used to evaluate the prognostic impact of clinical risk factors (age<vs≥52 y, ECOG≤VS>2, stage I-II *vs* III-IV, LDH normal *vs* high, extranodal sites, IPI (0-5), IPI-a (0-3).

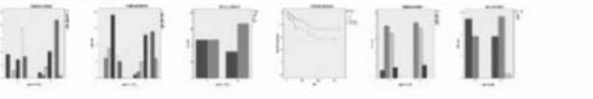
Results: In total, 95 patients were included for analysis, clinical caracteristics of our patients are summarized in Table I. Data collected were: year of diagnosis, age, gender, Ann Arbor stage, ECOG performance status, extranodal sites, S-LDH, IPI, age adjusted IPI (aaIPI). When patients older (52 years or older), or younger than 52 years, were analysed separately, the same risk factors remained no significant. Sex was not found to be significantly correlated with adverse outcome in univariate analysis according to the Kaplan-Meier method and associated log rank tests: women have a comparable overall survival to that of men in the same age group (75% *vs* 88,5% ; P=0,332). **n**In a relative survival multifactorial model adjusted for stage, ECOG performance status, serum lactate dehydrogenase and two or more extranodal sites, male gender was not found to be an adverse risk factor for patients younger than 52 years (RR 0.893, 95% CI 0.641-1.244). However, the impact of the above-mentioned prognostic factors was significantly correlated with age. In effect, using multiple regression Cox model, we found that Ann Arbor stage III and IV, as well as extranodal involvement of two or more sites, IPI are correlated to worse outcome, with 0.024 *vs* 0.087, 0.009 *vs* 0.844, 0.0001 *vs* 0.687 respectively, P (for age ≥*vs*<52 years), see Figure 1-6.

Table 1.

Table I

		ALL (N=95)			AGE<52 Y (N=46)			AGE≥52 Y (N=49)		
		MEN	WOMEN		MEN	WOMEN		MEN	WOMEN	
TOTAL		57 (60%)	38 (40%)	P	26 (56%)	20 (44%)	P	31 (63%)	18 (37%)	P
STAGE	I-II	20 (21%)	19 (20%)	0,148	12 (26%)	11 (24%)	0,569	8 (16%)	8 (16%)	0,202
	III-IV	37 (39%)	19 (20%)		14 (20%)	9 (19%)		23 (47%)	10 (20%)	
S-LDH	NORMAL	14 (14%)	15 (16%)	0,110	7 (15%)	7 (15%)	0,573	7 (14%)	8 (16%)	0,129
	HIGH	44 (46%)	23 (24%)		19 (41%)	13 (28%)		24 (49%)	10 (20%)	
ECOG	<2	49 (51%)	31 (32%)	0,576	24 (52%)	16 (35%)	0,262	15 (31%)	15 (31%)	0,414
	>2	8 (8%)	7 (7%)		2 (4%)	4 (8%)		6 (12%)	3 (6%)	
EXTRA NODAL	YES	33 (33%)	14 (15%)	0,099	12 (26%)	7 (15%)	0,476	19 (39%)	7 (14%)	0,146
	NO	26 (27%)	24 (25%)		14 (30%)	13 (28%)		12 (24%)	11 (22%)	
IPI	0	5 (5%)	3 (3%)	0,225	4 (9%)	2 (4%)	0,124	1 (2%)	1 (2%)	0,340
	1	11 (11%)	3 (3%)		7 (15%)	2 (4%)		4 (8%)	1 (2%)	
	2	13 (13%)	19 (20%)		7 (15%)	12 (26%)		6 (12%)	7 (14%)	
	3	9 (9%)	6 (6%)		5 (11%)	2 (4%)		4 (8%)	4 (8%)	
	4	14 (15%)	5 (5%)		3 (6%)	2 (4%)		11 (22%)	3 (6%)	
	5	4 (4%)	2 (21%)		-	-		4 (8%)	2 (4%)	
	NA	1 (10%)	-		-	-		1 (2%)	-	
IPI-aa	0	4 (7%)	3 (8%)	0,241	2 (4%)	1 (2%)	0,652	2 (4%)	2 (4%)	0,430
	1	10 (10%)	7 (7,3%)		5 (11%)	2 (4%)		5 (10%)	5 (10%)	
	2	13 (13,6%)	15 (15,7%)		9 (19%)	10 (21,7%)		4 (8%)	5 (10%)	
	3	23 (24%)	7 (7%)		6 (14%)	2 (4%)		17 (34%)	7 (14%)	
	NA	5 (5,2%)	1 (10%)		4 (8,7%)	-		1 (2%)	1 (2%)	

aaIPI, age adjusted IPI, DLBCL, diffuse large B-cell lymphoma, ECOG PS, Eastern Cooperative Oncology Group performance status, Extranodal, involvement of 2 or more extranodal sites, IPI, International Prognostic Index, NA, not available, S-LDH, serum lactate dehydrogenase, Stage, Ann Arbor stage.



Summary/Conclusions: our study clearly demonstrates the impact of age on the survival of patients with LGDB, however gender does not appear to influence patient outcomes.

Bleeding disorders (congenital and acquired)

E988

INCIDENTAL FINDINGS BY TARGETED EXOME SEQUENCING USED TO DIAGNOSE RARE INHERITED BLEEDING DISORDERS-PATIENT'S CHOICES ON INFORMATION AND OUTCOME

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Background: Whole exome sequencing (WES) has the ability to identify numerous mutations within a genome, many of which are not related to the phenotype in question, but they may have clinical ramifications. Incidental findings (IF) are pathogenic alterations in genes, that are not apparently relevant to the diagnostic situation, for which, the sequencing was intended. Scientific and ethics boards recommend, that the informed consent process ensures, that patients understand the possibility that IF will be detected and indicate which (if any) information on IF, they wish to receive. We have implemented WES to diagnose patients with inherited bleeding disorders. Our exome sequencing panel for bleeding disorders only examines specific genes, but with the additional use of search terms (thrombocytopenia, thrombocytopathia and bleeding) incidental findings may occur. Mutations associated with inherited thrombocytopenia may also be associated with haematological cancers. No previous study has described the outcome of IF in this diagnostic setting.
Aims: To examine patient`s choice on information regarding IF and to report the rate of IF.
Methods: Patients were examined by ISTH-BAT score and standard coagulation testing. Prior to WES analysis, patients were given oral and written information concerning the risk and possible seriousness of IF. The pretest information on IF included risk of detecting mutations associated with cancer or brain disease. Patients from Copenhagen were asked to make a pretest written choice of whether they wanted information on: A. All genetic results of clinical significance, including IF. B. No information on IF. C. Provision of IF with known clinical significance and the possibility of treatment or prevention of disease. Patients from Malmoe were asked to make a pretest written choice of whether they wanted information on: A. All genetic results of clinical significance, including IF. B. No information on IF. The Illumina HiSeq 2500 platform was used for targeted exome sequencing. Sequencing was performed on the HiSeq 2500 as paired end (PE) sequencing, 2x101 bases, resulting in approximately 100 M PE reads. Variants were called with a minimum of 10x coverage and exported as.vcf format with approximately 220,000 variants prior to upload in Ingenuity Variant Analysis (Qiagen). Common SNPs were subtracted.
Results: A total of 91 genes associated with bleeding were examined in 107 patients from Copenhagen and 37 patients from Malmoe. The following IF were made: 1. A heterozygous variant in *Telomerase reverse transcriptase (TERT)* (His412Tyr) in a 34-year female with macro-thrombocytopenia. The variant had previously been reported to be associated with reduced telomerase activity, dyskeratosis congenital and aplastic anemia. A telomer analysis performed by an outside lab, showed that leukocytes did not have short telomeres or low telomerase enzymatic activity. It was concluded, that the variant was a non-pathogenic polymorphism and the patient informed correspondently. 2. A heterozygous missense mutation in *SERPINC1* (Pro73Leu) in a 40-year female without thrombosis. It was previously described as a founder mutation in the Finnish population, associated with type II antithrombin (AT) deficiency and increased risk of thrombosis. Her AT III level was normal; no additional function testing was done. It was concluded that due to her haemorrhagic diathesis, she was not at high risk of thrombosis and informed correspondently. 3. A heterozygous *GATA2* mutation (Pro41Ala) previously associated with myelodysplasia and skin cancer in a 36-year female. Several relatives had a history of skin cancer and her aunt had leukemia. She was informed of the increased risk of skin and haematological cancer, offered regular follow-up and advised on skin protection.

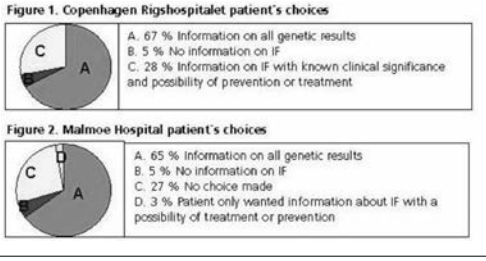

Figure 1.

Summary/Conclusions: IF were discovered in 3/148 (2%). In two separate cohorts of patients from Copenhagen and Malmoe, the choices on information were comparable. Ninety-nine patients (66%) wanted information on all genetic results and 7 patients (5%) declined all information regarding IF. The remaining 42 patients (29%) did either not make a choice or only wanted information, when a disease could be treated or prevented. Targeted exome sequencing of rare inherited bleeding disorders may disclose IF with major impact and/or require additional consultation/validation by an external laboratory or expert. It is of outmost importance that patients understand the consequences of IF, before giving informed consent to WES:

E989

HEMORRHAGIC EVENTS AND PRIMARY HEMOSTASIS DISORDERS DURING TREATMENT WITH IBRUTINIB

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Background: Ibrutinib is a first-in-class Bruton kinase inhibitor (BTK) registered for the treatment of Chronic Lymphocytic Leukemia (CLL), mantle cell lymphoma (MCL) and Waldenström Macroglobulinemia (WM). Increased risk of bleeding events are associated with ibrutinib, mostly grade ≤2. Probable mechanism for this risk could be the role of BTK in platelet activation via GPIb, GPVI and FVW.
Aims: To present our experience regarding hemorrhagic events and hemostasis tests alterations in CLL patients (pts) treated with ibrutinib.
Methods: 10 pts with relapsed CLL were started on ibrutinib between december/2012 and march/2016. Median age was 69 years (range: 42-83 years). 8 of them were male. Median exposure to the drug was 17 months (range 1-38). After 9 to 36 months from initiation of treatment (median 22 months) 6/10 were thoroughly interviewed about personal history of bleeding events and the following laboratory work was performed: factor VIII level, FvW antigen and activity, platelet function analysis (PFA-100^{TM}) with ADP and epinephrine, and aggregation studies with collagen, ADP and arachidonic acid (AA). Along this tests all patients had blood counts with platelets more than 100 x 10e9/L.
Results: 2 out of 10 pts had bleeding events (grade 1 or 2), consisting in spontaneous bruising, in the first weeks of treatment. 4 pts underwent minor invasive procedures with no complications, withholding the drug at least 3 days before and after. 2 pts were on concomitant antiplatelet (1) or anticoagulant (1, LMWH, due to AF) and had no bleeding events. All pts tested (6/6) had normal or slightly elevated levels of F VIII, FvW:Ag and FvW:ristocetin cofactor activity. 2 pts had abnormal PFA-100^{TM} and abnormal aggregations with every agonist. One case had normal PFA-100 ^{TM} while aggregations with collagen and AA were mildly altered.
Summary/Conclusions: Our limited experience shows a moderate risk of minor bleedings consisting with a platelet disfunction. Despite the fact that we have no baseline tests done, alterations in primary hemostasis tests, including PFA-100^{TM} and specially aggregometry could be expected early on after exposure to ibrutinib.

E990

REGULATION OF CD72 EXPRESSION ON CD19+CD27+ MEMORY B CELL BY CD40L IN PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: CD72, a co-receptor of B cell receptor, regulates B cell activation and is involved in B cell-related autoimmune diseases. Despite the knowledge that expression of CD72 on peripheral B cell is aberrant in patients with some autoimmune diseases, it is uncertain whether this aberrant expression is restricted to specific B cell subsets in primary immune thrombocytopenia (ITP). Furthermore, the mechanisms that regulate CD72 expression on peripheral B cell in ITP are unknown.
Aims: This study aimed to examine CD72 expression on subsets of B cells and uncover the regulatory mechanisms of CD72 expression on peripheral B cell in ITP.
Methods: Frequencies of B cell subsets and expression of CD72 and CD40L were determined by flow cytometry in patients with active ITP, patients in remission and health controls. To activate B cells, the following reagents were added at the initiation of culture in vitro, anti-IgM, CD40L, LPS. The following cytokines were used to evaluate their effect on CD72 expression: interleukin-4 (IL-4), IL-10, IL-21, B-cell-activating factors (BAFF). The level of CD72 mRNA was assessed by real-time PCR. The concentration of plasma CD40L was measured by ELISA. Specific anti-platelet autoantibodies were measured by the Pak Auto method.
Results: Patients with active ITP had a significantly higher frequency of CD19+B cells, CD19+CD27+ memory B cell and lower frequency of CD19+CD27- naive B cells than did controls and patients in remission. The mean fluorescence intensity of CD72 on circulating B subcells is upregulated in active ITP patients compared to that recorded in controls and patients in remission. In active ITP patients, the expression of CD72 on CD19+ CD27+B cells was inversely corre-

lated with platelet counts, but there is no difference among groups with different age and gender. Patients with anti-platelet autoantibodies had significantly higher expression of CD72 on CD19+CD27+B cells. In vitro, analysis of B cell activation revealed that the CD40L could induce CD72 significant upregulation on B cells and CD19+CD27+ B cells both in patients and controls. The mRNA levels of CD72 in peripheral blood mononuclear cells activated by CD40L were increased. When used in combination with CD40L, IL-10 and BAFF further enhanced the expression of CD72 on CD19+CD27+B cell, whereas IL-21 reduced CD72 upregulation. CD40L expression on CD4 T cells and plasma CD40L levels were significantly higher in active ITP patients compared to controls. Significant increases in plasma CD40L were correlated with CD72 expression on CD19+CD27+ B cell in ITP patients.
Summary/Conclusions: These data provide evidence that active ITP patients have higher CD72 expression on increased CD19+CD27+ memory B cells, which is associated with platelet counts and autoantibody. Soluble higher CD40L found in active ITP patients might contribute to the increased levels of CD72 on CD19+CD27+ B cell.

E991

THROMBIN GENERATION AND WHOLE BLOOD VISCOELASTIC ASSAYS IN THE MONITORING OF HAEMOPHILIA WITH INHIBITORS

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Background: During the past decade numerous studies using thrombin generation (TGA) and viscoelastic tests (TEG) have been introduced in clinical laboratories, looking for a new perspective on monitoring patients with severe haemophilia complicated with inhibitors.
Aims: We attempted to obtain a more reliable image on global hemostasis of patients with haemophilia A (HA) and high titre inhibitors treated with bypassing agents (BPAs) by using these two new laboratory tools, TGA and TEG.
Methods: Seven persons with HA and high titre inhibitors treated with by passing agents during 12 bleeding episodes, 5 of them in invasive surgical conditions have been evaluated. Reaction time (R), kynetics (K) and maximal amplitude (MA) on thrombelastograph System 5000, and lag time, peak, time to peak (ttpeak), endogenous thrombin potential (ETP), start tail and velocity index have been the focused parameters, interpreted also in connection with the clinical outcomes. The same procedure was performed on 15 healthy volunteer control group.
Results: While traditional coagulometric assay failed giving information on the global hemostatic potential, TEG displayed significantly prolonged R and K ($p<0.01$), with prompt improvement after BPAs administration. The thrombogram revealed also significantly low values ($p<0.001$) for peak and velocity index and prolonged time to peak and start tail, also, with significant ($p<0.01$) long lasting (more than 4 hours) improvement after BPAs administration.
Summary/Conclusions: In our modest experience, the combined use of TEG and TGA has been a useful source for surrogate markers, able providing more informative and reliable data on the impact of BPAs on the hemostatic results in an attempt of a more safe and effective personalised therapy in haemophilia complicated with inhibitors.

E992

ACQUIRED HAEMOPHILIA: A MALAYSIAN OUTLOOK

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Background: Acquired Factor VIII inhibitor is a very rare bleeding condition due to the spontaneous development of autoantibodies directed against factor VIII.
Aims: To identify at the demographics, presenting features and bleeding characteristics of patients with Acquired Hemophilia referred to the Haematology Unit at Ampang General Hospital, over a 10 year period.
Methods: The list of patients with acquired factor VIII inhibitor who were treated from June 2005 to May 2015 was identified and their case notes were then retrieved from the medical records of the hospital.
Results: Between 2005 and 2015, 28 patients from Kuala Lumpur were diagnosed with acquired factor VIII inhibitors. The population of Kuala Lumpur is 1,501,800 (Population Statistics 2003) giving an incidence of 1.8 cases /million/year.The patient demographics are shown in Table 1.Ten patients (36%) had an underlying associated disease. One was postpartum with history of psoriasis, two had systemic lupus erythematosus, three with bullous pemphigoid, two with thyroid disease, one rheumatoid arthritis and one with a malignant lung nodule. In the remaining 18 patients (64%) were idiopathic. All patients had abnormal bleeding with a total of 54 haemorrhagic episodes. 14 patients had life or limb threatening bleeding: postpartum haemorrhage, iliopsoas haematoma, compartment syndrome, arterial puncture, gastrointestinal bleed and intracranial bleed.

Table 1. Demographics of patients with acquired FVIII inhibitor.

Age(yrs) Mean (range)	58.5(27-80)
Gender Male / Female	16 /12
Inhibitortitre(BU)Median(range)	31.5(1.1-1404)
Median Inhibitor titre (BU) in life/limb threatening bleed	9.7
Median Inhibitor titre (BU) in subcutanoues bleed	51.5

Table 2. Sites of Haemorrhages on Presentation.

Site of bleeding	No. of haemorrhages	Percentage (%)
Ecchymoses	28	52
Soft Tissue Haematomas	5	9
Haematuria	3	5
Intracrannial Bleed	1	2
Iliopsoas Hematoma	3	5
Other Intramuscular hematoma	3	5
Compartment Syndrome	1	2
Postpartum Haemorrhage	1	2
GI Bleeding	5	9
Bleeding post procedure / arterial puncture	3	5
Bleeding from injury	1	2

Summary/Conclusions: Over a 10-year period we treated 28 patients with acquired hemophilia with an estimated incidence of 1.8/million/year. The median age of was 58.5 years, with a male preponderance(57%). It was associated with an underlying disease in 36% of cases. Life or limb threatening haemorrhages were seen in 50% of patients. Factor VIII level and inhibitor titre at presentation did not predict severity of bleeding.Mortality rate was 15%; all patients died of complications related to bleeding and treatment. It is important to recognize and diagnose this disorder, as appropriate treatment will give a favorable outcome; failing which it can be fatal.

E993

CONTRIBUTION OF HEMODILUTION, FIBRINOLYSIS, ACIDOSIS, AND HYPOTHERMIA TO DERANGEMENT OF CLOT FORMATION IN THE *IN VITRO* MODEL OF TRAUMA-INDUCED COAGULOPATHY

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Background: Trauma-induced coagulopathy (TIC) develops often after major trauma and may be diagnosed before any therapeutic intervention is provided. Pathophysiology of TIC includes tissue injury and severe hypoperfusion which cause endothelial activation of the protein C pathway and release of tissue plasminogen activator (tPA) resulting in systemic anticoagulation and hyperfibrinolysis. Subsequent hemodilution due to massive transfusions as well as hypothermia and acidosis exacerbate the hemostatic derangement and may lead to uncontrollable postoperative hemorrhage in severely traumatized patients.
Aims: To investigate the relative contribution of hemodilution, fibrinolysis, acidosis, and hypothermia to the derangement of clot formation in the in vitro TIC model.
Methods: The study was approved by the local research ethics committee. Citrated venous blood was obtained from adult healthy volunteers who signed an informed consent form. The in vitro TIC model included hemodilution combined with hyperfibrinolysis, acidosis, and hypothermia. Hemodilution (40%) was produced by mixing the blood with TRIS/saline solution. Fibrinolysis was induced by treating the blood with 165 ng/mL tPA. Acidosis (pH 7.0–7.1) was produced by treating the blood with 1.2 mg/mL lactic acid. Hypothermia (31°C) was achieved by setting the appropriate temperature while running the test. Intact blood tested at 37°C served as control. Clot formation was evaluated using rotational thromboelastometry (ROTEM) device (Pentapharm, Germany). Clotting time (CT, s), maximum clot firmness (MCF, mm) and lysis onset time (LOT, s) were measured using the EXTEM test with 1:50 pre-diluted reagent and presented as Mean±SD.
Results: In control, CT was 349±66 s, and none of the factors (hemodilution alone or in combination with tPA-induced fibrinolysis, hypothermia, and/or acidosis) affected this parameter. In control, MCF was 64±3 mm. Hemodilution reduced MCF to 54±4 mm ($P<0.001$ *vs* control). Combination of hemodilution and hypothermia produced the same effect. Combination of hemodilution and hyperfibrinolysis reduced MCF to 46±7 mm ($P<0.001$ *vs* hemodilution alone). Combination of hemodilution, hypothermia, and hyperfibrinolysis had the greatest inhibitory effect on MCF reducing it to 38±8 mm ($P<0.001$ *vs* combination of hemodilution and hyperfibrinolysis). Acidosis did not modify the effect of the other factors on MCF. No clot lysis was observed in control. Hemodilution did not lead to clot lysis unless hyperfibrinolysis was induced; in these conditions, LOT was 1766±324 s. When additionally combined with hypothermia, LOT was prolonged to 2380±530 s ($P<0.001$). Acidosis had no effect on LOT at either blood temperature.
Summary/Conclusions: Differential effect of TIC constituents on clot formation is revealed. Latent processes that precede clot formation (CT) were not affected by any of the factors studied. Clot strength (MCF) was reduced in the presence of hemodilution, further reduced if combined with hyperfibrinolysis, and maximally reduced if additionally combined with hypothermia. tPA-initiated clot lysis was delayed by hypothermia. Acidosis had no effect on clot formation. The data presented may be helpful to better understanding the pathogenesis of TIC and elaboration of individually-tailored treatment strategy.

E994

DIRECT ORAL ANTICOAGULANTS THERAPY AFTER INTRACRANIAL HAEMORRHAGE WHILE ON VITAMIN K ANTAGONISTS

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Background: Restarting antithrombotic therapy after an intracranial haemorrhage while on vitamin K antagonists (VKA-ICH) treatment is challenging in many cases. When cardioembolic risk is high (CHADS2-Vasc ≥3) antithrombotic therapy should be offered to these patients. The lower rate of intracranial bleeding of direct oral anticoagulants (DOACs) compared to warfarin reported in recent phase III randomized trials for patients diagnosed of non valvular atrial fibrillation (NVAF), probably make these drugs a safer option to treat VKA-ICH patients.
Aims: The objective of this retrospective, observational study is to evaluate the efficacy and safety of restarting oral anticoagulation treatment with DOACs in NVAF patients with a high cardioembolic risk and a previous VKA-ICH episode.
Methods: From October 2012 until January 2016 a total of 23 patients at the Complejo Hospitalario de Navarra were treated with DOACs after a VKA-ICH episode while on treatment with the VKA acenocoumarol (sintrom®). We analysed the following variables: INR on admission, the time in the therapeutic range (TTR), the rate of spontaneous or posttraumatic ICH, the use of reversal drugs on admission, the median days to restart oral anticoagulation, and the cumulative incidence of a second ICH following the restart of anticoagulation treatment.
Results: 23 patients with intracranial hemorrhage during treatment with VKA restart anticoagulation with a DOAC at our institution. The median age was 78 (68-89), 56.5% were male. All patients had been treated with a VKA antagonist for NVAF with a median CHADS2VASc score of 4 (2-7) and a median HASBLED of 3 (1-5). The mayority of ICH events ocurred spontaneously (65.2%). In contrast, 34.8% ICH occurred after trauma. Only one patient in our cohort had a lobar ICH. International normalized ratio (INR) on admission was infratherapetic in 26% (n=6), therapeutic in 52.2% (n=12) and supratherapetic in 21.7% (n=5). The mayority of cases were managed at the Emergency room with non activated prothrombin complex concentrate (PCC) (73.9%) as reversal agent with a median dose of 24.6 UI/kg (9.68-37.5) in combination with 10 intravenous mg of vitamin K. (Table 1). The median days to restart oral anticoagulation with a DOACs after an ICH was 42 (4-521). 17.4% rivaroxaban (n=5), 4.3% dabigatran (n=1) and 73.9% apixaban (n=17). With a median follow up of 32.88 months (3-88.58) the cumulative incidence of a second ICH was 7.1% (standard error 6.9%). Only 2 patients in our cohort had recurrent spontaneous ICH, one under rivaroxaban 15 mg once daily and a second one while on apixaban 2.5 mg bid, at 19 and 43 months after restarting DOACs, respectively. No thromboembolic event was recorded during the follow up in our cohort. (Table 2).

Tables.

Table 1: Patient characteristics

	ICH (n=23)
Sex, male (%)	13 (56.5%)
Age , median (range)	78 (68-89)
CHADS2 VASc, median (range)	4 (2-7)
2-3 points (%)	10 (43.5%)
≥ 4 points (%)	13 (56.5%)
HASBLED, median (range)	3 (1-5)
INR on admission, range	2.15 (1.6 - 3.5)
Therapeutic values (%)	12 (52.2%)
Infratherapeutic values (%)	6 (26%)
Supratherapeutic values (%)	5 (21.7%)
TRT	
≥ 65% (%)	13 (56.5%)
< 65% (%)	4 (17.4%)
Trauma (%)	8 (34.8%)

Table 2: DOAC treatment

Switch to (%)	
Rivaroxaban	5 (17.4%)
Dabigatran	1 (4.3%)
Apixaban	17 (73.9%)
Therapeutic doses (%)	18 (78.3%)
Restart of DOAC, days, median (range)	42 (4- 521)

Summary/Conclusions: The use of direct oral anticoagulants after an ICH is a safe option for long-term anticoagulation treatment in patients with non-valvular atrial fibrillation at high risk of ischaemic stroke. However, further studies are required to assess longer-term efficacy and safety of DOAC after an ICH.

E995

BEVACIZUMAB AS TREATMENT IN REFRACTORY HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Background: Hereditary Hemorrhagic Telangiectasia (HHT) is a dominantly inherited vascular disorder characterized by recurrent epistaxis, cutaneous telangiectases and visceral arteriovenous malformations (AVMs) that affect many organs: lungs, gastrointestinal tract, liver and brain. Diagnosis is definitive if at least 3 of the following are fulfilled: spontaneous and recurrent epistaxis, telangiectasia, family history and visceral lesions. Although the disease is highly penetrant, during the first years of life, only 50% of individuals have symptoms. Although 80% of patients have gastric or small bowel telangiectases, only 25-30% of patients develops symptomatic gastrointestinal bleeding. Treatment of these patients is based on iron therapy and secondary use of medications such as Hormone Replacement Therapy, antifibrinolytic, tamoxifen, interferon, sirolimus and endoscopic cauterization. There are few clinical studies that clarifies therapeutic measures in these patients, and that is why the best available scientific evidence is based on case reports.
Aims: This is the case of a patient with refractory HHT, who presented severe gastrointestinal bleeding, which was successfully treated with Bevacizumab
Methods: A 67 year-old man with history of Chronic Obstructive Pulmonary Disease, hypertension, diabetes mellitus, hypothyroidism, right-sided heart failure and HHT which was diagnosed 11 years ago. HHT was initially characterized by epistaxis, skin telangiectases and occasional events of gastrointestinal bleeding. The patient had received repeated nasal and esophagogastric cauterizations, but there was scarce response and it was necessary recurring blood transfusions. Diagnosis was confirmed, and multiple palate, esophagus, stomach and duodenum bleeding angiodysplasias were found. Laboratory tests demonstrated iron deficiency and anemia. The patient was on parenteral iron supplementation every week, without obtaining any benefit.
Results: The patient was admitted every week in emergency service with hemorrhagic shock (Hemoblobin (Hb) levels between 3.5 to 6 g/dL), receiving between 3-4 blood units every week. The patient received oral thalidomide 200 mg/day during 2 months with no benefit, persisting low Hb levels despite numerous transfusions, and he was diagnosed with hepatic and pulmonary AVMs. Bevacizumab was initiated 5 mg/kg every 14 days for 6 doses. Hb rose to14 g/dl during the first week of treatment and it has been stable since then, the patient has been independent of blood transfusions for 8 months, decreasing hepatomegaly and right heart failure.
Summary/Conclusions: Bevacizumab is a humanized recombinant monoclonal antibody against VEGF, which plays an important role in angiogenesis. It inhibits endothelial cell proliferation thus preventing vessel growth and causing regression of existing vessels. HHT is characterized by high serum and tissue levels of VEGF, that is why Bevacizumab is a reasonable agent to consider for the treatment of HHT. To our knowledge, this is one of the first cases reported of intractable HHT with gastro-intestinal hemorrhage efficiently treated using bevacizumab. Some cases of epistaxis with anemia successfully treated with bevacizumab has been reported. Others works have reported that administration of bevacizumab in patients with HHT associated with severe hepatic AVMs and high cardiac output, was associated with reversal of high-output cardiac failure related to hepatic shunting. Nevertheless, longer follow-up studies are necessary to determine the duration of HHT efficacy and whether maintenance therapy is required.

E996

PREDICTION OF INHIBITORS IN UKRAINIAN HAEMOPHILIA PATIENTS

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Background: Replacement therapy in hemophilia patients (pts) is complicated by the factor VIII (IX) inhibitors among 30% of hemophilia A pts and 3-5% of hemophilia B pts. The treatment of bleeding and elimination of inhibitors is complicated, costly and not always successful.
Aims: To develop a simple score that stratifies hemophilia pts according to their risk of developing inhibitors.
Methods: 135 pts with hemophilia A and B was divided into two groups: I -74 pts with inhibitor and II –61 pts without inhibitor. We analyzed 16 risk factors to develop inhibitor: type of hemophilia (A or B), severity, age, age at diagnosis, hereditary or sporadic, family history of inhibitors, intensive treatment at initial treatment- days of exposure (ED) /1 episode), age at first exposure to FVIII/IX, reason for first treatment with FVIII, FVIII/IX product type, prophylaxis or "on demand" treatment, factor concentrate switching, significant and life-threatening bleeding localization; surgery: I-urgent or selective; II - major or minor, the purulent complications. To examine the relationship between risk factors and the likelihood of inhibitor development, we used regression models of discrete choice. Specifically, we estimate the range of one factor binomial choice models to study the individual effects of each factor on the probability of inhibitor development. In addition, we analyze the joint impact of risk factors using multiple logit regression that allows to explore the effects and importance of each factor, controlling for the presence of other factors. Adequacy of logit models was conducted using chi-square test, and the significance of regression coefficients was based on Student Wald statistics. Finally, we calculated the theoretical values of probability of an inhibitor development for each patient and ranges (95% CI) of predicted risk of inhibitor development in pts with the value of the dependent variable "yes" and "no".

Results: The first model includes the following factors: age, clotting factor concentrate switching, FVIII/IX product type, purulent complications and the number of ED / 1 episode of bleeding. At 5% significance level, the type of surgery (minor/major) as well as positive "inhibitory" history are added. At the 10% significance level, the model additionally contains a variable characterizing the urgency of surgery (urgent or planned). In this approach, the factors that determine the type of hemophilia, age, diagnosis and life-threatening bleeding are not significant and therefore, we do not include them in the final multivariate regression. The number ED / 1 episode has the highest impact on the probability of inhibitor development: if it increases, the likelihood of inhibitor development increases by 27%. For patients who changed the type of concentrates, the likelihood of inhibitor development increases by 23%. The effect of the FVIII/IX product type, the age and the type of the surgery - is negative and significant, while purulent complications and "inhibitory" history result in the increase in the likelihood of inhibitor development by 21% and 12%, respectively. This multivariate logit model allows to predict the likelihood of developing an inhibitor for a pts based on the information about the values of each of these factors.
Summary/Conclusions: According to our model, the factors associated with treatment have the highest impact on the probability of inhibitor development. Based on the results, reducing the frequency of inhibitors can be achieved by changing the approaches to the treatment of pts with hemophilia.

E997

ACQUIRED ISOLATED PROTHROMBIN DEFICIENY IN A PATIENT WITH FOLLICULAR LYMPHOMA SUCCESSFULLY TREATED WITH IMMUNOCHEMOTHERAPY

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Background: Acquired haemophilia is rare bleeding disorder, that is caused by autoantibodies against clotting factors. Approximately 50% of cases are associated with either malignancies, autoimmune diseases or pregnancy, respectively. However, half of the cases remain idiopathic. While in the vast majority autoantibodies are directed against factor VIII leading to acquired haemophilia A, acquired prothrombin (factor II) deficiency is a rare event and information on treatment outcome is scarce.
Aims: Here we report on a case of a patient with follicular lymphoma and an acquired isolated prothrombin deficiency suffering from severe bleeding complications, who was successfully treated with immunochemotherapy.
Methods: A 73-year old female patient was diagnosed with a grade I-II asymptomatic follicular lymphoma stage IV (intermediate risk according to FLIPI) in July 2015. In November 2015 the patient was admitted to our hospital due to macro haematuria and pain in the distal right thigh. The patient did not remember any trauma but MRI showed an intramuscular haematoma. Laboratory testing revealed decreased Quick values (32%) and a prolonged activated partial thromboplastin time (aPTT: 64 sec.) as compared to August 2015 (Quick 100%; aPTT 26 sec.) Since the patient received no anticoagulant treatment and diet-related vitamin K deficiency could be ruled out, single factor analyses were performed and showed an isolated prothrombin deficiency, which was confirmed by repeated analyses. Lupus anticoagulants were negative, as were analyses with regard to von-Willebrand-Jürgens syndrome. Moreover, the patient developed a haemoglobin relevant gastrointestinal bleeding with gastroscopy showing a diffuse bleeding in the fundus reflecting the haemorrhagic diathesis due to the coagulopathy. The patient received repetitive prothrombin substitution with prothrombin complex concentrates leading to transient stabilization of the coagulation system. Eventually she was subjected to successful surgical revision of the muscular haematoma. Although inhibitory antibodies were not detectable we assumed an association of the coagulopathy with the concomitant FCL and initiated an immunochemotherapy with rituximab and bendamustine in December 2015.

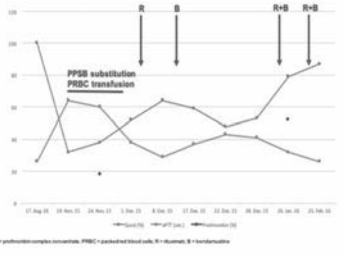

Figure 1.

Results: Repetitive evaluation of coagulation parameters and prothrombin activity showed a successive improvement of the respective parameters after the first cycle of treatment (Figure 1). Since December 2015 no prothrombin substitution was necessary and prothrombin activity increased from 18% in November 2015 to 52% by January 2016. Currently, the patient has no signs of haemorrhage or relapse of the coagulopathy with continuous improvement of the global coagulation parameters.
Summary/Conclusions: Although rare events, isolated clotting factor deficiencies should be considered in cases of unexplained coagulopathy in patients with malignancy in general and malignant lymphoma in particular. Treatment of the underlying lymphoma by immunochemotherapy can reverse the haemophilic state in patients with NHL.

E998

THE BLEEDING SPECTRUM OF 21 EGYPTIAN PATIENTS SUFFERING FROM CONGENITAL AFIBRINOGENAEMIA: A 10-YEAR RETROSPECTIVE STUDY

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Background: Congenital afibrinogenaemia has an estimated prevalence of one in 1,000000. The afibrinogenemic patients' phenotype is mainly characterised by bleeding but thromboses, bone pain and delayed wound healing have also been reported
Aims: To describe the phenotypic spectrum and bleeding incidence of an Egyptian afibrinogenaemic cohort
Methods: All patients with a confirmed congenital afibrinogenaemia (based on fibrinogen levels and genotype) were included. Patients were followed regularly every 1-12 months over a period of 1 to 10 years. All bleeding episodes and fibrinogen replacements were recorded.
Results: Twenty-one patients (13 males, 8 females) with a mean age of 12.9 years from 15 consanguineous families were included.They were followed for a median period of 9 years.The bleeding phenotype was characterized by umbilical bleeding (65%), oral bleeding and epistaxis (45%), central nervous system haemorrhage (25%), joint bleeds (25%), hematochezia (10%), peritoneal bleeding (10%), hematuria (5%), spinal hemorrhage (5%), muscle bleeds (5%). Most women in child bearing period reported menorrhagia (75%). In addition, 15% of patients experienced a delayed wound healing and all had post-traumatic bleeding. Half of patients (57%) had atypical bleeds in the form of spontaneous unexplained severe bone pain in forearms and lower limbs, relieved by fibrinogen replacement. Overall, the incidence of severe bleeding varied from 1 to 5 bleeds/pt/years although one patient had weekly hemorrhages. No patients had arterial or venous thrombosis. All patients were on demand therapy but one with recurrent cerebral bleeding on biweekly prophylaxis. Replacement fibrinogen included cryoprecipitate 15-20 mg/kg/d for mild to moderate bleeds and 30mg/kg/12-24 hours for severe/serious bleeds for a period ranging from 1-5 days.
Summary/Conclusions: Our cohort showed a wide phenotypic spectrum with high prevalence of cerebral bleeding. The absence of thrombosis is possibly explained by the young age of patients. We confirmed that bone pain is a frequent complication of afibrinogenaemia. Optimal management of this symptom require further investigations.

E999

HEALTH RELATED QUALITY OF LIFE IN GREEK CHILDREN WITH HAEMOPHILIA AND THEIR CAREGIVERS

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Background: Health-related Quality of Life(HRQoL) has become an important outcome in every day clinical practice.
Aims: The aim of the study was the assessment of health-related quality of life (HRQoL) in children with haemophilia and their caregivers in Greece, using a disease-specific questionnaire and the investigation of demographic and clinical factors that affect their HRQoL.
Methods: The study was conducted in two national pediatric haemophilia centers, in Thessaloniki and Athens [and included 45 children and their parents] The Greek version of the Hemophilia-Specific Quality of Life Index (Haemo-QoL) questionnaire was administered to 45 pediatric patients aged 4 to 16 years and their parents. The latter filled in a questionnaire with demographic data (child's and parent's age, residence, number of siblings-whether sick or not, marital status, education level, employment) and clinical data of the patient, namely type of haemophilia, severity, presence or history of inhibitor, type of treatment, number of haemarthrosis over last 12 months, presence of chronic pain). Statistical analysis of the results was performed using SPSS 19.0.
Results: Overall, patients and caregivers had a mean age of 10.46±4.3 and 39.51±7.4 years respectively. 86.7% of patients had haemophilia A and 77.8%

were on prophylaxis treatment. HRQoL scores were found to be widely below 40 for children and below 50 for their caregivers in all dimensions. "Family" and "Treatment" had the highest scores (mean scores 57.7 and 46.2 respectively), suggesting reduced quality of life in these dimensions in age group 4-7. The most impaired domain of Haemo-QoL in age group 8-12 was "Perceived Support" (mean score 40.63), while the older children were more impaired in "Family" and "Treatment", but much less so than the younger ones (mean scores 36.1 in both domains). The least impaired dimensions were "Self-view", "Other people" and "Relationships" among the three groups. Cronbach's alpha coefficient was assessed for every subscale and in total, suggesting sufficient/very good internal consistency reliability of the Greek version of Haemo-QoL. Among Haemo-QoL subscales, "Perceived Support" in age group 8-12 and "Sports/School" and "Relationships" in the adolescent group were found strongly associated with the number of haemarthrosis over the previous 12 months.

Summary/Conclusions: The use of a disease specific QoL tool showed that children and their caregivers report a relatively good quality of life, with "family" and "treatment" being the most impaired especially in the younger age group. The number of haemarthrosis over the previous year is a clinical characteristic which impairs some of the aspects of quality of life, especially in the adolescent group. These data are important for the future planning of treatment regimens as well as the psychological support of patients and their families.

E1000

ANTIPHOSPHOLIPID ANTIBODIES IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA

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Background: Presence of antiphospholipid antibodies (APAs) has been observed in children with immune thrombocytopenic purpura (ITP), but their role for the outcome of disease is controversial.

Aims: The aim of this study was to evaluate the relation between APAs and the clinical course, labarotory findings, response to treatment and prognosis of ITP in childhood.

Methods: The cross-sectional study included to patients with newly diagnosed ITP aged between 0-18 years, in the period of time between March 2014 and March 2015, at our centre. Clinical and laboratory findings, medical treatments, and the course of the diseases were recorded for all patients. At the time of diagnosis, anti-phospholipid antibodies including lupus anticoagulant, anticardiolipin antibodies (aCL), anti-beta-2 glycoprotein I antibodies were studied. The patients who have positive results for APAs at diagnosis were examined again for APAs at 12th week of follow-up period.

Results: Forty children (21 (52.5%) females and 19 (47.5%) males) with newly diagnosed with ITP were enrolled the study. APA levels were positive at 12 patients (30%) at the time of diagnosis. Among the APA positive patients, only one patient had all of three APAs, one patient had both aCL IgM and LA, 10 patients had single positive results for APAs (figure 1). Among APA positive patients, the positivity of anti beta 2 glycoprotein I, aCL and LA were 58.3%, 16.7%, and 8.3% respectively. After 12 week, only 3 of these 20 cases were still positive for APA. One of them was positive both LA and anti beta 2 glycoprotein I, and two of them were positive for anti beta 2 glycoprotein I IgG. There was not a significant difference between APA positiveness (at the time of diagnosis and control) and gender groups, platelet counts, IgG levels or course of disease. At the time of diagnosis, mean age of APA positive patients was significantly higher than in APA negative patients ($p<0.05$). According to age interval; there was three patients aged between 0-1 year and none of them were APA positive. At the time of diagnosis, APA positiveness was found in 7 of 28 (25%) patients aged between1-9 years, and 5 of 9 patients (55.5%) aged between 9-18 years. There was no relationship between APAs and treatment response or outcome of disease.

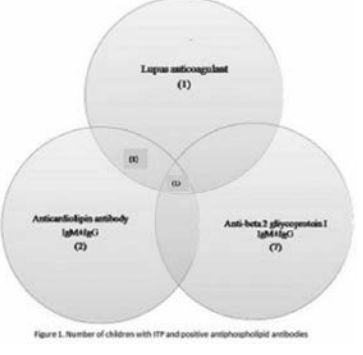

Figure 1. Number of children with ITP and positive antiphospholipid antibodies

Figure 1.

Summary/Conclusions: Unlike in adults, pediatric studies about APAs and ITP are limited. APAs may be present in children with ITP. It may be related to underlying viral infections or idiopathic, and more common in adolescence. The persistence of APAs may contribute to thrombotic complications in the future. More comprehensive studies are needed to determine the relationship between APAs and İTP.

E1001

FUNCTIONAL PLATELET ACTIVITY ANALYSIS IN CHILDREN WITH BLEEDING

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Background: Diagnosis in a bleeding child without thrombocytopenia or clotting factor deficiency is always challenging. In these cases using a platelet flow cytometry (PFC) method might be helpful because it can detect several glycoprotein deficiencies on platelet surface and confirm the diagnosis of platelet dysfunction. Here we present a case series of patients with bleeding due different platelet disorders, established by a modification of PFC method-functional platelet activity analysis (FPAA).

Aims: to asses the role of a modification of platelet flow cytometry (PFC) method-functional platelet activity analysis (FPAA) in children with bleeding.

Methods: 32 patients (1-17 yo) with mild to severe bleeding without von Willebrand disease, factor XIII deficiency, or other coagulopathy were included. Whole blood platelets were studied by FC either in native or activated with collagen-related peptide (0.18 µg/ml) and thrombin receptor activating peptide (12.5 µM). Fluorescencently labeled antibodies against CD42b, CD61 and PAC-1, phosphatidylserine (PS) and CD62p were used; dense granule (DG) release was studied using loading with mepacrine.

Results: After FPAA from 32 children 2 pts were excluded because of normal results and 1 because of preanalytical issues. From those 29, 4 pts were diagnosed with CD61 deficiency, and 25-with different granule defects. Isolated deficiency or mobilization defect of dense and alpha granules was found in 9 and 4 children, respectively. Combined storage pool deficiency/mobilization defect (CSPD/MD) was diagnosed in 8, while we found 1 combination of CSPD/MD and PAC-1 deficiency, and 3 combinations of CSPD/MD and PS deficiency.

Summary/Conclusions: Different platelet disorders could be found in up to 93,5% of patients with bleeding without clotting factor deficiency. Most of the alterations include storage alpha/dense granule pool deficiency/mobilization defect or CSPD/MD.

E1002

SAFETY AND EFFICACY OF B-DOMAIN DELETED 3RD GENERATION RECOMBINANT FACTOR VIII (GREENGENE FTM) IN KOREAN PATIENTS WITH HAEMOPHILIA A: DATA FROM A POST-MARKETING SURVEILLANCE STUDY

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Background: New B-domain deleted 3rd generation recombinant factor VIII (GreenGene F™, beroctocog alfa) was launched in 2010.

Aims: This clinical trial evaluated the safety and efficacy of GreenGene F™ in patients with haemophilia A over a period of 12 months.

Methods: From July 2010 to July 2014, a total 136 hemophilia A patients were enrolled in the post marketing surveillance (PMS) study and were analyzed for safety of the drug. Among them, 114 patients were analyzed for efficacy. Subjects with differing haemophilia A severities and medical histories were monitored during 12 months of prophylactic and/or on-demand therapy. Efficacy was rated by their doctors with 4 scales as excellent, good, moderate, or no effect.

Results: Among 135 evaluable subjects, 85 (63.0%) had severe haemophilia, 35 (25.9%) had moderate haemophilia and 15 (11.1%) had mild haemophilia. Excellent/good efficacy rate was 91.3% for hemostasis and 89.4% for hemorrhage prevention. Twelve subjects reported 13 adverse drug reactions (ADRs), which were recovered without sequelae. The frequent ADRs were gastrointestinal disorders, nervous system, vascular disorders and general disorders. In 113 previously treated patients (PTP), two patients (1.8%) developed inhibitors after intensive FVIII treatment for surgery and hematuria, respectively.

Summary/Conclusions: The results of this PMS study support that GreenGene F™ is safe and efficacious in the treatment and prevention of patients with hemophilia A. The results of this study are consistent with the previously published GreenGene F™ studies.

E1003

FACTOR XI DEFICIENCY IN PREGNANCY: THREE CASE REPORTS

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Background: Management of pregnant women with factor XI deficiency (F11D) poses a challenge to the clinician because of both the variabile uterine bleeding during labor and the risk associated with factor replacement. Hemoleven® is a factor eleven (FXI) concentrate processed by deep filtration and ion exchange chtomatography, with double viral inactivation performed using solvent detergent and nanofiltration. It represents a good treatment for protecting against bleeding, but the potential risk of thrombosis shoul be considered.
Aims: We present the cases of three women with F11D and a history of bleedings, where the efficacy and safety of Hemoleven® treatment was assessed during pregnancy and labor.
Methods: During labor, the drug was administered together with tranexamic acid.
Results: In case 1 the woman was diagnosed as F11D when she was 10, with a plasma FXI level less than 1% and an omozygote phenotype. The 34 year old primagravidae had an uneventful pregnancy, and she was admitted at 39+1 weeks of gestation. When cutting the umbelical cord, antihaemorragic prophylaxis was performed with 1 g of tranexamic acid, intravenously injected, and 1000 UI of Hemoleven. 1 g of tranexamic acid was then administered every 8 hours, from the first to the eighth day post-partum, while 1000 UI of Hemoleven were given during the second and the fifth day post-partum.In case 2, a 26 year old primagravidae had an uneventful pregnancy, but she was admitted at 38+4 weeks of gestation due to a very big condylomatosis. She was diagnosed as F11D at the age of 17, with a plasma FXI level of 42% and an heterozygote phenotype. Despite FXI level, she had an hemorragic phenotype. Caesaren section was planned and performed at the day of admission, in general anesthesia. 30 minutes before the delivery started, 1000 UI of Hemoleven were infused, and 1 g of tranexamic acid was administered intravenously ad the beginning of the procedure. After caesarean section, 1 g of tranexamic acid was given every 8 hour till the seventh day.In case 3, a 36 year old woman had an uneventful second pregnancy, and she was admitted at 39 weeks of gestation. She was diagnosed as F11D at the age of 31, with a plasma FXI level of 30%. Vaginal delivery was induced with oxitocin. After umbelical cord excision, 1000 UI of Hemoleven were infused and 1 g of tranexamic acid was given intravenously. Subsequently, 1 g of tranexamic acid was then administered every 8 hours, from the first to the seventh day post-partum.
Summary/Conclusions: Neither post-partum haemorragy nor thrombotic events were evident in all the three cases, thus confirming safety and efficacy of clotting factor eleven concentrate. Hemoleven® dose and duration of treatmen was alway carefully decided before delivery, with a management plan shared between haemostasis experts, obstetrics, and the patients. Considering our experience, the benefit/risk ratio of Hemoleven® should be weighted and management tailored to each individual patient.

E1004

SERUM CYTOKINE AND CHEMOKINE LEVELS IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia (ITP) is an acquired immune disorder derived from disruption of platelet production and removal from blood circulation, usually as a consequence of autoantibody and cytotoxic T-lymphocyte development against them, which ultimately results to the phagocytosis of the trapped platelets from monocytes. Onset of ITP in children is often triggered by viral infections, infectious diseases or vaccination. In pediatric patients with ITP, as in adults, impaired immunity by deregulation of the balance of Th1/Th2 (T-helper) and of Tregs (regulatory T cells) is referred. The cytokines which are secreted by activated lymphocytes control the immune system either by stimulating or inhibiting the activity of cells but simultaneously they are involved in the production of antiplatelet antibodies or other cytokines and/or chemokines.
Aims: The aim of our study was to determine the serum levels of specific Th cytokines and chemokines in children with ITP and to correlate them with clinical parameters.
Methods: The group of patients consisted of 18 children with ITP (8 male,10 female) of a mean age at investigation 5.46 years. ITP was chronic in 6/18 children, while acute/persistent was in 12/18 (duration of thrombocytopenia>12 or<12 months, respectively). None but one patient had received any treatment during the last 3 months. The control group was constituted by 37 children of preschool age. Serum levels of cytokines IL-1a, TNF-a, IL-1b, IL-10, IL-12p40, hsIL-6 and chemokines of macrophages such as IL-8 (CXCL8) and MCP-1 (CCL2) were measured in patients and controls using the cytokine-chemokine-MAP technology which is a bead based multiplex assay using the Luminex. Inform consent was obtained from the parents of both groups.
Results: There was no significant difference in the concentrations of IL-1a, Il-1b, IL-10, IL12p40 and the chemokine IL-8 among the two groups, except of the concentration of TNF-a which was found quite lower among patients and controls. Furthermore, only the levels of TNF-a differed remarkably between children with acute/persistent compared to children with chronic ITP ($p<0.05$). A significant reduction of hsIL-6 (1.0±0.2 *vs* 1.6±0.2pg/mL, $p<0.05$) and MCP-1 (252.7±20.0 *vs* 408.5±22.2pg/mL, $p<0.001$) was noticed in children with ITP.
Summary/Conclusions: The current study did not seem to confirm the hypothesis of the overall increase of proinflammatory cytokines in children with ITP. The higher values of TNF-a in the initial phase of the disease could be interpreted as a response to inflammation. The finding of the dramatically reduced chemotactic protein MCP-1 (CCL2), possibly reveals deficient response in the ability to control inflammation, a conclusion reinforced by a recent study in murine models of human ITP[1]. The exact role of various cytokines/chemokines in the deregulation of the immune system, sush as occurs in autoimmune diseases, remains to be investigated, particularly in children which are often on inflammatory process. Well-designed studies with a larger number of children with ITP and larger subsets of patients at different stages of the disease, as well, which will investigate a wider range of cytokines, may provide useful answers in the future.

Reference

1. Leontyev *et al.* Transfusion, 2014; 54:2871.

LB2246

COMBINED DEFICIENCY OF FACTOR V AND VIII CAUSED BY TWO NOVEL MUTATIONS AT MCFD2 GENE

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Background: Combined deficiency of coagulation factor V and VIII (F5F8D) is a rare autosomal recessive disorder characterized by a mild-to-moderate bleeding tendency with the reduction of the two factors in plasma. Mutations in LMAN1 and MCFD2 gene account for all F5F8D patients.
Aims: To identify the molecular mutation causing F5F8D in a Chinese pedigree.
Methods: The activated partial thromboplastin time(APTT), prothrombin time (PT) and coagulate activities of coagulation factors of the proband and her parents were detected for diagnosis. PCR was used to amplify the exons and intron/exon boundaries of LMAN1 and MCFD2 genes . The amplicons were sequenced directly and aligned with software chromas 2.
Results: The proband's APTT, PT, FV:C and FVIII:C was 75s, 17s, 9.9% and 5.9%, respectively, while those parameters of her parents were all within the normal range. Sequencing the 13 exons of the LMAN1 gene and 4 exons of the MCFD2 gene from the patient revealed two novel missense mutations. The first one was a heterozygous mutation: g.35T>A, causing the Leu12Glu in exon2 of MCFD2. The second one was a homozygous mutation: g.398A>T, leading to the Asp133Val in exon 4 of MCFD2. The two mutations Leu12Glu and Asp133Val were identified in the compound heterozygous state in her parents.
Summary/Conclusion: The two novel mutations might be the molecular pathological mechanism causing F5F8D in this pedigree.

Bone marrow failure syndromes incl. PNH - Biology

E1005

FALSE HOMOZYGOUS HLA GENOTYPING RESULTS DUE TO LOSS OF HETEROZYGOSITY IN ACQUIRED APLASTIC ANAEMIA

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Background: The pathogenesis of acquired aplastic anaemia is driven by T cell-mediated destruction of hematopoietic progenitors. Clonal evolution is known to occur in around 70% of patients. This includes around 12% with loss of heterozygosity (LOH) of chromosome 6p, involving the human leukocyte antigen (HLA) locus. Loss or down-regulation of HLA class I antigens is a way to escape cytotoxic T-cell surveillance. LOH can appear even without previous therapy. In acquired aplastic anaemia it is well studied as a biological phenomenon. LOH appears to have no impact on the course of the disease nor does it predict response to treatment, as results are contradictory in this matter.
Aims: The aim of this case report is to draw attention on possible false HLA genotyping in acquired aplastic anaemia prior to allogeneic hematopoietic stem cell transplantation.
Methods: Repeated HLA genotyping was performed on peripheral blood using flow multiplex DNA typing and sequence-based typing.
Results: We report false HLA genotyping results due to loss of heterozygosity in a 59-year-old female patient of Caucasian origin, presenting with very severe aplastic anaemia. At diagnosis we obtained heterozygous results (A*24, A*33, B*07, B*14, C*07, C*08, DRB1*13, DRB1*15, DQB1*03, DQB1*06). Bone marrow karyotype was normal. After treatment with rabbit anti-thymocyte globulin+cyclosporine, not reaching a partial response at 6 months, we repeated HLA genotyping in order to search for an unrelated donor, because there was no HLA compatible sibling. This time we obtained homozygous results (A*24:02, B*07:02, C*07:02, DQ*06:02,DR*15.01). Repeated testing confirmed loss of HLA genotype heterozygosity. New bone marrow karyotype didn't show alterations in chromosome 6. Monosomy 7 was found, compatible with development of high risk myelodysplastic syndrome-associated cytogenetic abnormalities.
Summary/Conclusions: When homozygous HLA genotype results are obtained in acquired aplastic anemia, confirmatory testing on other somatic cells (eg. buccal swab) is needed to avoid hematopoietic stem cell transplantation with an unmatched donor.

E1006

PREVALANCE OF CHROMOSALLY-INTEGRATED HERPESVIRUS 6 IN PATIENTS WITH APLASTIC ANEMIA

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Background: Aplastic anemia (AA) is a rare heterogeneous bone marrow failure syndrome. It is hypothesized that in most cases AA is an immune-mediated disease. One third of patients, especially those who do not respond to immunosuppressive therapy, have shortening of telomeres in there leucocytes. Mutations in genes responsible for telomere repair proteins are present in some of these patients. Human herpesvirus 6 is one of the herpesviruses which establish latent infection. It is shown that reactivation of latent HHV-6 can have direct suppressive effects on hematopoietic progenitors which can be the cause of bone marrow suppression after hematopoietic stemcell transplantation. Besides latent infection, HHV-6 can integrate in the human DNA at the telomeric region which results in germ-line transmission of the HHV-6 genome. Chromosomally integrated HHV-6 (ciHHV-6) can be found in less than 1% of the population.
Aims: We performed an exploratory study to investigate the prevalence of ciHHV-6 in patients with AA in order to determine whether ciHHV-6 represents a risk factor for developing AA.
Methods: Between 2008 and 2015 101 patients were treated for AA at the University Medical Center Groningen in The Netherlands. Whole blood samples of 53 of these patients that were initially used for clinical purposes were available for analysis by quantitative PCR (qPCR) for the presence of HHV-6 DNA.
Results: Of the 53 samples 11 patients had detectable HHV-6 DNA. Ten patients had a low HHV-6 viral load with median concentration of $1.9x10^2$ copies/ml (range $1.2x10^6$-$2.3x10^4$ copies/ml). One patient had a high viral load with $5.2x10^6$ copies/ml suggestive of ciHHV-6. Viral chromosomal integration was subsequently proved by demonstrating HHV-6 DNA in hair follicles of this patient. In our cohort the prevalence of ciHHV-6 was 1.9%, which is not above the expected incidence of the general population.
Summary/Conclusions: The prevalence of ciHHV-6 in patients with AA is similar to that of the general population. It is therefore unlikely that ciHHV-6 is a risk factor for AA.

Bone marrow failure syndromes incl. PNH - Clinical

E1007

PROSPECTIVE MULTICENTRIC EVALUATION OF THE CURRENT MEDICAL INDICATION FOR PAROXYSMAL NOCTURAL HEMOGLOBINURIA DIAGNOSTIC SCREENING

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Background: Although consensus guidelines have been proposed in 2010 for the diagnostic screening of paroxysmal nocturnal hemoglobinuria (PNH) by flow cytometry (FCM), so far no study has investigated the efficiency of such medical indications in multicentric *vs.* reference laboratory settings.
Aims: To evaluated the efficiency of the current consensus medical indications for diagnostic screening of PNH by FCM
Methods: Overall, information about 3,938 samples from an identical number of individuals prospectively submitted between January 2011 and December 2014 for diagnostic screening of PNH by flow cytometry was collected at 24 flow cytometry laboratories in Spain which participate in the PNH-External Quality Assurance Program of the Iberian Society of Cytometry (1,718 samples) plus one reference laboratory in Sao Paulo, Brazil (2,220 samples). The following GPI-associated markers were analyzed: FLAER (analyzed in 87% of the cases), CD14 (98%), CD16 (37%), CD24 (93%), and/or CD157 (5%). In those cases with GPI-deficient mature neutrophils and monocytes, expression of CD59 (100% of cases) was also analyzed on red blood cells.
Results: Overall, diagnostic screening based on consensus medical indications was highly efficient (567 PNH^+/3,938 screened cases; 14% PNH^+ samples) both in the multicenter setting in Spain (10%) and reference laboratory in Brazil (16%). Although GPI-deficient cells were found within all age groups, a significantly higher frequency of PNH^+ cases was observed among the screened PB samples from individuals ≤40 years *vs.* older cases (18% *vs.* 11%; $p<0.001$) Estimated annual incidence of new PNH cases was be of ≈2.5 cases/million individuals per year. When patients with previously diagnosed hematological associated disorders (mostly AA and MDS) were excluded from the analysis, the annual incidence of PNH was of 0.6 cases per million individuals per year. The highest frequency of PNH^+ cases was observed among patients screened because of bone marrow (BM) failure syndrome (33%), particularly among those with mainly aplastic anemia (243/541; 45%) and to a less extent also myelodysplastic syndrome (26/266; 10%). Among the other individuals studied, the most efficient medical indications for PNH screening included: hemoglobinuria (35/73; 48%), unexplained cytopenias including anemia (88/393; 22%), non-immune hemolytic anemia (71/382; 19%), and thrombosis associated to (non-hemolytic) anemia and/or another cytopenia (10/73; 14%). PNH^+ cases was less commonly observed among patients with chronic myeloproliferative neoplasms (1/21; 4.8%), unexplained cytopenias in the absence of anemia (39/772; 5.1%) or unspecified anemia (17/468; 3.6%). In contrast, only a minor fraction of the patients who had been submitted for PNH testing because of unexplained thrombosis in the absence of cytopenia were positive (3/800; 0.4%).
Summary/Conclusions: In summary, our results demonstrate that the current medical indications for PNH screening by FCM are highly efficient, although

improved screening algorithms are needed for patients presenting with thrombosis and normal blood cell counts.

E1008

POSSIBLE RISK FACTORS FOR THROMBOSIS IN CHINESE PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disease caused by an acquired mutation of the X-linked PIGA gene on the hematopoietic stem cell. The most common cause of morbidity and mortality in PNH is thrombosis, but the exact mechanisms involved in thrombus formation are still unknown, and predisposing factors for thrombosis in PNH patients have yet to be defined.
Aims: To identify high risk factors and susceptibility genes leading to thrombotic formation in PNH.
Methods: Totally, 95 patients with PNH diagnosed between 2009 and 2015 were enrolled in the study. Clinical data like sex, age, hemoglobin level, reticular cell percentage, white blood cell and platelet count, LDH level, CD59- and FLAER-granulocytes percentage, thrombophilia risk factors like level of protein C, protein S, antithrombin III, APC resistance, blood fat, phospholipid antibody were evaluated. Samples from patients were genotyped for the reported 28 alleles in 21 genes including MTHFR, PROC, PROS, F2, F5, prothrombin and other genes which are reported as high risk factors for venous thromboembolism (VTE) by polymerase chain reaction fragment length polymorphism methods (PCR-RFLP).
Results: Of the 95 PNH patients, 19 (20%) patients had at least 1 episode of thrombotic event. Only 3 patients had arterial thrombosis and 16 patients had venous thrombosis. The medium age of patients with thrombosis was 42-year-old, similar to those without (42-year-old, p=0.9947). Male: female ratio was 1.71 in thrombosis group, 1.17 in non-thrombosis group (p=0.6072). Patients with thrombosis had the same disease pattern compared to those without. Although there was no difference in level of hemoglobin (p=0.2512), white blood cell count (p=0.4681), platelet count(p=0.6185), reticular cell count (p=0.6296)and LDH level (p=0.4511) between patients with thrombosis and those without, patients with thrombosis showed higher percentage of CD59-granulocytes (p=0.038)and FLAER- granulocytes (p=0.036) compared to those without. The routine thrombophilia screening tests did not show any difference either between PNH patients and normal controls, or between patients with or without thrombosis. Patients with the TC genotype (rs2519093 in the ABO gene) were approximately 18.5 folds prone to thrombus formation than those with the CC genotype (p<0.0001). In addition, the T allele was found to be a significant risk factor for thrombosis (OR 6.447, 95%CI 2.815-14.76, p<0.0001). No association was detected between other SNPs and risk to thrombosis.
Summary/Conclusions: Compared with non-thrombotic patients, PNH thrombotic patients have similar clinical features except that they have bigger PNH clone. And for the first time, our results suggested that the rs2519093 in the ABO gene confers risk to thrombosis in PNH. Therefore, rs2519093 polymorphism may represent a potential genetic biomarker in PNH patients for thrombus formation.

E1009

POLYMORPHISM OF THE COMPLEMENT RECEPTOR 1 GENE IN CHINESE PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired genetic disorder of the bone marrow, eculizumab is an effective monoclonal antibody inhibits terminal complement activation through block the distal complement pathway, but the hematologic response to eculizumab is variable. Previous report showed patients with PNH who have rare allele (L) of the *complement receptor 1* (*CR1*) gene, either heterozygotes (H/L) or homozygotes (L/L), displayed more sub-optimal responder to eculizumab compared with common allele (H/H).
Aims: We investigate polymorphism of the *CR1* gene in Chinese patients with PNH in order to determine its potential impact on eculizumab efficiency.
Methods: DNA was extracted from peripheral blood mononuclear cells from 95 Chinese patients with PNH. Rs2274567 and rs3811381 in *CR1* gene were genotyped by polymerase chain reaction fragment length polymorphism methods (PCR-RFLP). Hemolysis factors including hemoglobin level and LDH level were investigated and thrombotic percentage was evaluated between different genotypes.
Results: Of the 95 patients, frequencies of the rare rs2274567 allele (12%) is much lower than Caucasian PNH population previous reported (31%, P<0.0001), and rare allele frequencies of rs3811381 is also significantly lower (12% *vs* 29%, P<0.0001) in Chinese patients. 72(76%) patients were H/H, while 23 (24%) patients were H/L genotype for rs2274567 polymorphism. There were no difference between the two genotypes in hemoglobin level (83.7g/L *vs* 72.8g/L, p=0.0917) and LDH level (1312U/L *vs* 1022U/L, p=0.2682). As for the rs3811381 genotype, there were 71 (75%), 22 (23%) and 2 (2.1%) patients with H/H, H/L, L/L genotype separately. There was no difference among the different genotype groups in the hemoglobin level (83.2g/L, 74.8g/L and 75.5g/L, respectively, p=0.4278) and LDH level (1312U/L, 997U/L,1233U/L, respectively, p=0.5171), either. Although not significantly, the thrombotic percentage tent to increase from H/H to H/L (17% to 30%, p=0.2288) for rs2274567 polymorphism and H/H to H/L to L/L genotype (17%, 27%, 50%, respectively, p=0.3201) for rs3811381 genotype.
Summary/Conclusions: Very rare H/L or L/L genotype of *CR1* was found in Chinese people with PNH. Frequencies of Chinese rare alleles in *CR1* gene were significantly lower than that of Caucasian population, which may indicate a favorable response to eculizumab treatment. No difference of hemoglobin level or hemolysis was found among different genotype groups, but the percentage of thrombosis seemed to increase from H/H, H/L to L/L.

E1010

EPIDEMIOLOGIC OVERVIEW OF CONGENITAL DYSERYTHROPOIETIC ANEMIA-UPDATED DATA FROM THE GERMAN CDA REGISTRY

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Background: Congenital dyserythropoietic anemia is a very rare hereditary disorder, leading to ineffective hematopoiesis, anemia and secondary hemochromatosis. According to the classification of Heimpel and Wendt (1968) at least four subtypes (CDA I-III, CDA variant) can be distinguished. Diagnosis is based upon morphological examination of blood and bone marrow. Genetic testing can confirm the diagnosis in many cases: CDA I-CDAN1, C15ORF41; CDA II - SEC23B; CDA III-KIF23; variant-KLF 1, GATA-1. Familial cases are autosomal recessive (CDA I, CDA II), autosomal dominant (CDA III) or variable (CDA variant).
Aims: The aim of this study was to analyze the prevalence of this very rare inherited anemia.
Methods: The German CDA registry was initiated in 1990 by Heimpel and coworkers and includes cases from all over the world. All patients known to the study group since 1967 have been documented. Data analysis is based upon an ACCESS database. Diagnosis was confirmed using the criteria published previously by Heimpel and Anselstetter (2003). From a minority of patients results from genetic testing are available. We analyzed the prevalence of CDA-subtypes in different continents. CDA IV is summarized within the CDA variant group.
Results: At the reporting date (13.02.2016) a total of 989 cases from 811 families were registered. Geographical analysis shows 751 patients are registered in Europe, 90 in America, 21 in Oceania (Australia, New Zeeland), 57 in Africa/near east (including Israel) and 70 in Asia. The most frequent subtype is CDA II, followed by CDA I. Most cases are reported in Italy, Spain, Great-Britain, Germany and Israel (data not shown). Detailed information is given in table 1.

Table 1.

Region	CDA I	CDA II	CDA III	Variant	Total
Europe	145	437	32	137	751
America	12	38	13	27	90
Oceania	8	10	0	3	21
Asia	27	32	3	8	70
Africa/Near East	37	18	0	2	57
Σ	229	535	48	177	989

Summary/Conclusions: Prevalence of congenital dyserythropoietic anemia in the different countries is very heterogeneous, reflecting the rarity and the lack of awareness of the disease. Nevertheless the diagnosis is under reported due to numerous cases with only mild symptoms misdiagnosed as myelodysplastic syndrome or unclassified hemolytic anemia.

E1011

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) SCREENING IN PATIENTS WITH UNEXPLAINED ANEMIA: A SINGLE INSTITUTION EXPERIENCE

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Background: Referral to hematology for work up and management of anemia is common. Paroxysmal nocturnal hematoglobinuria (PNH) is a clonal disorder in which cells deficient in glycosylphosphatidyl inositol (GPI) anchor are lysed by complement. PNH can present with direct antiglobulin test (DAT) negative

hemolysis, thrombosis, unexplained cytopenias, and iron deficiency secondary to ongoing hemolysis. Evaluation for PNH clones is recommended for patients with DAT negative hemolysis or cytopenias that remain unexplained after thorough work up. PNH screening is important to identify patients who could benefit from eculizumab therapy, which improves quality of life and overall survival while reducing hemolysis, transfusion requirement, and thrombosis.
Aims: This study sought to evaluate how frequently patients with unexplained anemia were being screened for PNH.
Methods: The study looked at patients with unexplained anemia referred to hematology at St. Paul's Hospital between 2010 and 2015. Demographic information, clinical features (including history of arterial/venous blood clots and history of red blood cell [RBC] transfusion), and list of key investigations such as bone marrow biopsy (BMBx) were collected for each patient. Baseline laboratory data relevant to hemolysis were obtained including lactate dehydrogenase (LDH), bilirubin, reticulocyte count, haptoglobin, and DAT testing. Flow cytometry for expression of FLAER, CD24, CD14, and CD59 on neutrophils, monocytes, and RBC was used as high resolution testing of PNH.
Results: A total of 540 patients were included in the study. The study group was comprised of those with anemia not yet diagnosed (NYD, n=318, including 9 with DAT negative hemolysis and 92 with unexplained iron deficiency despite endoscopic gastrointestinal investigation), pancytopenia NYD (n=49), and anemia of chronic disease (n=173). Of these patients 112 (20.7%) underwent BMBx, 48 (8.9%) had a history of RBC transfusion, and 24 (4.4%) had prior thrombosis. For hemolysis investigations, 445 (82.4%) patients had testing done for LDH, 459 (85.0%) for total bilirubin, 425 (78.7%) had reticulocyte counts, and 219 (40.6%) patients had haptoglobin levels done. A total of 131 (24.2%) patients had a suggestion of possible hemolysis based on these measurements. PNH testing was done in 56 (10.4%) patients, corresponding to 12.3% of anemia NYD, 44% of DAT-negative hemolysis, 21.7% of unexplained iron deficiency, 20.4% of pancytopenia NYD, and 4.0% of anemia of chronic disease. One patient with anemia NYD had a positive PNH screen with a small (0.017%) detectable clone of uncertain significance. Compared to those who did not undergo PNH testing, those who were screened for PNH were more likely to have a history of thrombosis (16.1% *vs* 3.1%, p=0.0003), underwent BMBx (44.6% *vs* 18.0%, p=0.0001), received RBC transfusions (21.4% *vs* 7.4%, p=0.0018),and to have elevated reticulocytes (26.8% *vs* 7.6%, p=0.0001), LDH (25.0% *vs* 12.2%, p=0.0126), and low haptoglobin (21.4% *vs* 2.9%, p=0.0001).
Summary/Conclusions: Anemia is a common referral to hematology, but initial investigations may not uncover a cause. Although hemolytic parameters are evaluated in most patients with unexplained anemia, PNH is tested for only in a minority of cases (10.4%) despite potential indicators of hemolysis in 24.2% of those with unexplained anemia. As effective therapy for PNH is now available, increased screening could identify patients who would benefit from treatment and should be considered.

E1012

EFFICACY AND SAFETY OF DEFIBROTIDE FOR TREATMENT OF HEPATIC VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME (VOD/SOS) IN ADULT AND PEDIATRIC PATIENTS FOLLOWING CHEMOTHERAPY

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Background: Veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is typically thought of as an unpredictable and potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT) conditioning, but also may occur following primary chemotherapy (CT) without HSCT. Severe hepatic VOD/SOS (associated with multi-organ dysfunction [MOD]) may be associated with >80% mortality. Endothelial cell (EC) damage is a critical pathophysiologic factor; preclinical data suggest that defibrotide (DF) stabilizes ECs with direct, as well as EC-mediated, restoration of the thrombo-fibrinolytic balance. DF is approved in the European Union for treatment of severe hepatic VOD/SOS in adult and pediatric patients post-HSCT. In the United States (US), a new drug application was accepted for priority review in 2015. DF has been available in the US on an investigational basis via a treatment protocol (T-IND) study.
Aims: To assess the efficacy of DF for treatment of VOD/SOS in pediatric and adult subgroups following primary CT with MOD or without MOD (off-label), from the T-IND program.
Methods: Eligibility for the T-IND program initially required VOD/SOS diagnosed by Baltimore criteria, with MOD, following HSCT. The protocol was amended to include patients with VOD/SOS without MOD, following CT, or as diagnosed by modified Seattle criteria (using a 5% weight gain threshold). All patients provided informed consent and received open-label defibrotide as a 2-hour intravenous infusion at 6.25 mg/kg every 6 hours (25 mg/kg/day) for a recommended ≥21 days. Survival at Day+100 following CT was measured using Kaplan-Meier (KM) estimates.
Results: Among 857 T-IND patients with VOD/SOS who received ≥1 dose of DF as of April 18, 2015, 101 (11.8%) had undergone primary CT (without HSCT), of whom 54.5% were male, 66.3% were white, and median (range) age was 7.0 (0, 69.0) years. This group included 81 (80.2%) pediatric patients (aged ≤16 years; 59 [72.8%] aged 2–11 years) and 19 (18.8%) adults (aged >16 years) (age not available for 1 patient); 48 (47.5%) patients had MOD (40 [49.4%] pediatric patients and 8 [42.1%] adults), and 52 (51.5%) did not have MOD (41 [50.6%] pediatric patients and 11 [57.9%] adults). For all patients and for the subset without MOD, the most commonly used chemotherapy agents included vincristine, cytarabine, and cyclophosphamide. KM-estimated Day+100 survival is reported in the Table. Overall, 62 (61.4%) patients reported ≥1 adverse event (AE), including 22 (21.8%) with AEs judged at least possibly related to DF, most commonly (≥3.0%) hypotension (4.0%) and nausea (3.0%). Two deaths (pleural hemorrhage and pulmonary hemorrhage plus hypotension) were possibly related to DF.

Table 1.

Day+100 Survival, % (95% confidence interval)	All Ages*	Pediatric (≤16 years)	Adult (>16 years)
All VOD/SOS	77.0% (67.5%, 84.1%) N=101	81.3% (70.9%, 88.3%) n=81	63.2% (37.9%, 80.4%) n=19
With MOD	71.4% (56.6%, 82.0%) n=49	72.5% (55.9%, 83.7%) n=40	75.0% (31.5%, 93.1%) n=8
No MOD	82.3% (68.7%, 90.4%) n=52	90.0% (75.5%, 96.1%) n=41	54.5% (22.9%, 78.0%) n=11

*Age not available for 1 patient, included in All Ages only.

Summary/Conclusions: KM-estimated survival rates at Day+100 post-CT for patients treated with DF were 81% in pediatric patients and 63% in adults, which were clinically encouraging findings. As has been observed in post-HSCT patients, pediatric patients may have higher survival rates than adults post-CT. DF was generally well tolerated in this study and had a safety profile consistent with previous studies. Support: Jazz Pharmaceuticals

LB2247

ALXN1210, A LONG-ACTING C5 INHIBITOR, RESULTS IN RAPID AND SUSTAINED REDUCTION OF LDH WITH A MONTHLY DOSING INTERVAL IN PATIENTS WITH PNH: PRELIMINARY DATA FROM A DOSE-ESCALATION STUDY

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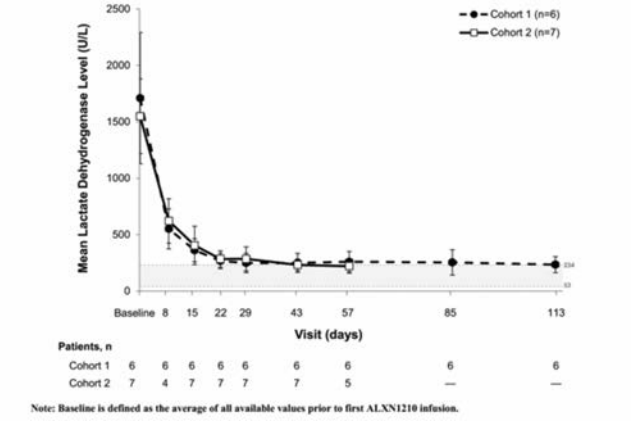
Background: ALXN1210, a rationally designed humanized monoclonal antibody to C5, was engineered for an extended duration of complement inhibition. A previous study in healthy volunteers showed immediate, complete, and sustained inhibition of C5. The half-life (t½) of ALXN1210 was >30 days-substantially longer than eculizumab-which facilitates an extended dosing interval of ≥1mo.
Aims: ALXN1210-PNH-103 is a Phase 1b, multicenter, open-label, intrapatient dose-escalation study (NCT02598583), evaluating the safety, tolerability, and efficacy of 2 IV maintenance dosing regimens of ALXN1210 in patients (pts) with paroxysmal nocturnal hemoglobinuria (PNH) never treated with a complement inhibitor.
Methods: In this interim analysis, 2 cohorts of pts ≥18 y were investigated: pts in Cohort 1 (C1) received either 400- or 600-mg induction doses followed by a 900-mg maintenance dose q4w; pts in Cohort 2 (C2) received 600- and 900-mg induction doses, followed by an 1800-mg maintenance dose q4w. The primary objective was to assess safety and tolerability of ALXN1210. The primary efficacy outcome was change in lactate dehydrogenase (LDH) level. Other endpoints included change in blood transfusions and in hematologic parameters related to PNH.
Results: A total of 13 pts consented and enrolled (Table). Median duration of exposure was 3.7 mo for C1 (n=6) and 1.8 mo for C2 (n=7). All pts showed rapid reductions in LDH levels, which were observed at the first evaluable time point (day 8). Decreases in LDH were sustained over 4-mo dose intervals, as shown by mean reductions from baseline in LDH of 85.6% in C1 on day 113

and 83.8% in C2 on day 57, the latest evaluable time points (Figure). Among 5 pts with ≥1 transfusion in the year before treatment (2 in C1, 3 in C2), only 1 patient (in C1, who had received 12 units of packed red blood cells in the prior 6 mo) received a transfusion (2 units) while on ALXN1210. Multiple doses of ALXN1210 resulted in no serious adverse events (SAEs), no infusion site reactions, and no drug discontinuations or AEs leading to withdrawals. The most common treatment-emergent AE (TEAE) was headache (2 pts). Investigators judged 69% of TEAEs to be unrelated to treatment. All related AEs resolved with ongoing ALXN1210 treatment.

Table 1. Baseline demographics and disease characteristics.

		Cohort 1 n=6	Cohort 2 n=7	Overall n=13
Race				
Asian	n (%)	6 (100)	6 (86)	12 (92)
White/Caucasian	n (%)	0	1 (14)	1 (8)
Female Gender	n (%)	2 (33)	5 (71)	7 (54)
Age at First Infusion (Years)	Mean (SD)	41.1 (10.86)	43.6 (13.49)	42.4 (11.91)
	Median	43.6	40.6	41.5
	Min, Max	24.5, 55.9	24.9, 62.3	24.5, 62.3
LDH (U/L)	Mean (SD)	1709.9 (582.10)	1549.4 (330.4)	1623.5 (450.22)
	Median	1744.8	1424.3	1541.0
	Min, Max	779.3, 2392.7	1165.5, 2057.7	779.3, 2392.7
LDH (xULN)	Mean (SD)	7.3 (2.49)	6.6 (1.41)	6.9 (1.62)
	Median	7.5	6.1	6.6
	Min, Max	3.3, 10.2	5.0, 8.8	3.3, 10.2
RBC clone size (%)	Mean (SD)	27.1 (9.68)	29.5 (13.54)	28.3 (11.29)
	Median	25.2	28.1	27.3
	Min, Max	17.4, 43.6	15.6, 53.8	15.6, 53.8
Pts with Transfusion in Year Prior to Enrollment	n (%)	2 (33)	3 (43)	5 (38)

LDH=lactate dehydrogenase; RBC=red blood cell; xULN=multiples (fold) of the upper limit of normal.



Note: Baseline is defined as the average of all available values prior to first ALXN1210 infusion.

Figure 1. Mean (SD) LDH values over time, by cohort.

Summary/Conclusions: ALXN1210-PNH-103 is the first study to demonstrate safety and efficacy of ALXN1210 in pts with PNH. ALXN1210 treatment resulted in rapid reductions in LDH levels in 100% of pts, which were sustained through 2-4 monthly dosing intervals, consistent with the extended t½ of ALXN1210. There was a notable decrease in the need for blood transfusions. These preliminary LDH data suggest that rapid, complete, and sustained complement inhibition with ALXN1210 results in highly effective blockade of intravascular hemolysis. A Phase 2 study examining monthly and longer dosing intervals of ALXN1210 is ongoing.

LB2248

12 WEEKS SAFETY AND EFFICACY RESULTS OF A NOVEL C5 INHIBITOR COVERSIN IN PNH WITH RESISTANCE TO ECULIZUMAB DUE TO COMPLEMENT C5 POLYMORPHISM

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Background: Paroxysmal Nocturnal Haemoglobinuria (PNH) is a rare acquired life-threatening disease characterized by complement induced haemolysis and its sequelae and a high incidence of thrombosis. The monoclonal antibody eculizumab binds to C5 and prevents its activation and cleavage into C5a and C5b and is an established treatment for PNH and aHUS. However, for patients with the rare amino acid polymorphism p.Arg885His or pArg885Ser, which interferes with the binding and efficacy of eculizumab (Nishimura et al, 2014, Langemeijer et al, 2015), there is still no effective treatment. A new small protein complement inhibitor named Coversin is in Phase 1-2 clinical development. Coversin also prevents cleavage and activation of C5 but binds to C5 at different site than eculizumab. In vitro Coversin inhibits C5 activation in both wild type C5 and C5 with a polymorphism at the Eculizumab binding site. Coversin studies in healthy volunteers proved safe and demonstrated inhibition of terminal complement activation.
Aims: We report pharmacokinetics (PK), pharmacodynamics (PD), safety and preliminary efficacy data of the novel C5 inhibitor Coversin in a severely haemolytic PNH patient with a C5 polymorphism.
Methods: Coversin was administered by s.c. injection at an ablating dose of 0.57 mg/kg on day 1, followed by a maintenance dose of 0.14 mg/kg per day thereafter. Peripheral blood samples were drawn for PK/PD. Protocol specified doubling of the dose and /or shortening of the dose interval were allowed on the basis of clinical symptoms and CH50 levels to achieve adequate and sustained complement inhibition.
Results: The patient is a 30 year old male with PNH, (granulocyte clone size: 90%) and severe haemolysis (LDH 3 to 17 x UNL), transient renal failure, extreme fatigue and symptoms of muscle dystonia and no history of thrombosis. He remained severely haemolytic during eculizumab treatment despite adequate drug levels and no antidrug antibodies. Other causes of haemolysis were excluded. The patient was shown to have a p.Arg885Ser polymorphism rendering him resistant to eculizumab therapy. There was a good initial response to an ablating dose of 0.57mg/kg Coversin with CH50 levels decreasing below 8 U Eq/ml, which is the lower limit of qualification of the ELISA assay. Clinical symptoms and laboratory markers of haemolysis improved during the maintenance treatment of 0.14mg/kg every 24 hours. However, 6 days into the treatment, the patient again experienced haemolysis-associated symptoms with dark urine hours before the next s.c. injection and no further decrease of his LDH. The same occurred after doubling of the dose to 0.29 mg/kg per day. Dividing the dose into 0.14 mg/kg injections Q 12 hours resulted in stable complement inhibition with CH50 levels <8 U Eq/ml and no breakthrough symptoms. The LDH decreased to approximately 1.5xUNL. For the first time since he was diagnosed with PNH in 2009 the patient feels well with no symptoms of fatigue and no muscle dystonia. There have been no further haemolytic episodes. Twelve weeks after start of treatment, our patient did not have any drug-related adverse events, except occasional local and transient irritation at the injection site.
Summary/Conclusions: Over a period of 12 weeks Coversin has proven safe and effective in the first PNH patient treated with the drug.

LB2249

A PHASE 1 MULTIPLE-DOSE CLINICAL STUDY OF RA101495, A SUBCUTANEOUSLY ADMINISTERED SYNTHETIC MACROCYCLIC PEPTIDE INHIBITOR OF COMPLEMENT C5 FOR TREATMENT OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired, clonal hematopoietic stem cell disorder caused by a deficiency in glycosylphosphatidylinositol (GPI)-linked proteins on cell surfaces. Patients with mutations in the phosphatidylinositol glycan class A gene are unable to produce functional, protective, GPI-linked proteins, resulting in the accumulation of specific complement proteins on the surface of red blood cells (RBCs) and subsequent RBC lysis by the membrane attack complex (MAC). Inhibition of complement activation at the level of complement C5 is a clinically validated approach for the treatment of PNH. RA101495, a synthetic macrocyclic peptide, binds to C5 at a unique site not targeted by currently available therapies and allosterically inhibits C5 cleavage into C5a and C5b, preventing production of a key component of the MAC. RA101495 also inhibits the assembly of MAC by blocking the interaction between C5b and C6.
Aims: A Phase 1 multiple-dose clinical pharmacology study in healthy human volunteers designed to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of RA101495 following once daily subcutaneous (SC) injections over 7 days.
Methods: The study was single-center (Australia), randomized, double-blinded, and placebo (PBO)-controlled. After obtaining written informed consent, all subjects received daily SC doses of 0.2 mg/kg RA101495 or matching PBO for 7 days while housed in a clinical pharmacology unit. Safety was assessed by intensive clinical monitoring, and daily blood samples were obtained at -15 minutes, 3 hours, and 6 hours relative to each day's dose for determination of RA101495 concentrations by liquid chromatography/high resolution mass spectroscopy and ability to inhibit complement-mediated RBC lysis in an *ex vivo* antibody-sensitized sheep erythrocyte hemolysis assay. All subjects received prophylaxis for *N. meningitides* infection with ciprofloxacin and vaccination.
Results: A total of 6 subjects were enrolled into the study (4 RA101495 and 2 PBO). Plasma concentrations showed a steadily increasing exposure over the 7 days of dosing in the 4 RA101495-treated subjects (see Figure). From these

data, the half-life of RA101495 appears to be 7 days. Preliminary results show that, for the subjects with measurable plasma levels of RA101495, the mean percent inhibition of hemolysis compared to baseline reached ≥95% beginning at the first time point, 3 hours after dosing on Day 1, and continued throughout the 7 days of dosing; all individual subjects showed ≥90% reduction of hemolysis at all time points (Figure). The only safety finding noted was mild cutaneous injection site erythema in 2 of the 4 RA101495-treated subjects; there was no associated pain, tenderness, swelling, or induration and all events resolved rapidly following dosing.

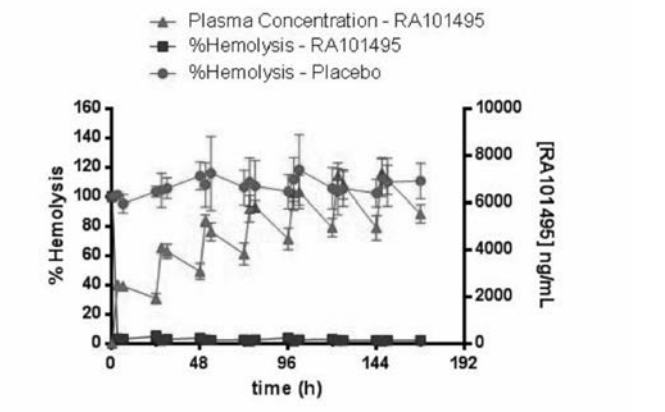

Figure 1.

Summary/Conclusions: RA101495 is a novel synthetic macrocyclic peptide inhibitor of C5-mediated hemolysis that is being developed as an alternative to intravenous monoclonal antibody therapy for the treatment of PNH. RA101495, currently being investigated for daily at-home SC self-administration, shows a rapid onset of activity, and appears to be safe and well tolerated. These preliminary data suggest low daily doses will achieve steady-state levels suitable for complete and sustained inhibition of complement and suppression of hemolysis, and, given the long half-life, that once-weekly dosing is possible.

LB2250

AN ORALLY ADMINISTERED SMALL MOLECULE FACTOR D INHIBITOR (ACH-4471) FOR TREATMENT OF PNH AND COMPLEMENT DISEASES: PRELIMINARY PHASE 1 RESULTS IN HEALTHY VOLUNTEERS

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Background: ACH-4471 is a novel, small molecule complement factor D (fD) inhibitor; to our knowledge, this represents the first clinical data reported for an oral inhibitor of this target. ACH-4471 prevents cleavage of complement factor B into Ba and Bb in the alternative pathway of the complement cascade, leading to blockade of C3 convertase production. Hence, unlike C5 inhibitors, ACH-4471 also prevents C3 fragment deposition on PNH cells and may confer a pharmacological advantage by protecting PNH cells from both intravascular and extravascular hemolysis.
Aims: To assess the safety and tolerability of single ascending oral doses of ACH-4471 in healthy volunteers and to evaluate its pharmacokinetic (PK) and pharmacodynamic (PD) profile and PK/PD relationship as measured by the serum AP activity *ex vivo*.
Methods: In this Phase 1a study, ACH-4471 was given as a single oral dose to healthy volunteers. Dose group 1 enrolled 6 active and 6 placebo subjects, while subsequent groups enrolled 6 active and 2 placebo subjects each. Each group was studied for 28 days after dosing. Active treatment subjects in the first 2 groups received 200 and 600mg ACH-4471 QD, respectively. Inhibition of serum AP activity was evaluated using the AP Wieslab assay, which tests ACH-4471 under stringent conditions in the presence of bacterial endotoxin (LPS), a potent AP activator. To date, 20 subjects (Groups 1 and 2) have been dosed and evaluated (19 males, 1 female) with a median age of 23.9 years (range 21.0 - 54.2). For both groups, all subjects were followed for AEs/SAEs through the last scheduled visit at Day 28. Blood samples were collected at predefined time points from Days 1 to 4 to determine plasma concentrations of ACH-4471.
Results: There have been no drug-related SAEs, TEAEs leading to study discontinuation, or study-drug related Grade 3/4 TEAEs. PK data indicate that peak plasma concentrations (C_{max}) are achieved by 1 to 2.5 hours after a single 200- or 600-mg dose, with the majority of exposure (measured as AUC) occurring over the first 24 hours. The terminal phase begins 16 hours after dosing and the mean terminal half-life is approximately 9 hours. PD data from the *ex vivo* evaluation of serum AP activity indicate rapid and nearly complete inhibition of AP activity after single 200- or 600-mg doses. Mean plasma concentrations of ACH-4471 necessary to achieve >80% or >90% AP inhibition were approximately 180 and 230ng/mL, respectively. Simulations indicate that these steady-state C_{trough} levels could be maintained by twice daily (BID) dosing with the current capsule formulation; ongoing formulation development efforts may allow for once daily dosing. Plasma Bb concentration, an *in vivo* biomarker for the inhibition of fD activity by ACH-4471, decreased dose-dependently with a nadir 9 hours after dosing and a gradual recovery to original levels by 48 hours. Taken together, these results indicate that administration of ACH-4471 results in potent inhibition of AP activity.
Summary/Conclusions: ACH-4471 was well-tolerated at the single dose levels examined to date with no safety trends. PD results indicate that ACH-4471 potently inhibits AP activity. PK modeling predicts that the current formulation of ACH-4471 will provide sustained suppression of AP activity with BID dosing, and future formulations may be effective with QD dosing. A 14-day multiple ascending dose study of ACH-4471 is planned to start in 2Q2016.

Chronic lymphocytic leukemia and related disorders - Biology

E1013

THE PI3K DELTA SELECTIVE INHIBITOR, IDELALISIB, MODULATES CYTOKINE PRODUCTION IN INNATE IMMUNE CELLS STIMULATED THROUGH TOLL-LIKE RECEPTORS

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Background: Idelalisib (IDELA) is an oral, selective PI3Kd inhibitor approved for the treatment of relapsed chronic lymphocytic leukemia (CLL). A subset of patients develop severe diarrhea/colitis which requires interruption of IDELA therapy and in some instances, corticosteroid treatment (Coutre et al., Leuk Lymphoma, 2015). Toll-like receptors (TLR) play a critical role in innate immunity by detecting pathogen associated molecular patterns (PAMPs). Responses to signaling through TLR drive not only an immediate innate cell response to pathogens, but also enable cells of the innate immune system to shape an adaptive immune response. PI3Kd has been shown to modulate signal transduction through TLR4 and TLR1/2 in murine bone marrow derived dendritic cells (Aksoy et al., Nat Imm, 2012). Additionally, PI3Kd has been shown to modulate TLR2, TLR4, TLR5, and TLR9 signaling in murine bone marrow derived macrophages (Plevy et al., Gastroenterology, 2010). Mice with a targeted inactivating mutation in the p110d subunit of PI3Kd spontaneously develop colitis at 8 weeks of age, and bone marrow macrophages from these mice secreted significantly more inflammatory cytokines (Plevy et al., Gastroenterology, 2010).
Aims: Evaluate the effects of clinically relevant concentrations of IDELA on TLR signaling in human monocyte derived macrophages (MDMs) as a potential mechanism contributing to clinically observed adverse events.
Methods: We utilized MDMs skewed to either a M1 or M2 phenotype by culture for six days in GM-CSF or M-CSF, respectively. MDMs stimulated with a TLR1/2 (PAM3CSK4), TLR4 (LPS), or TLR5 (flagellin) agonist in the presence of clinically relevant concentrations of IDELA were examined for cytokine production at early and late time points (MesoScale Discovery). Additionally, nCounter Human Inflammation Kit V2 (NanoString) was used to assess the effects on gene expression and Peggy Sue (Protein Simple) was used to analyze changes in protein expression on MDMs.
Results: Cytokine analysis revealed that both MDM1 and MDM2 cells produced inflammatory IL-12p40 when stimulated with TLR agonists. However, IL-12p40 production by MDM2 cells when stimulated with TLR1/2, TLR4 or TLR5 agonists was significantly increased in the presence of IDELA (Fig. 1A). Inflammatory cytokine upregulation was not seen in MDM1 cells. MDM2 cells produced immunosuppressive IL-10 in response to TLR1/2, TLR4 or TLR5 agonist stimulation. The IL-10 production was inhibited in the presence of IDELA (Fig. 1B). MDM1 cells produced little to no IL-10 in this context. IDELA exerted a differential effect on gene expression in MDM2 *versus* MDM1 cells. CXCR2 ligands were upregulated in MDM2 cells in the presence of IDELA plus TLR agonists to a much greater extent than MDM1 cells, while the anti-inflammatory IL-1RA was downregulated.

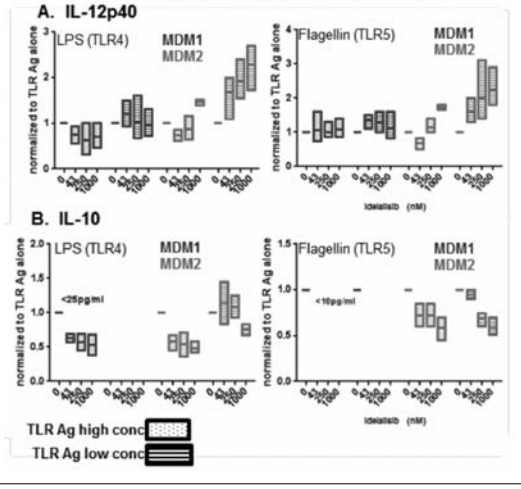

Figure 1.

Summary/Conclusions: The immunological challenge of the mucosal environment of the intestine is maintaining a balance between a tolerogenic state with the commensal microbiota and an ability to aggressively respond to potential pathogens. Under normal conditions, TLR signaling by enteric bacteria is protective (Rakoff-Nahoum et al., Cell, 2004). Here we show that IDELA modulates TLR signaling in MDMs, resulting in MDM2 cells producing higher amounts of inflammatory cytokines, and less immunosuppressive IL-10. This dysregulation of the tolerogenic cytokine balance and subsequent perturbations of the innate/adaptive immune system is consistent with proinflammatory cytokine imbalance which may result in colitis in PI3Kd deficient animal models.

E1014

MUTATIONS IN SF3B1 GENE ARE SELECTED INDEPENDENTLY ON ATM/TP53 STATUS AND REDUCE THE TIME TO FIRST TREATMENT IN CLL PATIENTS

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Background: Significant proportion of chronic lymphocytic leukemia (CLL) patients manifests abnormalities in the DNA damage response pathway (DDR) through defects in *TP53* or *ATM* gene. Interestingly, recurrent mutations in splicing factor 3 subunit b1 (*SF3B1*) have also been shown to partially impair DDR in CLL patients. Whilst *ATM* dysfunction seems to typically occur early in CLL pathogenesis, mutations in *TP53* and *SF3B1* have been reported to be mostly subclonal events originating later during the disease course.
Aims: (a) to assess whether *SF3B1* mutations' occurrence associates with adverse genetic lesions in *TP53* and *ATM* genes, (b) to determine the impact of all three types of defects on time to first treatment (TTFT) and overall survival (OS), and (c) to analyze changes in *ATM* and *SF3B1* mutation status during the disease course.
Methods: We used the yeast functional analysis FASAY coupled to Sanger sequencing for the identification of *TP53* mutations (analyzing thus exons 4-10), the next generation sequencing (NGS) on MiSeq instrument (Illumina) for the whole *ATM* gene screening (62 coding exons with splicing sites), and direct Sanger sequencing for the analysis of mutational hot-spot exons 14-16 in *SF3B1* gene. The median coverage in the NGS analysis was ~4000 reads, and we set up a uniform sensitivity of 10% for all three methodologies.
Results: We analyzed unfavorable cohort of 205 patients consisting predominantly (86%) of *IGHV* unmutated patients, with 64% of the patients being treatment-naïve. The *TP53* dysfunction was identified in 53/205 patients (26%), while both *ATM* and *SF3B1* mutations occurred in 49/205 cases (24%). In line with the expectations, we observed the frequent co-occurrence of *ATM* mutations with del(11q) (P<0.001), and the strong mutual exclusivity of *ATM* and *TP53* mutations (co-occurring in only one patient). By contrast, there was no significant association or mutual exclusivity between *SF3B1* mutations´ presence and *ATM* or *TP53* mutational status, neither among untreated patients nor in the whole cohort. For the TTFT and OS analyses, we firstly employed a hierarchical classification of *TP53* and *ATM* mutations and did not consider *SF3B1* mutational status; in addition, we limited this analysis to only *IGHV* unmutated patients. Compared to TP53-wt/ATM-wt patients having median of 18 months (m), the TTFT was apparently reduced in both *TP53*-mutated (6 m; P=0.002) and *ATM*-mutated patients (7 m). However, the latter group exhibited heterogeneous impact of mutations leading to only non-significant TTFT reduction (P=0.130). The OS analysis then showed a prominent (P<0.001) negative impact of *TP53* mutations (38 m) and a borderline significance of *ATM* mutations (67 m; P=0.058) compared to wt patients (90 m). As a sub-analysis, we assessed the effect of single *SF3B1* mutations: these led to reduced TTFT (11 m; P=0.029 compared to 18 m in wt patients), while did not show an impact on OS. The analysis of *ATM* and *SF3B1* throughout the disease course involving at least one relapse then disclosed that (a) 27/28 repeatedly analyzed *ATM* mutations (median time between analyses 40 m) and (b) 29/30 analyzed *SF3B1* mutations (33 m) were identified in both samplings. In addition, 7 out of 72 repeatedly analyzed *SF3B1*-wt samples showed a new mutation in this gene.
Summary/Conclusions: Mutations in *SF3B1* occur regardless of the ATM/p53 pathway dysfunction in CLL and can be selected by therapy. Their presence results in early therapy need but not in shorter survival. Supported by projects no. 65269705, TA0 TE02000058 and MUNI/A/1028/2015.

E1015

BIOLOGICAL AND CLINICAL FEATURES OF IMMUNOSUPPRESSIVE THERAPY IN PATIENTS AFFECTED BY LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: Large Granular Lymphocyte Leukemia (LGLL) is a rare and heterogeneous lymphoproliferative disorder characterized by the chronic proliferation of clonal Large Granular Lymphocytes (LGLs) with cytotoxic activity. Recently, activating STAT3 mutations have been discovered in 30-40% of T-LGLL patients. The treatment of LGLL is based on immunosuppressive drugs, such as Methotrexate (MTX), Cyclophosphamide (CTX) and Cyclosporin A (CyA), used

at low doses. These therapies have limited efficacy and the Overall Response Rate (ORR) is near 50%. Furthermore, *in vitro* studies on the molecular effects of these drugs on leukemic lymphocytes are not available in the literature and the biological rational of LGLL treatment has yet not been investigated.
Aims: The aim of this study was to analyze the *in vivo* efficacy of treatment on a cohort of patients referred to the Hematology Unit at Padua University. In addition, we performed *in vitro* study of the molecular effect of these drugs on pathological LGLs in order to better understand their efficacy/specificity, in particular considering their effect on STAT3 pathway.
Methods: Clinical characteristics and treatment response were collected from 17 LGLL patients. For *in vitro* analysis a cohort accounting 35 patients was studied. Cell cultures of patients' peripheral blood mononuclear cells (PBMCs) were set up with CTX (5μM), CyA (10μM) or MTX (100μM). On collected culture cells apoptosis by Annexin V assay and phosphorylation of STAT3 by Western Blot were investigated.
Results: Clinical results demonstrated that ORR for each drug ranged from 35.7% to 57.1%, CTX treatment showing the higher ORR independently from the therapy line. Fifteen out of the 17 treated patients were STAT3 mutated, indicating a strong correlation between STAT3 mutation and symptomatic disease. Interestingly, consistent with literature data, patients carrying Y640F STAT3 mutation showed a better ORR on MTX treatment as compared with patients with different STAT3 mutation (66% *versus* 14.2%). *In vitro*, CTX and CyA showed a strong increase of LGL apoptosis, leading to the complete disappearance of LGL clone within 3-6 days of culture. Anyway, concerning the specificity of the killing leukemic LGL cells, while sparing non leukemic PBMCs, our data showed that both CTX and CyA were also partially toxic for non-LGLs population. As regards MTX treatment, no increase of apoptosis was revealed, but, interestingly, we observed that MTX induced apoptosis when LGL were stimulated to proliferate by IL-15, suggesting a predominant effect on activated cells. Considering the key role of STAT3 activation in leukemic LGL survival, we observed a decrease of STAT3 phosphorylation levels only after CyA treatment. Finally, adding autologous plasma (10%) to cell culture the cytotoxic effect of drugs was partially reduced (~50%), suggesting that plasma might contain cytokines preserving LGL survival.
Summary/Conclusions: In summary, CTX showed a good efficacy *in vivo* and *in vitro*, even if in this last setting the compound did not exhibit a good LGL specificity. MTX might be more efficient when LGLs are proliferating and when LGLs carry activating mutations, even if it does not affect STAT3 activation. Finally, we demonstrated that CyA expresses its cytotoxic effect through a mechanism that down-modulates STAT3 activation. Our results contribute to get insights into the molecular mechanisms of immunosuppressive drugs used in LGLL treatment and suggest that STAT3 mutation/activation might represent a suitable target for therapy.

E1016

CD49D IS A BETTER PREDICTOR OF OVERALL SURVIVAL THAN THE NOVEL RECURRENT MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA: RESULTS FROM AN ITALIAN MULTI-CENTER COHORT

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Background: CD49d, the alpha-chain of VLA-4 integrin, was identified among the strongest predictors of overall survival (OS) in chronic lymphocytic leukemia (CLL), along with *IGHV* status and the 17p deletion (Bulian et al, JCO, 2014). In addition to *TP53*, the clinical relevance of mutations of *NOTCH1*, *SF3B1* and *BIRC3* recently emerged.
Aims: To test the clinical relevance of CD49d in subgroups defined by *NOTCH1*, *SF3B1*, *BIRC3* alterations.
Methods: The cohort was of 778 unselected CLL (treated cases, n=356, median follow-up 80 months with 173 deaths), all characterized for CD49d expression (CD49d^{high}, ≥30% positive cells by flow cytometry, n=229), *IGHV* status (unmutated, UM, n=262), karyotype abnormalities (5% cut-off, 13q-, n=308; +12, n=103; 11q-, n=64), at diagnosis, along with *TP53* mutations/deletions (hereinafter, disruption, n=84, including 58 17p-), *NOTCH1* mutations (n=81), *SF3B1* mutations (n=54), *BIRC3* disruption (n=59). Recurrent mutations were investigated by Sanger sequencing before therapy (at diagnosis in 65% of cases).
Results: i) CD49d^{high} associated with age ≥65 years (p=0.0001), Rai stage ≥I (p<0.0001), UM *IGHV* status (p>0.0001), absence of 13q- (p=0.0001), presence of +12 (p<0.0001), *NOTCH1* mutations (p<0.0001). **ii**) By univariate analysis, CD49d^{high} had impact as OS predictor (hazard ratio/confidence interval, HR/CI=2.62/1.94-3.54; p<0.0001). In multivariate analysis, CD49d^{high} was confirmed as independent prognosticator (HR/CI=1.88/1.36-2.60, p<0.0001), along with age≥65 years (HR/CI=3.90/2.68-5.67), Rai stage ≥I (HR/CI=1.80/1.30-2.51, p=0.0005), UM *IGHV* (HR/CI=1.84/1.31-2.60, p=0.0005), 11q- (HR/CI=2.21/1.39-3.51, p=0.0008), *TP53* disruption (HR/CI=3.65/2.15-5.30, p<0.0001), *NOTCH1* mutations (HR/CI=1.79/1.17-2.73, p=0.0068). Conversely, +12, *SF3B1* mutations, *BIRC3* disruption were all excluded from the final model. **iii**) The variable importance (VIMP) of biological markers as OS predictors was evaluated by a random forests approach. CD49d (VIMP=0.0410) was ranked among the most important OS predictors, followed by *IGHV* status (VIMP=0.0388), *TP53* disruption (VIMP=0.0352) and *NOTCH1* mutations (VIMP=0.0168); conversely, *SF3B1* mutations (VIMP=0.0002) and *BIRC3* disruption (VIMP=-0.0024) had no importance as OS predictors. iv) Post-treatment survival was evaluated to explore the impact of prognosticators as therapy response predictors. CD49d^{high}, UM *IGHV*, *TP53* disruption but not *NOTCH1* mutations were able to split cases into groups with significant different treatment responses (see Figure). This observation was confirmed by multivariate analysis where CD49d^{high} (HR/CI=1.55/1.08-2.23, p=0.0192), UM *IGHV* (HR/CI=1.70/1.17-2.49, p=0.0060), *TP53* disruption (HR/CI=2.67/1.79-4.00, p<0.0001) were maintained and *NOTCH1* mutations were excluded. iv) In Rai stage 0 cases (n=389), CD49d^{high} had impact as Time-to-First-Treatment predictor (HR/CI, 2.68/1.78-4.05, p<0.0001) by univariate analysis. In multivariate analysis, CD49d^{high} again emerged as independent prognosticator (HR/CI=1.61/1.01-2.56, p=0.0442), along with UM *IGHV* (HR=2.66/1.72-4.12, p<0.0001), +12 (HR/CI=3.36/2.00-5.62, p<0.0001), *TP53* disruption (HR/CI=1.97/1.03-3.78, p=0.0419), *BIRC3* disruption (HR/CI=2.70/1.24-5.89, p=0.0127), whereas age, *NOTCH1* mutations, 13q-, 11q-, were excluded.

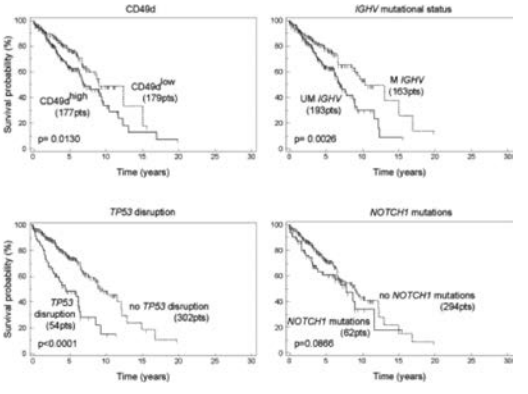

Figure 1.

Summary/Conclusions: CD49d, along with *IGHV* status, is the most powerful independent negative OS prognosticator in CLL also when *NOTCH1*, *SF3B1*, *BIRC3* alterations were evaluated. CD49d, *IGHV* status and *TP53* disruption may also have a role as therapy response predictors.

E1017

COMBINED SYK AND JAK INHIBITION BY CERDULATINIB INDUCES APOPTOSIS IN CLL AND OVERCOMES RESISTANCE TO IBRUTINIB

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Background: Despite recent advances in the treatment of chronic lymphocytic leukemia, robust and durable complete responses enabling extended periods of time off-therapy are not achieved. One possible reason for this may be explained pre-clinically; agents like ibrutinib and idelalisib induce cell cycle arrest, but not apoptosis, in CLL cells treated in vitro. Consistently, when cells from ibrutinib-treated patients are analyzed, no significant apoptosis is seen even after 3 months of therapy. CLL tumor cells receive growth and survival signals from not only the BCR, but also a variety of cytokine receptors which primarily utilize Janus kinases (JAK) to initiate the signaling cascade, culminating in the upregulation of BCL2 family members and preventing apoptosis. We have therefore been exploring the utility of combining BCR pathway suppression via SYK with JAK/STAT pathway suppression using a novel small molecule inhibitor, cerdulatinib. Cerdulatinib is a selective and potent inhibitor of SYK, JAK1, JAK3, and TYK2, currently in a phase I dose escalation study in patients with B cell malignancies.
Aims: The aim of these studies was to determine the anti-tumor activity of cerdulatinib in primary CLL co-cultures as well as molecular correlates of response.
Methods: Using a repository of previously-banked viable CLL primary tumors (n=60), we evaluated cell death in the presence of cerdulatinib on stromal cell supported CLL cultures. Viability assays were performed by evaluating PARP cleavage and propidium iodide uptake. Western blotting or FACS analysis was

used to determine cerdulatinib-impact on survival signaling networks and BCL2 family expression.
Results: In general, tumor specimens were sensitive to cerdulatinib, especially in cases with poor prognosis (e.g. mutation status, ZAP70 expression, cytogenetics). Importantly, sensitive tumor cells actually underwent apoptosis, which was not observed in direct comparisons with ibrutinib. The mechanism of apoptosis induction was demonstrated by western blotting to be cerdulatinib-induced down-regulation of MCL-1, which was associated with induction of tumor PARP cleavage. Apoptosis was induced by this agent irrespective of co-culture with known survival factors, including different stromal cell lines, or media supplementation with IL4, CD40 ligand, anti-IgM, or a combination of the three. Cerdulatinib anti-tumor activity significantly correlated with inhibition of pAKT S473 and pERK T202/Y204 within the tumor cells, and was associated with decreased BCR, JAK/STAT, and NF-kB pathway activation. Moreover, primary CLL samples resistant to ibrutinib remained sensitive to cerdulatinib.
Summary/Conclusions: The data suggest an alternate mechanism by which apoptosis may be induced in CLL, and provide a justification to continue clinical studies with cerdulatinb in this disease. Cerdulatinib has the potential to deliver a higher rate of complete responses in CLL, as well as provide another therapeutic option for patients who either did not respond to or relapsed on ibrutinib.

E1018

DNA REPLICATION CONTROL MECHANISMS INVOLVED IN CHEMORESISTANCE OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL), the most common leukemia in the Western hemisphere, is characterized by clonal expansion of B lymphocytes co-expressing CD5 and CD19 that arise from the lymph nodes and accumulate in patient's peripheral blood and bone marrow. Historically, standard first line treatment of CLL patients has been based on a DNA targeting agent, cyclophosphamide, nucleoside analogue, fludarabine and an anti-CD20 antibody, rituximab. Although the chemotherapeutic regime results in high rate of complete response, majority of CLL patients, nevertheless, relapse.
Aims: CLL genomes are characterized by, both, chromosome aberrations affecting the karyotypic integrity and elevated frequencies of nucleotide point mutations. Altered DNA replication, repair and recombination (3R) mechanisms may account for chromosomal instability and mutator phenotype that propels the development of chemoresistance towards genotoxic therapies. Here, we aimed to determine the expression of 3R genes and decipher their functional role in CLL.
Methods: For this purpose we assessed complete 3R gene expression profiles in 140 peripheral blood (PB) samples obtained from clinically annotated CLL patients and correlated the obtained data with patients' clinical outcomes. Next, in order to investigate the DNA replisome of the cycling CLL cells, we used an *in vitro* approach based on interleukin-2 (IL-2) and CpG-oligonucleotide stimulation to obtain proliferative primary patient leukemic cells. To determine the chemotherapeutic implication of the 3R gene of interest (GOI), we performed drug sensitivity screens in presence of fludarabine on two CLL cell lines established successively from the same patient that differed in the expression of the 3R GOI. Also, we used a mouse embryonic fibroblast (MEF) cell line and its heterozygous and homozygous knock-outs for the 3R GOI to confirm the obtained results. We characterized chemoresistance profiles using cellular viability screens, cell cycle and DNA synthesis analysis. In order to decipher the molecular basis of chemoresistance driven by our 3R GOI, we used drugs that partially recapitulate the mechanism of action (MOA) of fludarabine, namely, hydroxyurea (HU), an inhibitor of the ribonucleotide reductase (RNR) and aphidicolin, an inhibitor of replicative DNA polymerases.
Results: Our qRT-PCR data revealed deregulated expression of several 3R genes among which, alternative DNA polymerase nu (*POLN*). Interestingly, we observed that relatively high expression of *POLN* correlates with a shorter progression free survival (PFS) in patients that received the chemotherapeutic treatment. In addition, *in vitro* proliferation assays showed that primary CLL lymphocytes that were stimulated to enter the cell cycle and divide, exclusively upregulated *POLN*. Drug screens in two independent cellular models showed that upregulation of *POLN* contributed to fludarabine chemoresistance in terms of cellular viability and sustained DNA synthesis. Our chemoresistance data demonstrated that, unlike *POLN* knock-out cells, *POLN* expressing cells are able to continue synthesizing DNA in presence of fludarabine. These results support observed clinical correlation between *POLN* expression and PFS. In terms of the molecular mechanism of *POLN* driven chemoresistance, differential DNA synthesis profiles obtained in presence of fludarabine were recapitulated when cells were treated with hydroxyurea but not aphidicolin.
Summary/Conclusions: Finally, our data reveal deregulated expression of 3R genes in CLL, among which that of a specialized DNA polymerase nu (*POLN*). We show that *POLN* overexpression correlates with worse clinical outcome in CLL patients upon receiving therapy. Moreover, our *in vitro* results clearly show that upregulation of *POLN* observed in cycling CLL cells can drive fludarabine chemoresistance. In conclusion, we propose that, on the molecular level, *POLN* contributes to chemoresistance by sustaining the DNA synthesis and preserving cellular viability in presence of a low dNTP pool imposed by fludarabine.

E1019

ARSENICTRIOXIDE REGULATES CHRONIC LYMPHOCYTIC LEUKEMIA PROLIFERATION BY TARGETING XPO1/SURVIVIN PATHWAY SIGNALLING

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Background: Chronic lymphocytic leukaemia(CLL) is characterized by the progressive accumulation of clonal mature B-cells in the blood, bone marrow, and lymphoid organs and by a highly variable clinical course. Elevated expression of exportin 1 (XPO1/CRM1) has been reported in CLL and correlated with a poor prognosis and resistance to therapy. Inhibition of XPO1 can result in CLL cell death in vitro and increased survival in a CLL mouse model. The efficacy of XPO1 inhibitor for the treatment of diffuse malignant peritoneal mesothelioma may rely on interference with the function of survivin. Our previous work showed that arsenic trioxide(As_2O_3) induced apoptosis in a CLL cell linetogether with up-regulation of p53 and down-regulation of survivin. Taken together, these findings raise the question of whetherAs_2O_3 might inhibit CLL by inducing apoptosis via the suppression of XPO1/survivin pathway signalling.
Aims: We sought to investigate whether XPO1/survivin pathway signalling might be involved in the anti-CLL effect induced by As_2O_3.
Methods: The expression of XPO1 in the MEC1 cell line and primary peripheral blood cells from CLL patients was assessed by qPCR and compared with the expression in purified peripheral normal B-cells. XPO1 siRNA was used to knockdown XPO1 expression in MEC1 cells. XPO1 plasmid transfection was used to overexpress XPO1. Thesurvival and apoptosis rates of MEC1 cells induced by As_2O_3were determinedby 7-AAD,Annexin V-PI and caspase3/7 analysis. The expression of various genes associated with apoptosis, including Bax, PUMA, survivin, BCL-2, BCL-x and XIAP, were assessed by real-time PCR.XPO1 normal and XPO1 knockdown MEC1 cells were inoculated intravenously intoNOD/scid/γc^{null}(NSG) mice to establish a human leukaemia xenograft model. We assessed tumour characteristics, including organ infiltration, apoptosis, andexpression of survivin, ki67 and XPO1, as well as survival in our xenograft model. Next, we used this model to study the effects of As_2O_3 *in vivo*.
Results: XPO1 was expressed in the MEC1 cell line and in CLL patients. Expression data suggested that XPO1 was up-regulated inCLL patients compared with normal controls. As_2O_3 could inhibit the proliferation and induced apoptosis in MEC1 cells, and it down-regulated the expression of XPO1 and survivin in MEC1 cells.XPO1 suppressed by $As_2O_3$was associated with enhanced mRNA expression of the pro-apoptotic genes Bax, PUMA and survivin, and suppression of the anti-apoptotic genes BCL-2, BCL-x and XIAP.Overexpression of XPO1 in MEC1 decreased As_2O_3 -induced apoptosis.Inhibition of XPO1 by selinexorin MEC1 cellsresulted in increasedAs_2O_3 -induced apoptosis.Furthermore, siRNA knockdown of XPO1 strongly impaired *in vivo* engraftment of MEC1 cells following transplantation in NSG mice, conferring a survival benefit compared with mice transplanted with control cells.As_2O_3 could inhibit tumour growth and significantly increased the survival of mice bearing MEC1-derived xenografts by inhibiting XPO1 and survivin.
Summary/Conclusions: This is the first report to demonstrate thatAs_2O_3can induce apoptosis and inhibit proliferation in CLL. The mechanistic effect of As_2O_3 on CLLresults from the down-regulation of XPO1/survivin pathway signalling.

E1020

IBRUTINIB STIMULATES IL-10 SECRETION BY NLCS, MEDIATING A PARTIAL PROTECTION OF CLL CELLS FROM APOPTOSIS

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Background: In lymphatic tissues, CLL cells establish intimate contact with accessory cells, such as nurse-like cells (NLCs). NLCs have a pivotal role in CLL clone maintenance and support CLL cell survival, proliferation and protection from drug-induced apoptosis. Ibrutinib is a potent inhibitor of Btk kinase, able to counteract the pro-survival effects in CLL cells provided by microenvironment. A peculiar effect of ibrutinib includes reduced retention and homing of CLL cells to tissue compartments and a mobilization from microenvironmental niches into the peripheral blood.
Aims: Here, we investigated the biological effects and mechanism mediated by ibrutinib on CLL- NLCs crosstalk.

Methods: CLL-PBMCs were cultured in complete medium for 12 days and then treated with 1μM ibrutinib. NLC phenotype was analyzed upon ibrutinib exposure. IL-10 production was tested by real time PCR and CSA in NLCs. CD19+ CLL cells viability and analysis of signaling pathways in presence of IL-10 stimulation were evaluated after treatment with ibrutinib.
Results: First, we found that CLL cells cultured without NLCs are more sensitive to ibrutinib-induced apoptosis if compared to CLL cultured with NLCs. Indeed ibrutinib does not completely hamper the protective effect mediated by NLCs on leukemic cells when both CLL and NLCs are exposed to the drug ($p<0.05$ at 24h, 48h and 72h). Among the plethora of factors potentially involved in CLL desensitization to ibrutinib effect, NLCs were characterized by a strong up-regulation of IL10 and NAMPT known to be involved in CLL protection from apoptosis ($p<0.05$). We focused our attention on IL-10 and we demonstrated that treatment with ibrutinib for 24h improves NLCs secretion of IL-10. CD19+ CLL cells stimulated with IL-10 in a dose escalation experiment (from 0.1 ng/ml to 100 ng/ml) show good protection from apoptosis at different doses at different time-points. We then analyzed the ability of ibrutinib to abrogate the pro-survival signal induced by IL10 stimulation (1 ng/ml) in CD19+ CLL cells after 24-48h of culture. Ibrutinib does not completely antagonize the ability of IL-10 to protect CLL cells from apoptosis and to activate pSTAT3 and pERK signaling pathways. In accordance, ibrutinib is able to potentiate the immunosuppressive and permissive profile of NLCs stimulating the expression of CD163, CD206 and the activation of pSTAT6 with a concomitant inhibition of pSTAT1.
Summary/Conclusions: The ability of ibrutinib to effectively disrupt the crosstalk between CLL cells and NLCs is still unclear. Our data demonstrate that ibrutinib may also target NLCs, potentiating their permissive features through the secretion of unwanted survival factors such as IL-10.

E1021

LEUKEMIC CELL/MICROENVIRONMENT INTERACTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA: ROLE OF JAK2/STAT3 AXIS IN THE SURVIVAL OF NEOPLASTIC CLONE

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Background: Chronic Lymphocytic Leukemia (CLL) is characterized by the accumulation of mature clonal CD19+/CD5+/CD23+ B lymphocytes in peripheral blood, bone marrow, and lymphoid tissues. Despite their *in vivo* prolonged lifespan due to intrinsic defects, CLL leukemic cells rapidly undergo spontaneous apoptosis *in vitro*, highlighting the need of extrinsic signals delivered by the microenvironment. Several molecules, including those released by mesenchymal stromal cells (MSCs), signal through JAK (Janus Kinases)/STAT (Signal Transducers and Activators of Transcription) pathways. Constitutive activation of JAKs and STATs occurs at very high frequency in various hematopoietic malignancies and solid tumors. STAT3 tyrosine (Tyr) phosphorylation at residue 705 is known to be critical for the activation of JAK2/STAT3 pro-survival signaling, while the role of STAT3 Serine (Ser) phosphorylation at residue 727 has not yet been well described. In CLL, STAT3 is constitutively phosphorylated at Ser727 with respect to normal B lymphocytes, but the phosphorylation status at Tyr705 still remains unresolved.
Aims: We are aimed to evaluate JAK2/STAT3 pathway involvement in leukemic B cell survival and to study the cross-talk between JAK/STAT and BCR/Lyn axes. With the identification of new targets implicated in this cross-talk, and through the analysis of leukemic cell resistance to drug-induced apoptosis, we plan to counteract the favorable pro-leukemic cell stimuli.
Methods: B cells were collected from 34 controls and 76 CLL patients. Purified cells ($2x10^6$ cells/ml) were cultured, with/without mesenchymal stromal cells (MSCs), and treated with AG490 (10, 50 and 100μM) and Stattic (5, 7.5, and 10μM), specific inhibitors of JAK2 and STAT3 respectively, for 24, 48 and 72h. STAT3 expression and phosphorylation were evaluated by Western Blotting (WB) and Flow Cytometry (FC), and its localization was analyzed by confocal microscopy and subcellular fractionation. CLL and normal B cell viability was tested by FC with Annexin V/PI test. Lyn and SHP-1 phosphorylation was assessed by WB.
Results: We demonstrated that STAT3 is overexpressed in malignant B cells (Student's *t* test, $p<0.001$) and the protein is higher phosphorylated at Tyr705 with respect to the normal counterpart (Student's *t* test, $p<0.05$), thus showing its constitutive activation in CLL. We also found that the *in vitro* incubation of leukemic B cells with AG490 and Stattic, specific inhibitors of JAK2 and STAT3, respectively, induces a dose-dependent apoptosis of CLL B cells. We demonstrated that these inhibitors are able to induce apoptosis in CLL B cells reverting the resistance to cytotoxic agents induced by the MSCs, bypassing the provided pro-survival stimuli. In addition to JAK2/STAT3 inhibition, we showed that AG490 treatment on CLL cells can mediate the activation of SHP-1, decreasing its phosphorylation at Ser591, thus leading to inactivation of Lyn protein, by de-phosphorylation in its active site at Tyr396. Lyn, a Tyr-kinase, and SHP-1, a Tyr-phosphatase, are both involved in the prolonged lifespan of neoplastic CLL cells.
Summary/Conclusions: Bypassing the pro-survival stimuli provided by the tumor microenvironment, the ability of AG490 and Stattic to induce apoptosis in leukemic B cells, represents a starting point for the development of new therapeutic strategies in CLL. These findings also provide new insights on the cross-talk between JAK/STAT and BCR/Lyn axes in CLL.

E1022

LINKING PROTEOME AND GENOME FOR BIOMARKER DISCOVERY IN CLL-ESTABLISHMENT OF A CLL PROTEIN DATABASE

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Background: Chronic Lymphocytic Leukaemia (CLL) is notable for its clinical variability, suggesting a role for precision medicine. Attempts at understanding the biological basis of this variability have mainly focussed on genomic alterations and gene expression at the mRNA level. However, the molecular basis of CLL variability remains incompletely understood. We speculate that this is because the clinical phenotype is ultimately determined by gene expression at the protein level. We have therefore embarked on a global proteomic study of a large number of CLL trial samples for which whole genome sequencing data will be available through the Genomics England Ltd (GEL) CLL Pilot Project. We anticipate that our study will bridge the gap in our understanding between genotype and clinical phenotype.
Aims: Recently developed mass spectrometric approaches to highly parallel, label-free protein quantification provide a new opportunity to develop diagnostic, prognostic and predictive biomarkers. SWATH (Sequential Windowed Acquisition of all THeoretical fragments) is a data-independent approach which generates a mass spectral library of fragment ions from all detectable peptide precursors. The composite MS/MS spectra are deconvoluted by alignment with a high quality and exhaustive, tissue specific database, whereupon patient samples may be stratified based on the quantitative expression profile of thousands of proteins. Here, we describe generation of a CLL-specific protein database that will provide a permanent reference source for all future trial samples, as indicated in Figure 1.
Methods: Protein extracts were prepared from CLL cells from 14 individual patients in various stages of the disease. Lysates were pooled and digested before being subjected to cation exchange chromatography. Forty fractions were delivered into a TripleTOF 6600 mass spectrometer (SCIEX) via an Eksigent nanoLC 415 system (SCIEX). Data dependent acquisition was performed using 25 MS/MS per cycle to optimise for quality and 30 MS/MS per cycle to optimise for coverage, and the combined data were searched using ProteinPilot 5.0 (SCIEX) against the SwissProt database (Nov 2015, 20,193 human entries). Proteins lying within a 1% global false discovery rate were included in the database.
Results: The new CLL-specific database contains 7773 proteins. The database covers 50% of all human UniProtKB/SwissProt entries which have evidence at the protein level. Pathway analysis of B cell receptor (BCR) signalling based on the GeneGo pathway maps in the MetaCore database (version 6.14, build 61508; Thomson Reuters) showed that this CLL database covers over 87% of the proteins involved in BCR signalling. The quality of the database entries is further underlined by high representation in the Human Genome database (20,814 entries) in aspect of Gene Ontology biological processes and molecular functions, as determined by PANTHER over-representation tests.

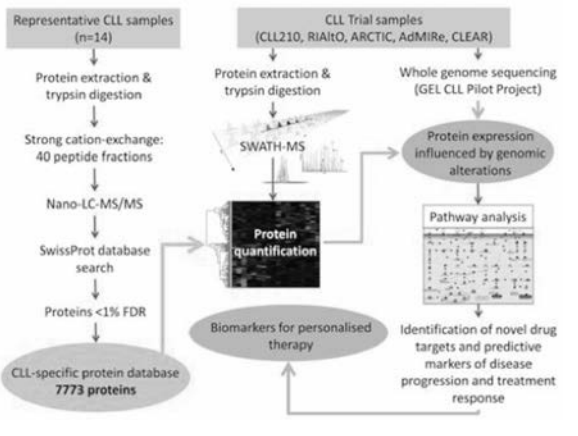

Figure 1.

Summary/Conclusions: Our global protein expression database for CLL cells is thus the most complete database of its type. By combining advanced computational biology to relate protein expression to whole genome sequencing data, the database will provide a framework for elucidating the effect of genomic alterations on protein expression. The construction of this comprehensive CLL database also provides the platform for applying SWATH/MS-based analysis of individual patient samples to identify novel drug targets and predictive biomarkers of disease progression and treatment response.

E1023

THE IMPACT OF THE TUMOUR MICROENVIRONMENT ON B-CELL RECEPTOR (BCR) EXPRESSION AND SIGNALLING IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Background: Chronic lymphocytic leukaemia (CLL) is thought to be driven following antigen engagement of the B cell receptor (BCR). This has been highlighted further by the BCR Kinase inhibitors Ibrutinib and Idelalisib, which have proved instrumental in the treatment of CLL. However, these inhibitors appear to only suppress the disease without being curative. A number of patients have developed resistance to ibrutinib following mutation of the *BTK* or *PLCγ2* gene, whilst other patients are unable to tolerate these drugs due to adverse events or progress whilst on therapy for unknown reasons. B-cell activating factor (BAFF) and interleukin-4 (IL-4) are known to increase CLL cell viability *in vitro*, and are thought to be present within CLL lymph nodes. Therefore we have investigated the role of BAFF and IL-4 in regulating BCR signalling in CLL cells and promoting resistance to ibrutinib and idelalisib.
Aims: To understand how IL-4 and BAFF regulate BCR pathway inhibition by ibrutinib and idelalisib.
Methods: Experiments were carried out using primary CLL cells. All isolates used contained >85% $CD19^+CD5^+$ cells. Protein expression was evaluated by flow cytometry or immunoblotting and signalling capacity was detected by calcium flux analysis in response to soluble αIgM by flow cytometry. Cell viability was assessed using annexin v/propidium iodide staining.
Results: As previously shown CLL cells treated with BAFF (500 ng/ml) or IL-4 (10 ng/ml) only for 48h significantly protected against basal apoptosis. This anti-apoptotic effect was further augmented when cells were treated with BAFF and IL-4 in combination. CLL cells treated *in vitro* with ibrutinib and idelalisib induced approximately 24.3% and 28.8% apoptosis respectively following treatment for 48h. However, apoptosis induced by the BCR-kinase inhibitors was reversed following pre-treatment with IL-4 or BAFF. CLL cells incubated *in vitro* with IL-4 for 24h significantly augmented sIgM expression and αIgM induced phosphorylated ERK and calcium flux. These IL-4 induced effects on sIgM expression were more prominent in Unmutated(U)-CLL samples, and could be reversed using the JAK3 inhibitor tofacitinib (CP-690550). Whilst, IL-4 had little effect on sIgD. Interestingly pre-treating CLL cells with IL-4 significantly reversed the ability of ibrutinib (1 μM) and idelalisib (1 μM) to inhibit αIgM-mediated signalling at 24h. In contrast BAFF treatment showed no clear effect on sIgM expression and downstream signalling. However, in a proportion of CLL samples treated simultaneously with BAFF and IL-4, BAFF reduced IL-4 specific increases in sIgM augmentation. Next we pre-treated CLL cells with tofacitinib (10 μM) for 1 hr prior to IL-4 treatment. Tofacitinib significantly reversed the effects observed with IL-4 and restored the ability of ibrutinib and idelalisib to inhibit αIgM induced signalling. This effect was specific to the inhibition of IL-4 since tofacitinib (≤10 μM) did not induce CLL cell death. Finally we showed that BAFF, and IL-4 to a greater extent, resulted in an increase in miRNA155 expression which has previously been shown to be associated with more progressive disease and enhanced BCR signalling.
Summary/Conclusions: Our data suggests that IL-4 augments sIgM recovery and down-stream signalling in CLL, which may compromise the effectiveness of BCR kinase inhibitors. However these effects were not observed for BAFF. Furthermore, co-treatment of BCR-kinase inhibitors in combination with tofacitinib may indicate a promising treatment strategy for the treatment of CLL.

E1024

INHIBITION OF USP7 INDUCES SELECTIVE CANCER CELL DEATH IN CHRONIC LYMPHOCYTIC LEUKEMIA THROUGH PTEN AND INDEPENDENTLY FROM P53 STATUS

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Background: Standard immune-chemotherapy allows to achieve 60-70% response rate in CLL patients, but complete remission remains uncommon. Unsatisfactory therapeutic options still exist for higher risk groups, and in particular those harboring *TP53* mutations or deletion.
Aims: We propose USP7, a known de-ubiquitinase with multiple roles in cellular homeostasis, as a potential therapeutic target in CLL.
Methods: Primary CLL cells were enriched in $CD19^+$ fraction using the Milteny anti-CD19 kit and were used to investigate USP7 levels. P5091 (USP7 inhibitor) efficacy was tested in CLL cell lines and patients. Proliferation and apoptosis were evaluated respectively with CTG technology and Annexin V-FITC/Propidium PE detection by flow cytometry. The specificity of the USP7 inhibitor was verified with a pool of 5 different siRNAs able to efficiently silence USP7.
Results: Our data show that the de-ubiquitinase USP7 is aberrantly expressed in CLL. In particular, USP7 is over-expressed in about 70% of CLL $CD19^+$ lymphocytes, both at the mRNA and protein levels. We also analyzed USP7 expression levels in an expansion cohort of a publicly available CLL patients (n=217) and 12 normal samples, where USP7 was over-expressed in CLL when compared to normal samples (****$p<0.0001$). We proved that USP7 is regulated at post-transcriptional level by miR-338-3p and functionally activated by Casein Kinase 2 (CK2) through phosphorylation at serine 18 residue. USP7 inhibition by P5091 as well as by specific siRNA, induces cell growth arrest and apoptosis mediated by the restoration of the nuclear pool of PTEN. Notably in primary CLL cells, P5091 treatment strongly promoted apoptosis. Moreover, USP7 inhibitor treatment of primary CLL was associated with increases ubiquitination of endogenous PTEN and consequently PTEN relocalization into the nucleus. Strikingly, TP53 deleted CLL samples and cells lines were equally subject to P5091 apoptosis induction, confirming that the USP7 inhibitor acts in a p53 independent manner. These data showed potent activity of P5091 against primary CLL.
Summary/Conclusions: We demonstrate the efficacy of a small molecule inhibitor of USP7, P5091, *in vitro* in cell lines and *ex vivo* in primary CLL samples in a P53-independent manner. Our preclinical study therefore, supports the evaluation of USP7 inhibitor as a potential CLL therapy.

E1025

DIRECT AND INDIRECT MODULATION OF MACROPHAGES IN THE TUMOR MICROENVIRONMENT UPON GENOTOXIC STRESS ALTERS PHAGOCYTIC FUNCTION

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Background: Resistance against chemotherapy is a central problem in the current treatment of B cell malignancies. Up until now, little is known about the impact of the tumor microenvironment on therapeutic outcome. This microenvironment consists of many different kinds of cells varying between tumors, significantly manipulating malignancy and enabling relapses after treatment. Macrophages are present in the microenvironment of most tumors-designated as tumor associated macrophages (TAM)-and are often correlated to a poor prognosis. We postulate that macrophages and their reprogramming are an essential element in the malignant progression of tumors and their response to DNA damage as occurring in chemotherapeutic treatment and are a promising target to improve therapy response.
Aims: We aim to define the functional characteristics of macrophages in their cross talk with malignant B cells and characterize their role for disease progression and antitumor therapy. Our particular interest lies on the investigation of the functional mechanistic and kinetics underlying macrophage repolarisation. Here, we intend to identify key regulators and their upstream signaling pathways by focusing on the DNA damage pathway.
Methods: To assess macrophage leukemia cell interaction we established *in vitro* and *ex vivo* co-cultures of macrophages and leukemic cells, specifically investigating macrophage antibody dependent cellular phagocytic (ADCP) capacities. Leukemic cell clearance and macrophage phenotype characterization are determined by flow cytometry. We addressed both primary CLL patient cells as well as humanized mouse model of BCL2/MYC double hit lymphoma.
Results: Functionally we prove that low dose application of the *in vitro* equivalent of Cyclophosphamide, Mafosfamide significantly increases the phagocytic function of macrophages. Besides this direct effect on macrophages, a combination of cyclophosphamide and alemtuzumab was demonstrated to induce a macrophage based increase in tumor clearance by cytokine secretion of stressed malignant B cells. Here, we could determine IL10 as an inducer of increased phagocytosis in tumor associated macrophages. Moreover we show that IL10 modulates differentiation of macrophages which strongly increases their ADCP compared to classical M1/M2 differentiated macrophages. Analyzing the functional contribution of the DNA-damage pathway we could identify pronounced secretion of IL10 in p53-deficient leukemia cells. We show *in vitro* that the DNA damage pathway plays an important role in the ASAP mechanism as a down regulation of various DNA damage key players in the leukemic cells diminishes the stimulating effect of Cyclophosphamide and antibody combinations.
Summary/Conclusions: Repolarisation of macrophages towards increased phagocytosis is essential in immunochemotherapies. We demonstrate that the DNA damage pathway is important for this stimulating effect and that the mech-

anism involves a complex interplay between macrophages and leukemic cells. The DNA damage of the genotoxic chemotherapy is increasing the phagocytosis activity of the macrophages directly as well as indirect by a cytokine secreted from stressed leukemic cells.

E1026

NF-KB INDUCES IL-6-MEDIATED STAT3-PHOSPHORYLATION IN CLL CELLS

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Background: In CLL cells STAT3 is constitutively phosphorylated on serine 727 residues whereas phosphorylation of STAT3 on tyrosine 705 residues cells is inducible. Cytokines, such as IL-6, or IgM antibodies that activate CLL cells' BCR, induce tyrosine phosphorylated (p) STAT3. However, while IL-6 induces tyrosine pSTAT3 within 15 minutes, IgM induces pSTAT3 within 2-4 hours. The reason for the delayed IgM-induced phosphorylation is unknown. Like STAT3, the transcription factor NF-κB is constitutively activated in CLL cells and stimulation of the BCR further activates NF-κB. Whether BCR stimulation upsurges NF-κB's transcriptional activity. Specificallhas not been elucidated.In CLL cells STAT3 is constitutively phosphorylated on serine 727 residues whereas phosphorylation of STAT3 on tyrosine 705 residues cells is inducible. Cytokines, such as IL-6, or IgM antibodies that activate CLL cells' BCR, induce tyrosine phosphorylated (p) STAT3. However, while IL-6 induces tyrosine pSTAT3 within 15 minutes, IgM induces pSTAT3 within 2-4 hours. The reason for the delayed IgM-induced phosphorylation is unknown. Like STAT3, the transcription factor NF-κB is constitutively activated in CLL cells and stimulation of the BCR further activates NF-κB. Whether BCR stimulation upsurges NF-κB's transcriptional activity. Specificallhas not been elucidated.
Aims: 1) To describe the BCR dependent, NF-κB-mediated gene expression profile of CLL cells. 2) To confirm our hypothesis that prolonged stimulation with IgM antibodies induces tyrosine pSTAT3 via NF-κB-mediated induction of IL-6 in CLL cells.
Methods: We incubated peripheral blood CLL cells in the presence or absence of IgM antibodies or IL-6, and harvested the cells at different time points. Total RNA was extracted using TRIzol, cDNA was synthesized with Super Script First synthesis System for RT-PCR, and NF-κB-target gene expression was quantified using RT-PCR. To measure the levels of tyrosine pSTAT3 we used flow cytometry and to assess binding of NF-κB (p65) to DNA we utilized an electromobility shift assay (EMSA) using an NF-κB-binding site labelled DNA probe.
Results: To study the transcriptional activity of NF-κB we used a PCR array that profiles the expression of 83 NF-κB-target genes. To reduce the 'noise' from stochastic variability in gene expression we first identified a core of genes that are expressed in cells from all patients' samples. To that aim we ranked the Ct values in each array and considered all genes that were amplified earlier than the cycle in the 75th percentile. Using this approach we identified 35 genes (42% of genes represented in the array) that were amplified in all 6 patients' samples. Annotation analysis revealed that the key pathways common to these 35 genes included 'Positive regulation of the NF-κB cascade', 'Inflammation' and 'Negative regulation of apoptosis'. Applying stringent criteria we identified 5 genes common to all cases that were amplified prior to the cycle representing the 25th percentile. Most amplified genes detected in all samples prior to stimulation (28/35, 80%) were also detected after 4 h of IgM stimulation, confirming that NF-κB is constitutively activated in CLL cells. However, 19 addition genes (19/83, 23% of the genes in the array) were detected in all IgM-stimulated but not in unstimulated cells. Remarkably, *IL-6* was detected in all cases only after IgM stimulation. Furthermore, observed an IgM-induced time-dependent increment in *IL-6* and *IL-8*, suggesting that IL-6 expression is dependent on stimulation of the BCR. Indeed IL-6 neutralizing antibodies significantly reduced the levels of tyrosine pSTAT3 in CLL cells incubated for 18 h with IgM antibodies. In addition, EMSA of CLL cells from 4 different patients showed that stimulation of the BCR with IgM antibodies increased the binding of NF-κB to DNA in a time-dependent manner. Furthermore, the JAK2 inhibitor Ruxolitinib attenuated the NF-κB-DNA binding, suggesting that 2-4 hour exposure to IgM antibodies induces activation of NF-κB, a process mediated in part by IL-6 that activates the JAK2/STAT3 pathway.
Summary/Conclusions: It has been well established that the BCR of CLL cells is stimulated in the bone marrow and lymph nodes. However, whereas the immediate effects of BCR stimulation have been excessively studied, the successive effect of BCR stimulation is poorly understood. We found that stimulation of the BCR induces tyrosine phosphorylation of STAT3 via NF-κB-mediated induction of IL-6, a process that requires protracted BCR stimulation. Although NF-κB is constitutively activated in CLL cells, continuous activation of the BCR further activates NF-κB. These findings suggest that agents, such as Ruxolitinib, that inhibit the successive effects of the BCR activation, would become effective therapeutic agents in CLL.

E1027

DIFFERENTIAL EXPRESSION PROFILE OF H/ACA RIBONUCLEOPROTEIN COMPONENTS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease. Telomere dysfunction has been proposed as an emerging prognostic factor in this pathology. Telomeres are essential to maintain chromosomal integrity and genome stability. They progressively shorten with repeated cell division, leading to telomere dysfunction and contributing to tumorigenesis. Telomerase is a ribonucleoprotein complex containing an internal RNA template (TERC) and a catalytic protein with telomere-specific reverse transcriptase activity (TERT). The human TERC gene consists of three major domains; among them the H/ACA domain is essential for telomere biogenesis. H/ACA ribonucleoprotein (RNP) complex is composed of four evolutionary conserved proteins, including dyskerin (DKC1), NOP10, NHP2 and GAR1. The first three proteins form a core trimer that directly binds to H/ACA RNAs. They are interdependent upon each other for stability and also regulate stability of the bound RNAs. GAR1 binds only to DKC1 and is needed for proper functioning of the H/ACA RNPs, but its absence does not reduce stability of the RNA.
Aims: In this study, we have evaluated the expression profile of the H/ACA RNP complex: *DKC1*, *NOP10, NHP2 and GAR1*, as well as *TERT* and *TERC* mRNA levels, in patients with CLL. Results were correlated with telomere length (TL), genetic alterations, mutational status of *IGHV* (immunoglobulin heavy chain variable region) genes, and clinico-pathological characteristics of the disease.
Methods: The study was performed on mononuclear cells isolated on a Ficoll-Paque Plus density gradient from peripheral blood samples of 60 patients with CLL at diagnosis (34 males; mean age: 66.8 years; range: 44-88 years) and 14 normal controls. All individuals gave their informed consent and the study was approved by the local Ethics Committee. Gene expression and absolute TL measurement was carried out by real-time quantitative PCR. *IGHV* gene rearrangements and mutational status were analyzed by RT-PCR and bi-directional sequencing. Cytogenetic and FISH analysis were also performed.
Results: Gene expression analysis showed increased mRNA levels of *TERT, NHP2 and GAR1,* as well as decreased expression of *TERC, DKC1* and *NOP10* in CLL patients compared to controls (p=0.043). A significant correlation between *GAR1-NHP2*, *GAR1-NOP10* and *NOP10-NHP2* transcription levels (p=0.0001) was detected, supporting a strong interaction among them. Patients with short TL had higher expression of *TERT* (p=0.033) and *DKC1* (p=0.02) than those with long telomeres. The analysis taking into account cytogenetic and FISH alterations showed higher mRNA levels of *GAR1* and *NHP2* in patients with two or more anomalies (p=0.02) compared to those with no/one alteration. An upregulation of *TERT, TERC, GAR1 and NOP10* expression in unmutated *IGHV* CLL patients was also observed. No association between gene expression and clinical parameters was found. However, a tendency to a short treatment free survival in patients with increased *TERT* expression was detected.
Summary/Conclusions: Our findings show a global modification in the expression of telomere associated genes in CLL, being, to our knowledge, the first analysis of *TERC, NOP10, NHP2 and GAR1* in this pathology. Their association with higher number of genetic alterations and unmutated *IGHV* mutational status suggests a role for these telomere-associated genes in genomic instability and telomere dysfunction in CLL.

E1028

REDOX SIGNALING HYPERSENSITIVITY DISTINGUISHES CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH FAVORABLE PROGNOSIS

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Background: Chronic lymphocytic leukemia (CLL) patients exhibit a variable clinical course, with some patients having indolent disease and others experiencing a more accelerated course, treatment resistance and dismal outcome. The B-cell receptor (BCR) signaling is a key determinant of heterogeneous clinical behavior of CLL and is a target for therapeutic interventions. Endogenously produced H_2O_2 is thought to fine tune the level of BCR signaling by reversibly inhibiting phosphatases. However, relatively little is known about how CLL cells sense and respond to such redox cues.
Aims: In this study, we used phospho-specific flow cytometry to compare BCR signaling responses to H_2O_2 in prognostic groups of CLL patients.
Methods: The phosphorylation levels of five proteins downstream of the BCR signaling, namely SYK, NF-κB p65, ERK1/2, p38 and JNK, were analyzed at the single-cell level in 26 CLL cell samples using phospho-specific flow cytom-

etry. In accordance with the Declaration of Helsinki, all patients provided written informed consent for the collection and use of their samples for research purposes. The local Ethics Committee (Comitato Etico per la Sperimentazione, AOUI) approved sample collection. Protein phosphorylation was measured in the basal condition and following stimulation with H_2O_2. Circulating B cells from healthy individuals were analyzed as controls. The two-sample Wilcoxon's rank sum test was used to compare protein phosphorylation in groups of patients. Time to first treatment (TTFT) was calculated from the date of diagnosis to the date of initial therapy. TTFT curves estimated using the Kaplan- Meier method for the respective groups of patients were compared using the log-rank test.
Results: In CLL cells, stimulation with H_2O_2 induced a statistically significant increase in phosphorylation of all analyzed signaling proteins with the exception of SYK. Moreover, the extents of responses to H_2O_2 were significantly higher in CLL than normal B cells for all signaling proteins but SYK. Comparison of H_2O_2 signaling response in prognostic groups of patients defined by *IGHV* mutational status, CD38 or ZAP-70 expression, showed that median phosphorylation response of ERK1/2 to H_2O_2 was significantly higher in the patient subset defined by the mutated *IGHV* status (M-CLL) (*P*=0.031). No significant correlations were observed between H_2O_2 responsiveness of BCR signaling proteins and ZAP-70 or CD38 expression. Kaplan-Meier curves showed statistically significant slower progression (longer time to first treatment, TTFT) in patients with higher p-ERK1/2 and p-NF-□B p65 responses to H_2O_2, indicating that lower responsiveness of these signaling proteins to H_2O_2 correlated with more rapid progression [median TTFT was 41.6 and 115 months for patients with lower and higher NK-kB p65 responsiveness to H_2O_2, respectively (log-rank test *P*=0.0011); median TTFT was 38.7 and 117.0 months for patients with lower and higher ERK1/2 responsiveness to H_2O_2, respectively (log-rank test *P*=0.0008)] (Figure 1). Interestingly, the ability of H_2O_2 responsiveness signaling to define prognostic groups is comparable to that of *IGHV* mutational status (log-rank test *P*=0.0003).

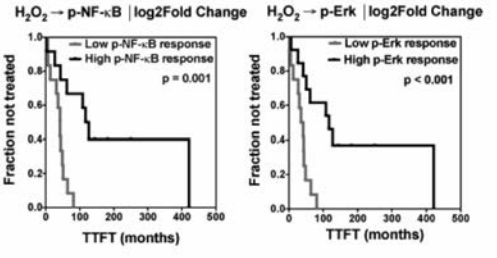

Figure 1. Kaplan-Meier curves of TTFT for subgroups of patients defined by response to H_2O_2.

Figure 1.

Summary/Conclusions: This study reveals that a novel H_2O_2 signaling response distinguishes a prognostic group of CLL patients with favorable prognosis. Specifically, higher H_2O_2 responsiveness of ERK1/2 or NF-kB is predictive of longer TTFT, thus highlighting ERK and NF-kB as biologically and clinically relevant signaling nodes in CLL.
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E1029

OFATUMUMAB AND LENALIDOMIDE PREFERENTIALLY INHIBIT B-CELL RECEPTOR SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS WITH MUTATED IGHV

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Background: Lenalidomide has clinical activity in chronic lymphocytic leukemia (CLL) patients exerting pleiotropic activity on the immune system. The combination of lenalidomide with the anti-CD20 monoclonal antibody ofatumumab induces durable responses in patients with relapsed/refractory CLL. In addition to cell- and complement-mediated B-cell depletion induced by anti-CD20 antibodies and to immunomodulatory activity of lenalidomide, both drugs have been shown to directly inhibit survival and proliferation of malignant B cells. Signal transduction mediated by the B-cell receptor (BCR) promotes CLL survival and proliferation and is considered a key determinant of CLL clinical behavior and target for therapeutic interventions. However, the hypothesis that ofatumumab and lenalidomide could target BCR signal transduction has never been examined in CLL.
Aims: In this study, we used phospho-specific flow cytometry to investigate the direct effects of lenalidomide and ofatumumab on BCR signaling in CLL cells from different prognostic groups of treatment-naive patients.
Methods: The phosphorylation levels of four proteins downstream of the BCR signaling, namely SYK, ERK1/2, PLC-γ, and NF-κB p65, were analyzed at the single-cell level in 9 CLL cell samples treated *in vitro* with single-agent ofatumumab, single-agent lenalidomide or their combination, using phospho-specific flow cytometry. Phosphorylation was measured in the basal condition and following BCR stimulation with anti-IgM, resulting in overall 576 signal readouts. The repeated measures Anova with Dunnett's multiple comparison test was used to compare drug effects on protein phosphorylation.
Results: Ofatumumab and lenalidomide induced different effects in ex-vivo CLL cells with different prognostic features as defined by the *IGHV* mutational status. In the *IGHV*-mutated (M) subset, treatment with ofatumumab, alone or in combination with lenalidomide, induced a significant reduction of SYK and ERK1/2 basal phosphorylation. Remarkably, in the same CLL group, lenalidomide significantly inhibited ERK1/2 basal phosphorylation. On the contrary, in the patient group defined by unmutated *IGHV* status (UM), treatment with ofatumumab and lenalidomide induced no significant changes in basal phosphorylation of BCR proteins. As expected, BCR stimulation with anti-IgM antibodies induced a signaling response in CLL cells that was statistically significant for ERK1/2, PLC-γ, and NF-κB p65. In the M subset, treatment with ofatumumab, alone or in combination with lenalidomide, significantly inhibited the anti-IgM signaling response of SYK and ERK1/2. Interestingly, lenalidomide significantly reduced the anti-IgM-induced signaling response of SYK. In contrast, in the UM CLL subset, ofatumumab and lenalidomide induced no significant changes in anti-IgM responses of BCR proteins.
Summary/Conclusions: This study suggests that ofatumumab and lenalidomide can influence the BCR signaling in the basal state as well as under stimulation, with differential effects in distinct prognostic subsets of CLL. Although a study on a larger patient set is needed to extend and confirm these results, overall they indicate that ofatumumab and lenalidomide can inhibit BCR signaling in the M subset of CLL whilst have no significant effect in UM CLL, potentially contributing to the capacity of these drugs to differentially inhibit disease-progression in patients with CLL.
Funding. This study was funded by Fondazione Cassa di Risparmio di Verona, Vicenza, Belluno e Ancona and AIRC (grant #6599).
Acknowledgments. We thank GSK and Celgene for providing ofatumumab and lenalidomide.

E1030

DECREASED EXPRESSION OF WNT3, A CANONICAL WNT PATHWAY LIGAND, IS FREQUENT IN CHRONIC LYMPHOCYTIC LEUKEMIA PROGRESSION AND IDENTIFIES PATIENTS WITH SHORT TREATMENT-FREE SURVIVAL IN MUTATED IGHV SUBSET

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Background: Wnt ligands drive several distinct pathways-the best explored are Wnt/β-catenin (canonical) and Wnt/Planar Cell Polarity (PCP) pathways. We have shown recently that ROR-1 driven Wnt-5a-activated WNT/PCP pathway controls migratory properties of chronic lymphocytic leukemia (CLL) cells and *WNT5A* positivity correlates with aggressive CLL (Janovska *et al.*, Clin Cancer Res 2016). Several reports suggested that the canonical Wnt/β-catenin pathway, known to drive malignant transformation of multiple cell types, also participates on CLL pathogenesis. This was based on the high expression of canonical Wnt ligands and LEF1, and mutations in Wnt pathway genes. There is, however, a lack of direct evidence showing that CLL cells can respond to canonical Wnt ligands by activating β-catenin-dependent transcription. On the other hand, the observation that *WNT3*, encoding for the canonical Wnt ligand, is among the most up-regulated genes, supports the role of Wnt/β-catenin pathway in CLL.
Aims: To clarify the role of Wnt/β-catenin pathway in CLL by (1) detailed analysis of *WNT3* expression and (2) cell-line based study of the ligand-induced pathway activation.
Methods: B-cells from CLL patients and non-malignant controls were negatively separated with RosetteSep (StemCell) or MACS kits (Miltenyi Biotec). *WNT3* mRNA expression was analyzed with quantitative RT-PCR using TaqMan Gene Expression Assays (Applied Biosystems). The *WNT3* expression was correlated with clinical and biological parameters. Survival analyses were performed using Kaplan-Meier curves, Cox regression model and log-rank test; cut-off was assessed in CutOff Finder web application. Wnt/β-catenin pathway activity was analyzed by the SuperTopFlash reporter system (Promega).
Results: By analyzing 137 previously untreated patients we confirmed that *WNT3* gene is strongly overexpressed in CLL compared to normal B-cells (P=0.0001) and that its level is higher in patients with mutated IGHV (M-CLL; P=0.047). Further, we identified low *WNT3* level as a strong independent marker indicating shorter treatment-free survival (TFS) in M-CLL (P<0.0002). Time course analysis in 86 patients revealed that WNT3 declines with disease progression in a significant proportion of patients (decrease in 37%, increase in 4% of patients; P<0.0001), namely in a disease activity onset in untreated M-

CLL and in a disease recurrence after therapy in patients with unmutated IGHV (U-CLL). Functional tests showed that selected lymphoid cell lines (CLL-derived MEC1 and ROR1-positive REC1) failed to respond to the ligand-induced activation of the Wnt/β-catenin pathway while bone marrow stromal cells M210B4 were able to transduce a signal to TCF/LEF-dependent transcription.

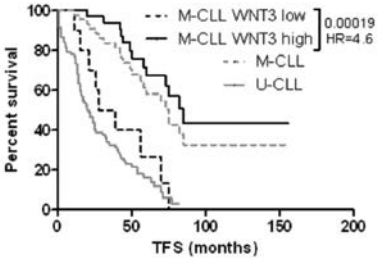

Figure 1.

Summary/Conclusions: Our results show that despite *WNT3* overexpression in CLL cells, its high levels are not associated to aggressive CLL. On the contrary, low *WNT3* independently identifies patients with short TFS in M-CLL and *WNT3* level decrease accompanies disease evolution in a significant proportion of patients. The data indicate that the Wnt/β-catenin pathway plays a more complex role in CLL pathogenesis than previously anticipated, opening a hypothesis that Wnt-3 ligand might be associated to early steps in CLL pathogenesis possibly via mediating interaction with the cells in the microenvironment.
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E1031

INNATE LYMPHOID CELLS ARE EXPANDED AND FUNCTIONALLY ALTERED IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Both innate and adaptive immune cells markedly affect CLL cells, yet recent findings also highlight reciprocal stimulation of immune cells by clonal B cells. Multi-directional signals derived from a triad formed by malignant cells, lymphocytes and innate immune cells could therefore critically contribute to shaping a supportive environment for the malignant clone. Elucidation of the immune disturbances and tumor microenvironment in CLL is essential for improving therapeutic possibilities and treatment of the CLL immunodeficiency. At the crossroad of innate and adaptive immunity, innate lymphoid cells (ILCs) have recently entered the stage as key players in the initial immune response and tumor surveillance. ILCs have lymphoid morphology but lack rearranged antigen receptors. In addition to NK cells, the ILC family consists of 3 non-cytotoxic groups, mirroring T helper subsets. Group 1 ILCs produce IFN-γ, whereas group 2 ILCs secrete IL-5 and IL-13. Group 3 ILCs produce IL-22 and IL-17, and are subdivided based on the expression of the natural cytotoxicity receptor NKp44. The role of ILC in CLL has not yet been clarified.
Aims: The aim of this study is to investigate the frequency and functionality of ILCs in CLL patients.
Methods: ILCs, defined as lineage$^-$CD127$^+$CD161$^+$ lymphocytes, were measured by flow cytometry in 21 untreated CLL patients and 8 age-matched healthy controls (HCs). We studied functionality of ILCs through measuring cytokine production by flow cytometry upon PMA/ionomycin stimulation in CLL patients (n=6) and HCs (n=6). To assess function after cytokine activation, proliferation and cytokine production was measured in ILC subgroups in 4 CLL patients and 3 HCs.
Results: The number of ILCs in the peripheral blood is significantly increased in CLL patients. Moreover, the ILC count correlates with the absolute leukocyte count in CLL patients, suggesting a rise in ILCs with disease progression. The absolute counts of both ILC1s and NKp44$^-$ ILC3s are significantly elevated in comparison to HCs. The activation status of ILCs, as measured by CD69 expression, is similar in CLL patients and HCs. Next, we compared the functionality of ILCs from CLL patients and HCs by measuring cytokine production. TNF-α production is significantly reduced in CLL patients, yet IFN-γ production is comparable between CLL patients and HCs. The production of type 2 (IL-13) and type 3 (IL-22) cytokine production by ILCs in CLL patients is similar to that in HCs. Finally, we investigated whether functional differences remained present upon cytokine activation. In parallel with direct cytokine measurements in the total ILC pool, cultured ILC1s from CLL patients produce less TNF-α, while IFN-γ production by cultured ILC1s was not affected. ILC1s from CLL patients tend to expand slower than their healthy equivalents, although the difference was not statistically significant. In contrast with ILC1s, the expansion and production of IL-17A and IL-22 of NKp44$^-$ ILC3s was not affected upon cytokine activation.
Summary/Conclusions: Taken together, we demonstrate that in patients with CLL, ILC homeostasis is disturbed. ILCs are expanded in CLL patients and their absolute numbers correlate with disease stage. In addition, ILC1 function is impaired which persists upon cytokine activation. These observations are in line with NK cell alterations and may represent a bystander effect, or reflect functional involvement of ILCs in CLL pathobiology. The extent to which ILCs affect disease progression and therapeutic response is yet to be identified.

E1032

IN VITRO EVALUATION OF BORTEZOMIB MOLECULAR EFFECTS IN LARGE GRANULAR LYMPHOCYTE LEUKEMIA

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Background: Large Granular Lymphocyte Leukemia (LGLL) is a chronic lymphoproliferative disorder characterized by clonal expansion of Large Granular Lymphocytes (LGLs) with cytotoxic activity. LGLs proliferation is maintained through the activation of several survival signaling pathways. Among these, one of the most important deregulated pathway is the JAK/STAT axis. STAT3 activation, mediated by IL-6 signaling or activating STAT3 mutations, promotes LGLs survival by inducing several anti-apoptotic genes transcription and represents a hallmark of this disease. Nowadays, LGLL therapy is based on immunosuppressive drugs, such as Methotrexate (MTX), Cyclophosphamide (CTX) and Cyclosporine A (CyA), showing however only a partial efficacy with an Overall Response Rate (ORR) of about 50-60%. Bortezomib (Bz), the first FDA-approved proteasome inhibitor used in Multiple Myeloma (MM) therapy, is not an approved treatment opportunity for LGLL, but the rare co-association of LGLL with MM offered the opportunity to retrospectively investigate the effect of this drug on leukemic LGLs. The literature reports that patients diagnosed with both LGLL and MM showed a decrease in the LGLs count after Bz treatment, suggesting that this drug may have an effect on inhibiting the LGL clone.
Aims: The aim of this study is to investigate the *in vitro* effects of Bz on PBMCs from LGLL patients, in order to understand its molecular mechanism of action in reducing the LGL clone.
Methods: Peripheral blood mononuclear cells (PBMCs) of 20 LGLL patients (8 out of 20 characterized by STAT3 mutations), isolated through Ficoll density centrifugation, were cultured and treated with Bz (5.2 nM) for 24 or 48 hours. Real Time-PCR, western blot (WB) assays and immunofluorescence assay were performed to analyze mRNA and protein expression. Cell apoptosis was evaluated by Flow cytometry, using Annexin V staining.
Results: We analyzed *IL-6* mRNA expression levels in patients-derived PBMCs by Real Time-PCR. Our results demonstrated that *IL-6* gene expression significantly decreases in Bz-treated cells, as compared to a not treated condition ($p<0.01$). The transcriptional data were supported by immunofluorescence assay, showing that this cytokine is mainly produced by the monocyte/macrophage lineage and that its expression is reduced after Bz treatment. To investigate the Bz effect on *IL-6*-induced JAK/STAT pathway, we performed WB analysis to evaluate STAT3 activation by measuring pSTAT3 Tyr705. Our results showed that pSTAT3 Tyr705 levels strongly decrease in Bz-treated condition, irrespective to the presence of STAT3 mutations. This pattern strictly differs from untreated PBMCs, in which STAT3 remains over-activated. Then, by Real Time-PCR and WB assays, we analyzed the expression of STAT3 downstream targets, focusing on the anti-apoptotic *MCL-1* and *BCL-2* gene expression. Our results demonstrated a significant reduction of the expression levels of both these genes following Bz treatment ($p<0.01$). Finally, we evaluated Bz effect on cell survival by Annexin V staining. Our results showed a significant time-dependent increase in LGL apoptosis after Bz treatment, as compared to a not treated condition. Moreover, we observed that this drug is highly specific in inducing leukemic LGLs apoptosis, not affecting the other PBMCs.
Summary/Conclusions: Our results provide evidence that Bortezomib might represent a new intriguing therapeutic option to be added to the immunosuppressive drugs already used in LGLL therapy.

E1033

A SHARP CONTRAST IN FUNCTIONALITY BETWEEN EBV- AND CMV-SPECIFIC CD8+ T-CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is characterized by a tumor induced T-cell dysfunction, which leads to impaired cytotoxicity, which is probably caused by an inability to form immune synapses and increased expression of exhaustion markers. However, we recently found that CMV-specific CD8$^+$ T-cells from CLL patients are functionally intact when compared to age-matched healthy

controls (HC). This finding challenges the concept of a global T-cell dysfunction in CLL. Whether intact functionality of CMV-specific T-cells is a rare exception or whether T-cell functionality is indeed more heterogeneous is currently unknown. Acquired T-cell dysfunction in CLL needs to be better understood in order to improve anti-tumor immunotherapies that rely on T-cell mediated effects.
Aims: To analyze T-cell function heterogeneity in CLL, we studied the immunophenotype and functionality of $CD8^+$ T-cells specific for Epstein-Barr-virus (EBV), another widely common chronic latent viral infection.
Methods: EBV-specific $CD8^+$ T-cells were analyzed using EBV tetramers and 14-color flow cytometry in 45 untreated CLL patients and 23 age-matched HC. We studied T-cell differentiation, expression of exhaustion markers (PD-1, CD244 and CD160), functional markers and homing markers. To study the functionality of EBV-specific $CD8^+$ T-cells, we determined cytokine production and polyfunctionality after stimulation with EBV-derived peptides. Furthermore, we analyzed *ex vivo* cytotoxicity of EBV-specific $CD8^+$ T-cells.
Results: We found that, when compared to HC, EBV-specific T-cells in CLL patients are further differentiated with a significantly smaller percentage of "early" effector memory cells (also called EM1; CLL=39.6% *vs* HC=57.68%). These results are mirrored by the expression pattern of the transcription factors T-bet and Eomes; 25.79% of EBV-specific T-cells of CLL patients display a $T\text{-}bet^{high}\text{-}Eomes^{high}$ phenotype *vs* 17.44% in HC, again pointing to an increased differentiation state. In comparison with HC, EBV-specific T-cells in CLL patients show higher expression of exhaustion markers CD244 and CD160, but not PD-1. However, there were no significant differences in granzyme B and K expression, suggesting an unaltered cytotoxic potential. On a functional level, no differences between CLL and HC were found with respect to production of the cytokines TNFα, IFNγ, IL-2 and MIP-1β and degranulation (measured as $CD107a^+$ cells) of EBV-specific T-cells after peptide stimulation. Polyfunctionality of EBV-specific T-cells of CLL patients was comparable with HC. Surprisingly, we found that EBV-specific T-cells of CLL patients display significantly less direct cytotoxic capacity compared to HC in an *ex vivo* killing assay (mean specific lysis of target cells CLL=6,83% *vs* HC=32,4%), indicating a functional impairment despite normal differentiation and cytokine production. We are currently performing experiments to study immune synapse formation of EBV-specific T-cells (which we will be able to present during the EHA meeting) to determine involvement of the immune synapse in this acquired defect.
Summary/Conclusions: Taken together, we conclude that EBV-specific T-cells show signs of functional impairment and are, in contrast to CMV-specific T-cells, not able to evade CLL induced T-cell dysfunction. In depth studies on differences in T-cell mediated viral responses in the context of CLL will yield important clues to develop strategies to overcome T-cell dysfunction in this disease.

E1034

IDENTIFICATION OF A STRUCTURALLY NOVEL BTK MUTATION THAT DRIVES IBRUTINIB RESISTANCE IN CLL BUT NOT RICHTER TRANSFORMATION

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Background: Ibrutinib (ibr), a first-in-class Bruton tyrosine kinase (BTK) inhibitor, has demonstrated high response rates in both relapsed/refractory and treatment naïve chronic lymphocytic leukemia (CLL). However, about 25% of patients discontinue ibrutinib therapy at a median follow-up of 20 months and many patients discontinue the treatment due to leukemia progression or Richter transformation. Mutations affecting the C481 residue of BTK disrupt ibrutinib binding and have been characterized by us and others as the most common mechanism of ibrutinib resistance. In addition, *BTK* mutations have been found in several Richter-transformed patients treated with ibr. In these patients, it is not clear whether *BTK* mutations are related to Richter transformation or ibr treatment or both.
Aims: As the use of ibr becomes more prevalent in CLL and other types of non-Hodgkin lymphoma (NHL), more patients are expected to develop resistance. Thus, a complete understanding of the mechanisms of ibr resistance is important for the development of strategies to prevent and treat ibrutinib resistance.
Methods: Serial samples were collected from a Richter transformed CLL patient who were treated with ibrutinib, responded and then relapsed. The samples were analyzed using Onco1K, a 1,200-gene next-gen sequencing panel with an average sequencing depth of 420x. The uncovered novel mutation was then validated with Sanger sequencing and characterized with structural modeling. The role of the mutation was further functionally defined with cell transfection followed by assays for cell-proliferation, BrdU-incorporation, and intracellular B-cell receptor signaling.
Results: A structurally novel mutation of Bruton tyrosine kinase was identified which was associated with CLL relapse but not Richter transformation. Functionally, cells carrying the mutant *BTK* show resistance to ibrutinib at both cellular and molecular levels to a similar extent as BTK^{C481S}.
Summary/Conclusions: Our study lends further insight into the diverse mechanisms of ibrutinib resistance that has important implications for the development of next-generation BTK inhibitors as well as mutation detection in relapsed patients.

E1035

CHECKPOINT KINASE 1 INHIBITION POTENTIATES APOPTOSIS AND INFLICTS MITOTIC FAILURE IN CLL-DERIVED MEC-1 CELL LINE

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Background: Treatment options for patients with TP53-mutated lymphoid tumors are very limited. In chronic lymphocytic leukemia (CLL), TP53 defects were not surmounted by chemoimmunotherapy regimens functioning in other patients, and also remain challenging for innovative small-molecule inhibitors of B-cell receptor signaling. Recently, the inhibition of ataxia-telangiectasia mutated and Rad-3 related (ATR) kinase operating in the DNA damage response (DDR) pathway has been identified as a potential alternative therapeutic strategy in CLL. ATR is a key molecule activating the DDR pathway through phosphorylation of checkpoint kinase 1 (Chk1) upon recognition of DNA damage.
Aims: (1) to test the Chk1 inhibition potential for sensitizing TP53-mutated CLL-derived MEC-1 and MEC-2 cell lines to nucleoside analogs (NAs) fludarabine, cytarabine and gemcitabine, and (2) to analyze appropriate cell death mechanisms in MEC-1 cells.
Methods: We used highly specific Chk1 inhibitor SCH900776 and measured (a) overall cell viability using a metabolic WST-1 assay, (b) DNA double strand breaks (DSBs) accumulation using pS139 detection on H2AX (γ-H2AX), and (c) apoptosis and cell cycle profile using annexin-V/PI and DNA content staining, respectively. In case of fludarabine, we also analyzed structure of mitotic chromosomes and employed a cell-live imaging for direct cell death monitoring.
Results: The MEC-1 cell line was significantly ($P<0.01$; two-way ANOVA) sensitized to all three NAs using 200 nM SCH900776, as evidenced by 72 h cell cultivation and concentration-dependent curves of viability. Interestingly, the serial sister cell line MEC-2 established from the same patient one year later completely lost the Chk1 inhibition-mediated sensitization to fludarabine. Analyzing further cell death mechanisms in MEC-1 cells, we observed that SCH900776 greatly facilitated γ-H2AX accumulation in the co-treatments with all three NAs and in all tested time intervals (4, 14, 24, and 48 h). Accordingly, apoptosis was clearly augmented in the co-treatments compared to single agent treatments with NAs. In the cell cycle analysis, an increase of sub-G1 (apoptotic) fraction in the co-treatments with Chk1 inhibitor resulted in all three NAs from diminished post-G1 populations (S and G2/M). The cytogenetic analysis for the co-treatment of SCH900776 with fludarabine disclosed a substantially reduced mitotic index (0.6% compared to 15.8% in control), and severe chromosome pulverization in cells undergoing mitosis (detected in 42% of analyzed mitotic cells). By contrast, this pulverization was only occasionally observed in single agent treatment with fludarabine (4%) and never present in control untreated MEC-1 cells. The cell-live imaging then disclosed several abnormal features during mitosis including (a) hardly recognizable metaphase plate, (b) inner cell mass asymmetric division between daughter cells, (c) accelerated time course of division process involving approximately 14 minutes (compared to 22 minutes in untreated cells), and (d) signs of apoptosis such as membrane blebbing.
Summary/Conclusions: The intrinsic resistance of TP53-mutated CLL cells to NAs can be significantly surmounted by Chk1 inhibition leading to cell cycle checkpoints abrogation and augmented DNA damage. Further testing of clinical candidate SCH900776 and other specific Chk1 inhibitors in CLL is justified and particularly appropriate among TP53 mutated patients.
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E1036

PROGNOSTIC SIGNIFICANCE OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 5 AND 5B EXPRESSION IN EPSTEIN-BARR VIRUS POSITIVE PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Signal Transducer and Activator of Transcription (STAT) proteins

are cytosolic transcription factors that, upon activation, dimerize and migrate to the nucleus to control gene expression. Among them, STAT5 consists of two isoforms (STAT5A and STAT5B) that are 90% identical and are encoded by two adjacent genes on 17q11.2. Aberrant STAT5 activity has been linked to tumorigenesis, and the implication of STAT5 in leukemias and lymphomas that are correlated to viruses has been long speculated. EBV-related lymphomagenesis has not been thoroughly studied in correlation to the JAK-STAT pathway, although there are in vitro data supporting a constitutive activation of STATs in EBV positive lymphoma cell lines.

Aims: We investigated the expression of total STAT5 and STAT5b in patients with Chronic Lymphocytic Leukemia (CLL) in correlation to the presence of Epstein-Barr Virus (EBV) and Latent Membrane Protein 1 (LMP1).

Methods: Peripheral blood samples from patients with CLL and healthy blood donors were obtained. EBV DNA quantification was performed by real time PCR using the EBV R-gene Quantification Complete kit (bioMérieux, Paris, France). A conventional Real time PCR was used for the detection of LMP1-mRNA and LMP1 expression was verified by ELISA (LMP1 detection kit, MYBiosource, San Diego, CA, USA). Western-blotting was performed for STAT5 and STAT5B in protein extracts. The secondary antibodies used were Affinity Purified Antibody Peroxidase Labeled Goat anti-Rabbit and anti-Mouse IgG (H-L) (Kirkegaard & Perry Laboratories, USA). Gel Pro Analyzer 4 was used to analyze the results. IBM SPSS statistics, v19.0 (IBM Corporation, NY, USA) was used for the statistical analysis of the results.

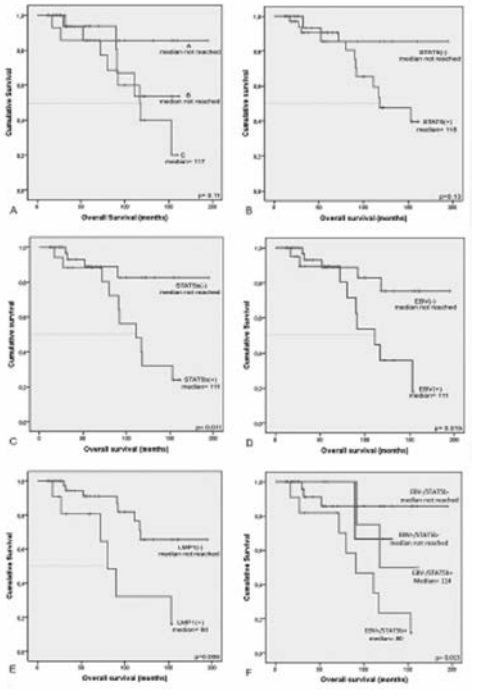

Figure 1.

Table 1. Correlation of EBV and LMP1 status with the expression of STAT5 and STAT5b.

Patients	EBV(+), N (%)	EBV(-), N (%)	P*
Expressing STAT5	17 (77.3)	21 (51.2)	0.044
Not expressing STAT5	5 (22.7)	20 (48.8)	
Expressing STAT5b	11 (50.0)	7 (17.1)	0.006
Not expressing STAT5b	11 (50.0)	34 (82.9)	
	LMP1(+)	LMP1 (-)	
Expressing STAT5	11 (91.7)	27 (52.9)	0.014
Not expressing STAT5	1 (8.3)	24 (47.1)	
Expressing STAT5b	7 (58.3)	11 (21.6)	0.011
Not expressing STAT5b	5 (41.7)	40 (78.4)	

**Pearson's chi-square test, 2-sided p*

Results: Sixty-three (63) patients with CLL and immunophenotypical comfirmed disease by peripheral blood at the time of sample collection were included in the study. The vast majority of the patients (81%) were treatment naïve, and the clinical stage according to the Binet staging system was: stage A 42.9%, B, 34.9%, and C 22.2%. Total STAT5 was expressed in 38 patients (60.3%) and STAT5b was expressed in patients only (18, 28.6%) but not in healthy subjects. Its expression was correlated to the detection of EBV (77.3% *versus* 51.2%, p=0.006) and the expression of LMP1 (58.3% *versus* 21.6%, p=0.011), (detailed results in Table 1). The expression of STAT5b and the presence of EBV and LMP1 were strongly negatively correlated to the overall survival (OS) of the patients (p=0.011, 0.015, 0.006 respectively). Double positive (for EBV and STAT5b) patients had the lowest OS (p=0.013), (Figure 1).

Summary/Conclusions: This is the first report of a survival disadvantage of EBV positivity in CLL, and the first correlation of STAT5b to OS. Moreover, double positivity (for EBV and STAT5b) was correlated to a worse prognosis, while double negative patients had the highest OS. The correlation of STAT5/5b expression with the presence of the virus and OS defines a subgroup of patients that may benefit from treatment with anti-STAT agents.

E1037

EX VIVO LYMPH NODE NATIVE MICROENVIRONMENT ASSAY SHOWS NOVEL ANTIPROLIFERATIVE ACTIVITY FOR IDELALISIB AND IBRUTINIB ON CLL CELLS

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Background: Survival and proliferation of chronic lymphocytic leukemia (CLL) cells is favored by the essential role of the tumor microenvironment (TME) that is similarly responsible at least in part for disease progression and drug resistance.

Aims: We planned to evaluate and predict the efficacy of therapeutic compounds *in vivo*, by reproducing in a co-culture system the different microenvironmental components that enable B cells to survive and proliferate, mimicking in particular the lymph node microenvironment where most of the crucial events of the pathogenesis of CLL occur.

Methods: To this purpose, cryopreserved peripheral blood (PB) mononuclear cells from CLL patients in need of treatment were tested with the Exvitech® proprietary automated flow cytometry-based platform. Different components have been evaluated to reproduce the ME and induce proliferation and survival of CLL cells: (i) 3 backbone stimulations: CD40L+CpG, CD40L+IL21, CpG+IL2; (ii) "Native Environment", defined as the plasma & erythrocyte/granulocyte fraction of a Ficoll gradient: (iii) the stroma cell line H5S, added at different ratios (1:10 or 1:100); (iv) both human and bovine fetal serum (at 10 or 20% total volume); (v) stimulatory B cell factors, including IL-21, soluble CD40L, BAFF, and B cell receptor stimulation (anti-IG).

Results: Of all the described combinations, the addition of CpG+IL2, HS5 (at 1:100 ratio), human serum 10%, and "native environment" from PB of CLL samples (pooled samples to prevent interpatient variability) were the best to promote CLL proliferation (30±3%) and viability (60±5%). We then tested dose responses of the novel BCR inhibitors, idelalisib (PI3kδ inhibitor) and ibrutininb (BTK inhibitor) in 29 and 25 cryopreserved progressive CLL samples, respectively (Figure) and showed that resting CLL cells were virtually unaffected by either drug (EC_{50} of 12,5μM and 28.3μM), suggesting a limited direct pro-apoptotic activity of the drugs. In contrast, marked inhibition of proliferation was observed (EC_{50} of 30 nM and 550 nM, respectively) in the presence of either inhibitor, leaving only a median of 5% CLL cells that continued proliferating even at the highest doses.

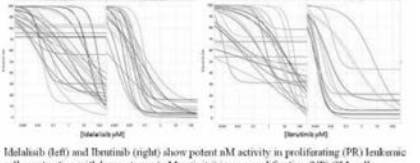

Figure 1.

Summary/Conclusions: We here report a novel *ex vivo* assay that incorporates TME stimuli thus more accurately simulating *in vivo* interactions and enabling high-throughput pharmacological characterization in more physiological conditions. This assay demonstrated a previously unreported anti-proliferative activity for both idelalisib and ibrutinib that may explain the efficacy of both drugs in patients.

E1038

THE RETINOID DRUG ACITRETIN UPREGULATES CD38 EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA AND MEC-1 CELLS AND REDUCES CELL HOMING TOWARDS THE CHEMOKINE CXCL12: POTENTIAL FOR EXPLOITATION IN THERAPY

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Background: CD38 expression is a robust prognostic indicator in Chronic Lymphocytic Leukemia(CLL) and has gained importance among the repertoire of markers utilised to aid management decisions in CLL. The CD38 molecule is also attracting interest as a mark for targeted therapies, namely anti-CD38 monoclonal antibody treatment. Retinoids modulate CD38 expression due to the presence of the Retinoic Acid Response Element (RARE) DNA sequence in intron 1 of the *CD38* gene. Several studies have investigated this effect in myeloid malignancies however little is known about retinoid effect on lymphoid malignancies.
Aims: In this study we demonstrate that the retinoic acid derivative, acitretin, upregulates the expression of CD38 on the MEC-1 cell line and on primary CLL cells from CD38 positive patients. We propose that by doing so, acitretin may render these cells more susceptible to the actions of anti CD38 monoclonal antibody drugs and thus have a potential role as an adjunct to such therapies. We also show that acitretin reduces CLL cell homing in response to the chemokine CXCL12, therefore potentially preventing cells from reaching the micro environment niche and may as a result, lead to shortened survival.
Methods: Ethical approval for the study was obtained from the ethical committee in Beaumont University Hospital. Peripheral blood samples were collected from 32 patients with a confirmed diagnosis of CLL and who had given written informed consent. MEC-1 cells were obtained from DSMZ, Germany. MEC-1 cells and cells from five CD38 negative and five CD38 positive patients were treated with 10μM acitretin for 24 and 72 hours. CD38 expression was measured by flow cytometric analysis at baseline and at the different time points. Results are expressed as fold difference in Mean Fluorescence Intensity (MFI) in treated *versus* untreated cells. Migration assays were performed using $2x10^6$ cells per well incubated with either 10μM acitretin or media, overnight in 1%FBS. Cells were subsequently transferred to chamber inserts overlying 10%FBS media containing 200ng/ml CXCL12 in all wells, except negative controls. Cells were allowed to migrate for 4hrs. Migration was calculated as percentage of cells in lower chamber to upper chamber.
Results: CD38 expression in MEC-1 cells was significantly upregulated in cells incubated with 10μM acitretin for 72 hours, (6.89 fold higher expression in acitretin treated cells compared to controls, n=3,**p=0.00075). In primary cells derived from patient samples that had CD38 expression ≥9% at baseline, acitretin produced an increase in CD38 expression that was 2.25 fold higher compared to untreated cells(n=5,* *p=.022*). However, in primary cells from patient samples with CD38 expression<9% at baseline, the effect of acitretin was only 1.53 higher CD38 expression in cells treated with 10μM acitretin at 72 hours. Significant modulation of CD38 expression in these cells was only achieved in the cells treated with 50μM acitretin for 72 hours (*p=.022). In cell migration, acitretin treatment resulted in a significant reduction in CLL cell homing toward CXCL12 in 14 out of 17 patient samples. Mean relative migration was reduced from 23.5% to 11.9% of input cells (11.6% reduction, ***p=.00018,* n=14, mean +/- SEM).These were cell samples from a varied group of patients that included 8(54%) from previously treated, relapsed patients and 6(43%) treatment naïve patient samples.
Summary/Conclusions: CD38 expression is significantly unregulated by acitretin in CLL cells expressing CD38 at baseline. We suggest this would prove advantageous in the setting of anti-CD38 monoclonal antibody therapy through improving efficacy of this drug, and should be studied further in CLL. Acitretin also reduced the homing ability of CLL cells towards the chemoattractant CXCL12, the mechanism and potential therapeutic implications of which needs to be further explored.

E1039

ARRAY CGH ANALYSIS REVEALS DELETION OF CHROMOSOME 22Q11 IN CLL WITH NORMAL KARYOTYPE AND NO FISH ALTERATIONS

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Background: Genomic aberrations have increasingly gained importance as prognostic markers in B-cell chronic lymphocytic leukemia (CLL). Recently, a number of additional studies have been completed that clearly demonstrate the feasibility of using array comparative genomic Hybridization (a-CGH) as a clinical tool to identify genomic alterations of prognostic importance in CLL.
Aims: aCGH is able to identify a significant percentage of genomic abnormalities that escapes conventional cytogenetics and CLL FISH panel due to the limitations of these methods.
Methods: Using Oligonuckleotide-based array CGH (CytoChip 4x180K-Illumina) we detected copy number changes in the tumor genomes of 23 CLL cases at diagnosis, with normal karyotype e no FISH alterations. The cytogenetic study was performed on nuclei and metaphases after stimulation with a combination of CpG-oligonucleotide DSP30 and interleukin 2 (IL-2). In Chromosome Banding Analysis (CBA) we analyzed a minimum of 20 metaphases for each patient. Interphase FISH was performed using a comprehensive set of commercially available probes, as follows: P53 (TP53)Deletion (17p13), ATM Deletion (11q22), D13S25 Deletion (13q14.3), MYB Deletion (6q23)[Cytocell], CEP-12 (Chromosome 12) [Abbott], XL IGH plus Break Apart Probe [MetaSystems]. A minimum of 100–200 interphase nuclei were evaluated per probe for each patient.
Results: A total of 21 of the 23 (91%) cases were successfully analyzed by Oligonuckleotide-based array CGH. We observed a submicroscopic deletion of chromosome 22q11, as the sole anomaly in 4/21 cases (19%). Patients with loss 22q11 showed progression disease, and the median Time to Treatment (TT) was 64.2 months (range 24.6 - 83.1 months). Loss 22q11 ranged in size from 0.68Mb-0.28Mb. The minimally deleted region included the ZNF280A, ZNF280B, GGTLC2, and PRAME genes. Moreover this genomic change had not been detected by standard cytogenetic and/or FISH analyses. Gunn et al. suggest that the incidence of 22q11 deletions was second only to loss of the 13q14 region, found in approximately 50% of CLL cases; few and divergent data were reported in literature regarding prognostic impact of this genomic abnormality in CLL.
Summary/Conclusions: Our results showed that submicroscopic 22q11 deletion is potentially significant genomic aberration in CLL and this alteration is being missed by the current routine techniques. Moreover, increasing the number of cases to analyze we can confirm the data obtained and to correlate the alteration observed to the clinical course.

E1040

EXPRESSION OF FCGAMMARIIB PREDICTS TIME TO TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The Fcγ receptor IIb (FcγRIIb) is a low-affinity Fcγ receptor that negatively regulates B-cell receptor (BCR) function in normal B-cells. In chronic lymphocytic leukemia (CLL) cells, FcγRIIb may exert this inhibitory effect when colligated with the BCR. Since CLL cells are activated *in vivo*, the clinical impact of FcγRIIb expression in this malignancy is worth of being investigated.
Aims: The aims of this study were to: (1) analyze the basal expression of FcγRIIb on CLL cells and normal B-cells; (2) investigate the relationship between FcγRIIb and well-known prognostic markers; and (3) explore the prognostic impact of FcγRIIb on clinical outcome of patients with CLL.
Methods: The study population included 174 patients with active CLL and 34 healthy donors. The median age at diagnosis was 66 years (34-88) and follow-up was 8.0 years (0-38.7). FcγRIIb expression was determined by flow cytometry using an Alexa488-conjugated mAb specific for human FcγRIIb within the following combinations: FcγRIIb/CD38/CD19/CD5 and FcγRIIb/CD49d/CD19/CD5. Results were expressed as the ratio between the MFI for FcγRIIb and the MFI for the corresponding isotype (MFIR). The Fisher's exact test and the Mann-Whitney U test were used for comparisons between groups. Kaplan–Meier survival and Cox regression analysis were performed to evaluate the correlation of FcγRIIb expression with clinical outcome. Best cut-offs for time to treatment (TTT) were determined by ROC curves.
Results: Both CD5+CD19+ leukemic cells and CD19+ normal B-cells expressed FcγRIIb, with no significant differences in MFIR between the populations [median MFIR: 46.4 (8.3-153.4) *vs* 49.4 (30.8-73.2), p=0.812]. FcγRIIb expression, however, was highly heterogeneous among CLL samples, with 4.5% showing very high expression (outliers). Within clones from the same patient, FcγRIIb MFIR was higher on CD38+ or CD49d+ cells than on CD38- or CD49d- cells, respectively (p<0.01). Eighty-one percent (142/174) of patients showed high (MFIR>30.8) and 19% had low FcγRIIb expression (MFIR<30.8). We did not find any association between FcγRIIb expression and well-known prognostic markers except CD49d (p=0.025). However, the correlation between FcγRIIb and CD49d expression was very weak (κ=0.085) showing concordance in only 39.7% of cases. At the time of analysis, 52 patients (29.9%) had required therapy. Patients with low FcγRIIb expression had a significantly shorter TTT than those with high FcγRIIb expression (median 46.07 *vs* 62.7 months; log-rank p<0.028). In univariate analysis, other prognostic parameters, such as clinical stage, serum B2M, elevated LDH, ZAP70, CD38 and CD49d expression, *IGHV* mutational status, cytogenetics, and mutations of *NOTCH1* and *SF3B1* significantly correlated with shorter TTT (p<0.01). In a multivariate analysis incorporating FcγRIIb expression, ZAP70, CD38 and CD49d expression, *IGHV* mutational status, cytogenetics, and mutations of *NOTCH1* and *SF3B1*, only low FcγRIIb expression (HR 2.66, 95%CI 1.22-5.80, p=0.014), unmutated *IGHV* (HR 3.02, 95%CI 1.30-7.02, p=0.010), and mutated *SF3B1* (HR 11.27, 95% CI 2.82-45.12, p=0.001) and *NOTCH1* (HR 4.79, 95%CI 1.31-17.48, p=0.018) retained prognostic significance for shorter TTT.

Summary/Conclusions: FcγRIIb expression can predict time to treatment in CLL, underlying a key role of the receptor in the progression of this malignancy. These results encourage further investigation to characterize the mechanisms by which FcγRIIb controls CLL cell activation and to uncover the relationship of FcγRIIb with other molecules, particularly CD49d.

E1041

FYN INHIBITOR INDUCES APOPTOSIS IN NK LEUKEMIA CELLS

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Background: Fyn protein, a member of Src-family kinase, has different biological functions including growth factor/cytokine receptor, T and B-cell receptor and integrin-mediated signaling, cell-cell adhesion, mitosis, differentiation of natural killer (NK) cells. Some studies show its involvement in hematologic malignancies. However, the tissue-specific pattern of Fyn mRNA expression indicate that it is more expressed in NK and T cells respect to other human tissues. NK cell neoplasms are a rare and heterogeneous group of disorders characterized by increased proliferation of cytotoxic NK cells. They include aggressive NK leukemia (ANKL) and chronic lymphoproliferative disorder of NK cells (NK-CLPD). ANKL has a fulminant clinical course frequently resulting in death within two months; NK-CLPD is typically indolent. Since no standard therapies for immature NK cell neoplasms have been established so far, new therapeutic options are needed. Tintori et al. found a new Fyn inhibitor by a structure-based drug design protocol followed by hit-to-lead optimization. The 4c pyrazolo[3,4-*d*]pyrimidine compound showed antiproliferative activity against different cancer cell lines.
Aims: The aim of this study was to determine the effect of 4c compound in NK leukemic cells.
Methods: Fyn mRNA level was evaluated in peripheral blood mononuclear cells (PBMC) from 10 healthy controls (HC) and 8 NK-CLPD patients by qRT-PCR; protein level was evaluated in PBMC from 3 HC and 3 NK-CLPD and in 2 ANKL cell lines (KHYG1 and NK92) by western blotting (WB). The 4c compound was tested in a time course MTS assay at increasing concentrations on HC-PBMC, KHYG1 and NK92. KHYG1 were treated with 4c at 4μM for 24h to evaluate apoptosis and cell cycle by cytometric analysis, as well as gene expression profile by Illumina HiScanSQ. Fyn immunoprecipitation and proteome profile arrays of apoptosis and phospho-kinase were also performed. Expression of procaspase3, active caspase3, P70 and Akt was validated by WB. 4c compound was tested also in 3 NK-CLPD e 1 ANKL patients. Cell viability was assessed by trypan blue dye count method and apoptosis was verified by cytometric analysis.
Results: qRT-PCR data showed a significant overexpression of *Fyn* level in PBMC of NK-CLPD patients compared to HC. Protein level was highest in NK-CLPD patients ($p<0.001$) and NK cell lines ($p<0.0001$) respect to HC. To verify the effect of 4c compound, we treated KHYG1 and NK92 with different concentrations, showing a reduction of cell viability (EC50= 4μM and 14μM respectively at 24h); on the contrary, there was no effect on HC-PBMC. Moreover, this treatment induced a significant caspase3 dependent apoptosis ($p<0.01$) and G2/M cell cycle arrest. Gene expression analysis of 4c treated or not KHYG1 identified 455 down-regulated and 326 up-regulated genes, most of which involved in cell death function, thus confirming the apoptosis data. To corroborate Fyn inhibition by 4c treatment, we detected a reduction of its phosphorylation and we also observed a decrease of phospho-Akt and phospho-P70, two proteins involved in apoptotic process and cell proliferation. Interestingly, 4c treatment reduced cell viability and activated caspase3 dependent apoptosis ($p<0.05$) in 3 NK-CLPD and 1 ANKL patients.
Summary/Conclusions: In this study we demonstrated a higher Fyn expression in NK leukemia patients compared to healthy controls. We also demonstrated an apoptotic effect of pyrazolo[3,4-*d*]pyrimidine 4c compound on NK leukemia cells and we provided *in vitro* preliminary evidences that Fyn is its possible cellular target.

E1042

RETROTRANSPOSONS SHAPED NON-REDUNDANT CLLU1 GENE MODULES

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Background: *CLLU1* was originally identified as a gene differentiating IgV_H-unmutated (IgV_H-UM) and IgV_H-mutated (IgV_H-M) chronic lymphocytic leukemia (CLL) and is now appreciated to represent a potent predictor of overall survival of CLL patients. Whilst *CLLU1* is located in a genomic region that is highly expressed in both germinal center B cells and CLL cells, indicating an open-chromatin structure, its transcription is highly restricted. *CLLU1* expression is markedly high in IgV_H-UM CLL, low in IgV_H-M CLL and negligible in normal B-cell subpopulations or any other hematological specimens tested. Alternative splicing of the *CLLU1* gene generates 7 transcript variants. Previous studies revealed a powerful linear relation between the expression levels of all the variants, signifying that transcription is orchestrated by a common promoter located upstream of exon 1. Accordingly, it was deduced that (epi)genetic aberrations, occurring during leukemogenesis, could trigger the inordinate activation of this promoter. DNA methylation microarray analysis identified *CLLU1* to present lower methylation levels in IgV_H-UM CLL cases compared to IgV_H-M CLL cases and to normal B-cells. It was shown that the larger proportion of methylated sites occupied *CLLU1* gene body, while a strong correlation between *CLLU1* body hypomethylation and gene expression was also established. Transposable elements (TEs) carry a markedly high density of CG dinucleotides and remain heavily methylated in normal cells. Aberrations in DNA methylation marks could allow for TEs to exert a significant impact on host gene expression. For example, long terminal repeats (LTRs) of endogenous retroviruses (ERVs) contain transcription regulatory elements that, in the sense orientation, are functional and could initiate transcription of adjacent human genes.
Aims: Document that non-redundant *CLLU1* gene modules were evolutionary provided by TEs. Provide a rationale for the intriguing transcriptional profile of the gene.
Methods: *CLLU1* genomic sequence was downloaded from the NCBI Gene database and scanned for the presence of integrated TEs by RepeatMasker. EMBOSS Cpgplot was used to identify genomic regions of unusual CG composition located within *CLLU1* body. PROMO Version 3.0.2 software, employing version 8.3 of TRANSFAC database, was used to evaluate transcription factor (TF) binding affinity of the region spanning 150 nucleotides (nts) upstream and downstream of *CLLU1* transcriptional start site (TSS), assumed to include important transcription regulatory elements. *CLLU1* locus syntenic alignments of numerous mammals were downloaded from the UCSC Genome Browser Database. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6.
Results: *CLLU1* exon 1 is located within a region reported from RepeatMasker to match a sense orientation MLT2B1 LTR, of the ERV-L family. The LTR extends 109 nts upstream of *CLLU1* TSS. *CLLU1* start codon was evolutionarily provided by a MIR element. *CLLU1* polyadenylation signal was also provided by a MIR element. *CLLU1* body contains CG-rich genomic content. EMBOSS Cpgplot analysis identified a CpG island in the gene body, which, however, is an AluY element of unusually high CG composition than a bona fide CpG island. Of note is the high Observed/Expected CG ratio within and adjacent to *CLLU1* coding DNA sequence. The region spanning 150 nts upstream and downstream of *CLLU1* TSS includes a plethora of high-affinity TF binding sites. Analysis identified multiple binding sites for TFs known to play a major role in CLL pathogenesis, namely PAX5, the NF-κB subunit RELA, IRF1 and ELK1. Binding motifs for known oncoproteins (JUN, MYB) as well as for XBP1, a potent TF factor in B cells, were also detected. Of note, XBP1 is known to immensely enhance the transcription of HTLV-I exogenous retrovirus. Phylogenetic analysis revealed that a 4 bp genomic deletion allowed the generation of an ELK1 binding site exclusively in the Hominid genome. Because the DNA sequence adjacent to the site presents null variation between Hominids and also because this genomic region includes no common single nucleotide polymorphisms in human, we assume the occurrence of a selective sweep fostering the creation of the motif.
Summary/Conclusions: Non-redundant *CLLU1* gene modules were provided by TEs thereby remain heavily methylated in normal cells and become susceptible to increased hypomethylation during leukemogenesis. This finding provides a mechanistic link between high *CLLU1* expression and poor clinical outcome prognostication since *CLLU1* body hypomethylation occurs predominantly in IgV_H-UM CLL cases. - In the Eμ-TCL1 transgenic mouse model, whilst *TCL1* mice are expected to develop an IgV_H-UM CLL-like disease phenotype at around 11 month, hypomethylation of TEs occurs as early as in 7-months-old mice. In addition, DNA methylation levels remain relatively stable over time among IgV_H-UM CLL cases, not affected by treatment. The above, combined with our findings, provide a rationale for *CLLU1* expression representing an inherent and constant feature of a particular CLL clone.

E1043

AMONG CLL PATIENTS WITH MULTIPLE FISH ABNORMALITIES, 13Q AND 11Q DELETIONS ENTAIL THE COMMONEST COMBINATION IN CONTRAST TO 11Q AND 17P DELETIONS, BEING THE COMBINATION WITH WORSE OUTCOME

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Background: Fluorescence *in-situ* hybridization (FISH) defines a hierarchy of genetic changes that predicts survival in CLL. However, multiple abnormalities (MA) may also occur and little is known about its distribution and clinical impact.
Aims: To assess the frequency and clinical outcome of 245 patients with CLL and MA detected by FISH in a database containing clinical and biological data.
Methods: Cases with more than one FISH abnormality were retrieved from the database. FISH abnormalities included in the routine CLL FISH panel (13q-, 11q-, 17p- and +12) were taken into account.
Results: MA were detected in 245 out of 1743 CLL cases (14%). FISH studies were performed at diagnosis (FISH-d) in 154 cases (61%), and during disease evolution (FISH-e) in 91 cases (38%). Among the 91 cases from the group of FISH-e, 26 cases (11%) had a prior FISH without MA, and 39 cases (16%) had received treatment for CLL before FISH. The most frequent associations in the group of FISH-d were the combinations of 13q- with 11q- observed in 52 cases (33.8%), followed by 13q- with 17p- (37 cases, 24%), and 13q- with +12 (28 cases, 18.8%). By contrast, in the group of FISH-e 13q- and 17p- were the most prevalent partners (28 cases, 31%) followed by 13q- and 11q- (25 cases, 27%) and +12 and 17p- (12 cases, 13%). More than 2 abnormalities were observed in a low proportion of cases: 17 (11%) in the group of FISH-d, and 7 (7.6%) in the group of FISH-e. Interestingly, most of them included 17p- (14/17 FISH-d; 6/7 FISH-e). The weirdest combination was 11q- and 17p-, only observed in 5 cases (3.2%) in the group FISH-d and 2 cases (2.1%) in the group FISH-e. Globally, 17p- was found in more cases during disease evolution (48 cases, 52%) than at the moment of diagnosis (66 cases, 42%). Moreover 17p- appeared as the major clone in a very low proportion of cases in both groups, whereas 13q- or +12 where observed more frequently as the major clone, suggesting 13q- or +12 as early events, in contrast with 17p-. Overall survival (OS) and time to first therapy (TTFT) were analyzed in the group of FISH-d, being respectively 68 months, and 25 months, for the whole series. The combination of 13q- and 11q- showed the larger OS (75 months), while CLL with 13q- and 17p- showed the shorter OS (37 months). Moreover the presence of 17p- was associated with a shorter OS (63 m *vs* 75 m, p=0.02). Regarding TTFT, CLL with 13q- and +12 showed a median TTFT of 53 months while the CLLs with 17p- and 11q- only had 4 months of TTFT (p=0.017) (Figure 1).

Figure 1. Overall Survival and time to first therapy in patients with multiple FISH aberrations at the time of diagnosis.

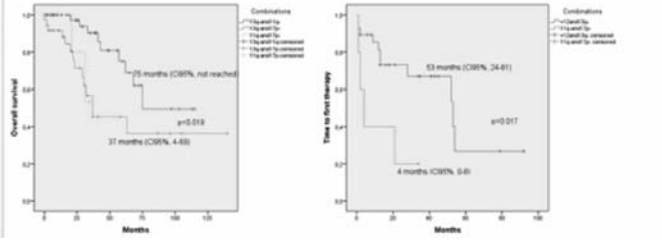

Figure 1.

Summary/Conclusions: MA were observed in 14% of CLL cases, representing a heterogeneous group. The distribution among combinations was unequal. The combinations of 13q- with 11q- and 17p- were the most frequent associations, in contrast to 11q- and 17p-, observed in a minority of the cases. 17p- often occurred during disease evolution involving minority clones. MA entailed poor prognosis when emerged at diagnosis, probably due to the high incidence of bad prognosis aberrations such as 17p- and 11q-. On behalf of Grupo Español de Leucemia Linfática Crónica (GELLC) and Grupo Cooperativo Español de Citogenética Hematológica (GCECGH).

E1044

FUNCTIONAL ROLE OF PI3K-DELTA IN MALIGNANT B CELLS

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Background: Among catalytic PI3K subunit isoforms, p110-δ is highly and selectively expressed in malignant compared to other cell types and involved in B cell receptor (BCR) signaling. Idelalisib, which specifically targets p110-δ, shows clinical efficacy e.g. for the treatment of chronic lymphocytic leukemia (CLL), but only modest direct cytotoxicity against malignant B cells *in vitro*.
Aims: To elucidate the mechanism of action of idelalisib and to dissect the contributions of p110-δ to both cell autonomous functions as well as interactions with the tumor micro-environment, engineered inhibitor-resistant p110-δ mutants were employed as chemical genetic tools. In addition cell line models expressing such mutants might anticipate a potential type of clinically acquired resistance.
Methods: According to structural alignment with known inhibitor-resistant p110-α mutants, p110-δ-I777M and –I825V were generated by site-directed mutagenesis as potentially inhibitor-resistant mutants. Inhibitor resistance was assessed by comparing phospho-Akt levels after inhibitor treatment in retrovirally transduced cell lines expressing wild type or mutant p110-δ. Transductants of the murine fibroblast cell line NIH3T3 were assessed for anchorage-independent growth and saturation density as markers of oncogenic transformation. The emphasis of our chemical genetic evaluation was on malignant B cells and employed the human Burkitt lymphoma and murine pre-B cell lines Ramos and BaF/3. Chemokine levels in culture supernatants were determined by enzyme-linked immunosorbent assay, while chemotaxis to CXCL12 was examined in trans-well migration assays.
Results: The murine fibroblast cell line NIH 3T3, which lacks endogenous p110-δ expression and sensitivity to idelalisib, gained sensitivity to idelalisib concentrations above 1 µM upon over-expression of p110-δ with regard to phosphorylation of Akt at serine 473. According to pAkt levels, the I777M mutation in p110-δ partly reversed the sensitivity to idelalisib of NIH 3T3 cells expressing p110-δ. Moreover expression of p110-δ-I777M increased the anchorage-independent growth and the saturation density of NIH3T3 cells. In Ramos and Baf-3 cells, which endogenously express p110-δ, additional expression of p110-δ-I777M led to markedly increased Akt phosphorylation and to resistance against 10-100 nM idelalisib. Expression of p110-δ-I777M rescued the secretion of the chemokine CCL-3 by Ramos cells upon stimulation of the B-cell receptor from inhibition by idelalisib. Chemotaxis of Baf-3 cells to the chemokine CXCL-12 was reconstituted by expression of p110-δ-I777M in the presence of inhibitory concentrations of idelalisib.
Summary/Conclusions: The novel engineered p110-δ-I777M mutant showed an unexpected gain of function and mediated resistance against idelalisib that can be exploited for the chemical genetic evaluation of p110-δ function. In malignant B cell lines, p110-δ was less involved in cell autonomous functions than in their interactions with the micro-environment, exemplified by chemokine secretion and migration.

E1045

ACTIVATION INDUCED DEAMINASE SPLICE VARIANTS EXPRESSED IN B CELL LEUKEMIA AND LYMPHOMA DO NOT RETAIN THEIR CATALYTIC ACTIVITY

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Background: Activation induced deaminase (AID) is essential for enhancing antibody affinity and effector functions in B cells during secondary immune response. It induces somatic hypermutation (SHM) and class switch recombination (CSR) in the immunoglobulin (Ig) genes. AID is a potent mutator -SHM frequency in the Ig genes is a million times higher than the frequency of spontaneous mutagenesis (10^{-3} -10^{-4} mutations/ cell/ generation). AID studies performed in mouse models have shown a correlation between AID activity and genomic instability. AID is aberrantly expressed in various B lymphoid malignancies and also in some solid tumors.
Aims: In this project, we set out to investigate the function of four alternatively spliced AID variants that differ from the full-length protein in the C terminal domain. These variants are present in an increased proportion in chronic lymphocytic leukemia (CLL), Ph-positive acute lymphoblastic leukemia (Ph-positive ALL) and mantle cell lymphoma (MCL).
Methods: Individual AID splice variants were cloned from peripheral blood CLL cells into an HA-tagged retroviral vector. Mature B cells purified from AID knockout spleens were stimulated with lipopolysaccharide and interleukin-4 and subsequently infected with retroviruses carrying individual AID splice variants. Cells were cultured ex vivo for a total of 4 or 5 days and then assessed for CSR to IgG1 by flow-cytometry or sorted based on high GFP expression marking the

infected cells. The level of SHM in the Ig switch region Sm was assessed by ultra-deep next generation sequencing. The amount of double strand breaks in the Ig Sm was quantified by ligation-mediated PCR and hybridization with an Sm probe.
Results: While full-length AID induced CSR, SHM and double strand breaks in the Ig genes, alternatively spliced AID variants behaved as catalytically inactive in all performed functional assays. Immunoblotting with an anti-HA antibody revealed that AID splice variants had lower protein expression compared to full-length AID. It is a well-established fact in the AID field that in most situations, AID levels correlate with its activity. Therefore, lower expression of alternatively spliced AID variants might have contributed to their loss-of function in our model system.
Summary/Conclusions: Alternatively spliced AID transcripts were previously shown to be expressed in CLL, Ph-positive ALL and MCL. We hypothesized that alternative splicing might modulate their activity and consequently change AID deamination levels in the Ig genes or its targeting in the whole genome. Results of AID splice variant analysis done in AID knockout mouse B cells stimulated ex vivo indicated their loss of function. Therefore, the role of alternatively spliced AID expressed in B-lymphoid leukemia and lymphoma remains unclear, and their function is to be further analyzed using human cell lines derived from B-cell malignancies.
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E1046

CHROMOSOMAL GAINS IN CHRONIC LYMPHOCYTIC LEUKEMIA: A RARE EVENT ASSOCIATED WITH A DISMAL PROGNOSIS

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Background: Hyperdiploidy is a common cytogenetic alteration in some hematological diseases such as B acute lymphoblastic leukemia or multiple myeloma, with prognostic value. However, the presence of chromosomal gains other tan trisomy 12 (+12) in chronic lymphocytic leukemia (CLL) has scarcely been reported before.
Aims: The aim of this study was to analyze, by FISH, the frequency and prognostic implications of chromosomal gains in CLL.
Methods: A retrospective review of 1,359 consecutive cases diagnosed with CLL referred for FISH analysis to a unique institution, between 2004 and 2012, was carried out. Chromosomal gains were considered when a gain of at least 3 out of the 5 FISH probes used (11q22/*ATM,* 12q13, 13q34, 14q34/*IGH*, and 17p13/*TP53)* was observed. In addition, Next Generation Sequencing analysis was carried out to investigate the mutational status of *TP53* (exons 4–11) gene.

Table 1.

Table I. Detailed fluorescence *in situ* hybridization (FISH) results for each case.

	Number of chromosomal gains (% of cells with the alteration)				
Probes	11q22/*ATM*	12q13	13q34	14q34/*IGH*	17p13/*TP53*
Case 1 (PB)	3n (23%); 4n (60%)	3n (23%); 4n (60%)	3n (23%); 4n (60%)	3n (23%); 4n (60%)	3n (23%); 4n (60%)
Case 2 (BM)	3n (6%)	3n (7%)	3n (5%)	3n (7%)	n (75%)
Case 3 (BM)	3-4n (25%)	3-4n (42%)	3-4n (37%)	3-4n (35%)	n (74%)
Case 4 (PB)	3n (14%)	3n (16%)	3n (13%)	3n (15%)	3n (12.5%)
Case 5 (PB)	n (36%)	3n (22%); 4n (14%)	3n (22%); 4n (14%)	Rearrangement (53%)	3n (22%); 4n (14%)
Case 6 (BM)	3n (12%)	3n (22%)	3n (23%); n (13%)	n (19%)	3n (13.5%)
Case 7 (PB)	3n (8%)	3n (12%)	3n (10%)	3n (10%)	3n (10%)

Abbreviations: PB= peripheral blood; BM= bone marrow; n= number of chromosomes

Results: 8 cases with chromosomal gains were identified out of the 1,359 CLL patients (0.59%), referred for FISH studies. One case was excluded from the analysis due to inadequate follow-up. Five cases (cases nº 3-7) presented chromosomal gains at diagnosis; the other two (case nº 1 and 2) during disease evolution and after having received treatment with chlorambucil. Four patients (57%) were female, and the median age at diagnosis was 74 years (range 63-86). The median white blood cell count at the time of diagnosis was 16 x 10^9/L (range, 7-67). No significant anemia or thrombocytopenia were detected in any case at diagnosis. It is noteworthy that the immunophenotype of three out of the seven cases suggested the diagnosis of atypical CLL. Serum LDH and $\beta_2$microglobulin levels at diagnosis were high in 57% (4/7) and 29% of the cases (2/7), respectively. Three out of seven (43%) patients presented with splenomegaly and hepatomegaly, and 29% with more than three enlarged lymph node regions. The presence of B symptoms was detected in 29% of the cases. Of note, at the time of diagnosis most cases were classified as early stages according to Binet classification (stage A 57%; stage B 29%; stage C 14%). *IGHV* mutation status was available in 57% of the cases being all of them classified as unmutated pattern. Besides chromosomal gains, additional chromosomal abnormalities were detected in 57% of the patients including: 17p- (2 cases), 11q- and *IGH* alteration (1 case), and 13q- and *IGH* deletion (1case). Detailed FISH results are shown in Table I. Only one patient (case nº 7) showed a missense *TP53* mutation (c.613T>G; p.Y205D) with a mutational burden of 95%. This variation was previously described as a mutation in the COSMIC database (COSM43844). This *TP53* mutated case did not show 17p deletion. Six patients (86%) required treatment during follow-up with a median TTFT of 1.4 months (CI95%, 0.8-1.9). Most treated patients (4/6, 66%) required two or more chemotherapy therapies (median 3, range: 1-4), due to refractoriness or relapse. Median duration of response to first line treatment was 7 months (0-36). With a median follow-up of 66 months (6-115), 5 patients have died due to: disease progression (3 cases), infection (1 case), and cardiovascular disease (1 case). Median OS from the time of diagnosis was 66 months (CI95%, 0-174), and from the time of chromosomal gains acquisition was 20 months (CI95%, 16-24).
Summary/Conclusions: Chromosomal gains are a rare event in CLL. Our results also suggest that the presence of chromosomal gains involves poor prognosis in these patients.
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E1047

TWO-DIMENSIONAL GEL ELECTROPHORESIS(2-DE) ANALYSIS REVEALS DIFFERENTIALLY EXPRESSED PROTEIN PATTERNS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AFTER THYMOSIN B4 (TB4) AND LENALIDOMIDE (LEN) TREATMENT

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Background: The proteomic approach is essential in the multi-disciplinary field of hematological research. Previously, using MALDI-TOF analysis we identified Tβ4, a G-actin sequestering protein involved in the regeneration of injured tissues and cell migration, as a downregulated protein in CLL patients, also confirmed by an independent GEP analysis comparing B-CLL cells and normal controls.
Aims: Here, using 2DE proteomic analysis we evaluated differential protein expression patterns after treatment with exogenous Tβ4 and/or Len, which has been shown to improve immune dysfunction in CLL by repairing actin polymerization and rapid signaling at the immunological synapse. We investigated whether purified B-CLL cells having high/low Tβ4 expression respond differently to migratory stimuli such as CXCL12/SDF1α.
Methods: Purified B-CLL lymphocytes were isolated from untreated Binet stage A CLL patients prospectively enrolled atdiagnosis (O-CLL1 clinicaltrial.gov identifier NCT00917540) and healthy controls. N=93 patients were evaluated for Tβ4 expression by Flow cytometry (FC, median^{RFI}=15.06, IQR:6.9–28.8).For migration assays, SDF1α (100nM) as chemoattractantwas used to determine the CLL migration index. B-CLL cells from two patient samples havinglow or highTβ4expression were pre-treated with Len (5mM) and then with Tβ4 (100nM)for 2DE analysis.
Results: CLL samples with the lowest Tβ4 expression (n=12) also had higher F-actin levels as evaluated by FC than normal controls (n=7) in a sample cohort (n=93). FC assessed Tβ4 expression in B-lymphocytes in CLL patients (n=93) *vs* controls (n=7). Tβ4^{RFI}values showed Tβ4 expression was heterogeneous (Tβ4^{RFI}=1.3–152.05;median^{RFI}=15.06) compared to controls (Tβ4^{RFI}=3.4–70.9;median^{RFI}=38.3). Baseline F-actin expression is also influenced by different levels of Tβ4 expression: CLL CD19$^+$CD5$^+$ samples with Tβ4^{high}also had higher G-actin expression, with a lower F/G-ratio, and conversely Tβ4^{low} samples, there wasa higher expression of F-actin, with a higher F/G-ratio (n=14), also confirmed by FC experiments. CD19+CD5+ CLL cells express high levels of functional CXCR4/CD184,which facilitates CLL cell migration beneath stromal cells. B-CLL strongly responded to the migratory stimulus SDF-1α. Furthermore, migration induced by SDF1α increased in CLL cells after Len (0.5 μM) treatment. Conversely, the CLL with Tβ4^{low} was more responsive to Len inhibition, resulting in a reduction in migratory potential towards SDF1α. Purified B-CLL lysates (n=2) were analyzed by 2DE analysis. Protein profile

analysis showed a number of protein spots ranging from 18 to 60kDa that were differentially expressed with respect to cells exhibiting Tβ4$^{low/high}$ expression. More importantly, treatment with Len or following Tβ4 replenishment also changed protein expression in function of the endogenous Tβ4
Summary/Conclusions: Our preliminary qualitative analysis suggests that the proteomic profile of CLL samples exhibiting higher or low Tβ4 levels also correspond to groups of proteins with a differential protein expression. Moreover, in the presence of Tβ4 or Len, or Tβ4/Len combination these proteins may respond with a strong inhibition or up-regulation of the expression of other proteins. Here,we show that 2DE evaluation of CLL profiles may be useful to identify changes in expression profiles of CLL proteins following treatment with different agents. These differences may translate into functional differences such as cell migration and cytoskeletal dynamics in the malignant clone.Special thanks to Celegene Corp and AIRC-CARICAL Regional Grant 16695.

E1048

A COMPARATIVE STUDY ON THE EFFECTS OF NUTLIN-3A IN B-CLL CHRONIC LYMPHOCYTIC LEUKEMIA CELLS WITH AND WITHOUT 17P DELETION

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Background: The p53-MDM2 interaction is emerging as a promising therapeutic target in chronic lymphocytic leukemia (CLL). It is well known that MDM2 oncoprotein inhibits the function of wild-type p53 by its degradation and that MDM2 inhibitors are mainly effective in CLL cases with wild type p53. However, there is no substantial data on the effect of MDM2 inhibitors on CLL cells with p53 mutation or 17p deletion (17p-).
Aims: The aim of this work is to have a deeper insight into the effect MDM2 inhibitor Nutlin-3a on CLL cells obtained from patients with or without 17p deletion under standard suspension culture conditions and in a microenvironment model using co-cultures of CLL cells with primary bone marrow stromal cells (BMSC). This includes evaluating the effect of Nutli-3a on cell viability, induction of apoptosis and the regulation of key p53 downstream targets.
Methods: Peripheral blood mononuclear cells (PBMC) were obtained from 25 B-CLL patients with (n=7) and without (n=18) 17p deletion and exposed to Nutlin-3a under suspension or co-culture conditions for 2-3 days. Dose and time kinetics were also performed and cell viability was evaluated by MTT assay. Induction of apoptosis was assessed by AnnexinV/propidium iodide (PI) staining and FACS analysis. In parallel, a selective effect of Nutlin-3a on CLL cells, T-cells and monocytes was investigated using specific markers for B cells (CD19), T-cells (CD3) and monocytes (CD14) in combination with PI. In addition, the effect of Nutlin-3a on the key p53 downstream targets involved in the regulation of apoptosis (MDM2, Bax, PUMA, Noxa and Mcl-1), cell cycle regulation (p21) or DNA repair (PARP) was assessed by Western blotting and RT-PCR.
Results: In vitro treatment with Nutlin-3a induced cell killing in CLL cells from patients with and without 17p deletion as demonstrated by MTT assays. The effect of the inhibitor was relatively higher on the samples from patients with wild-type 17p at low concentrations (0,5μM to 1μM) compared to patients with 17p deletion. However, at higher concentrations (1-10μM), Nutlin-3a showed a remarkable effect on both groups. A similar pro-apoptotic response to Nutlin-3a was detected by FACS analysis experiments where Nutlin-3a induced 2,5 fold increase in apoptosis rate in patients without 17p deletion compared to 1,6 fold increase in apoptosis in patients with 17p deletion. The pro-apoptotic effect of Nutlin-3a was comparable under suspension and co-culture conditions and was higher in CLL (CD19+) cells compared to T-cells and monocytes. Consistent with the increase in apoptosis rates, Nutlin-3a treatment induced the expression of p53 downstream target p21 mRNA and this was associated with PARP cleavage in both patient groups.
Summary/Conclusions: These data confirm the beneficial effect of Nutlin-3a in CLL and further suggest that MDM2 inhibitors might be effective in 17p wild-type and 17p deleted patients depending on the concentration of Nutlin-3a. Therefore, the clinical relevance of these data deserves further investigations.

E1049

DNA-DEPENDENT TRANSCRIPTIONAL REGULATORY GENES WERE HYPOMETHYLATED IN KOREAN CHRONIC LYMPHOCYTIC LEUKEMIA-A METHYLCPG BINDING DOMAIN NGS STUDY

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Background: Chronic Lymphocytic Leukemia (CLL) is the most common leukemia in Western countries, but it is very rare and shows relatively poor prognosis in Asian countries including Korea.
Aims: To investigate the differences in the pathobiology of Korean CLL, we analyzed the methylation profile and the expected affected pathways using the methylCpG binding domain NGS (MBD-seq).
Methods: Bone marrow aspirate of 9 CLLs and 5 control individuals were used for MBD-seq using MethylMiner Methylated DNA Enrichment Kit and Illumina Hi-Seq 2000. Differentially methylated regions (DMRs) were determined by filtering for each regions associated with |log2FC|≥1 and exact test p-values<0.05. Hierarchical clustering analysis, gene-enrichment and functional annotation analysis was performed.
Results: Pair-wise correlation coefficient matrix showed a high similarity was observed among either CLL group or control group, but not between the CLL group and the control group. Among 79,612 tested regions regarding to 18,434 genes with CpG islands, CLL group showed 2,078 DMRs (780 genes)-1,083 hypermethylated regions (350 genes) *vs* 995 hypomethylated regions (430 genes). In CpG islands analysis, the DMR profiling clearly separated CLL group from control group. The most frequently hypermethylated genes included *ANKRD20A2, CHERP, PRPF38A, C1orf123, FKTN, TRAFD1, SH3D21, MGAT1, NECAB3* and *UCKL1*. Gene ontology analysis showed genes related with protein localization, cellular macromolecule localization and protein transport were hypermethylated. The most frequently hypomethylated genes included *CBX4, OSR2, LMX1B TNRC18, ICAM5, GATA6, CDX2, NR3C2, STK3* and *HOXB8.* Gene ontology analysis showed genes related DNA dependent transcriptional regulation and RNA metabolic process regulation were hypomethylated. Pathway analysis showed genes related pathways in cancer or hedgehog signaling pathway were hypomethylated.
Summary/Conclusions: We performed the whole genome methylation profile analysis using NGS MBD-seq in Korean CLL for the first time. Our result demonstrated that Korean CLL has a distinct methylation profile. Genes related with protein localization, cellular macromolecule localization and protein transport were hypermethylated, and genes related DNA dependent transcriptional regulation and RNA metabolic process regulation were hypomethylated. Cancer pathway and hedgehog signaling pathway could be overexpressed due to hypomethylation in relevant genes.

E1050

INCREASED EXPRESSION OF PIM2 GENE IS ASSOCIATED WITH POOR PROGNOSIS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: *PIM2* gene belongs to *PIM* family, which encodes serine/threonine kinases involved in cell survival and apoptosis. *PIM2* gene is implicated in many neoplasms including solid tumors and acute myeloid leukemia. The relation between expression of *PIM2* gene and course of chronic lymphocytic leukemia`s (CLL) as well as outcome has not been fully defined.
Aims: The aim of the study was to evaluate the role of *PIM2* gene as a marker for CLL malignancy and the importance of *PIM2* gene expression as a prognostic factor in CLL.
Methods: Sixty-seven patients, 35 females and 32 males, aged 49-90 years (median age was 66 years) with de novo CLL were enrolled in the study. Thirty-two patients reached complete remission (CR). Bone marrow samples were collected in all patients at the time of diagnosis, whereas only samples with monoclonal lymphoid cells for more than 80% of total cellularity were assessed. In 16 patients bone marrow in CR phase was examined. The control group consisted of 14 healthy individuals. The expression of *PIM2* gene was analyzed by Taq Man RQ-PCR assay and PIM2 protein was detected by Western blot. All statistical analyses were performed using Statistica software.
Results: Median *PIM2* gene expression in CLL patients was significantly higher than in control group. CLL patients with high expression levels of *PIM2* gene (above the median) had significantly shorter progression free survival (PFS) than patients with low *PIM2* expression (p=0.001). In addition, it was found that patients who achieved CR, had significantly lower expression of *PIM2* gene than patients with no CR (p=0.002). Importantly, elevated *PIM2* gene expression assessed initially, was restored to normal level in all patients in CR. Moreover, the overexpression of the PIM2 kinase was detected in CLL patients in contrast to controls. *PIM2* expression in correlation to CLL Rai stages was evaluated also and it was noted that *PIM2* expression in stages IV and III was higher than in both stages I and II (p=0.002). Moreover, CLL patients with rapid lymphocyte doubling time (LDT) (<12 months) shown higher PIM2 expression than patients with lower LDT (≥12 months), p=0.003. Positive correlation between *PIM2* gene expression and the percentage of malignant lymphocytes with ZAP70 expression was found (r^2=0.3214 p=0.01).
Summary/Conclusions: We showed that increased expression of *PIM2* gene in CLL cells is associated with poor prognosis and resistance to chemotherapy in CLL patients.

LB2251

RECOVERY OF TUMOUR SUPPRESSOR FUNCTION OF NOTCH3 BY AN ASPERGILLUM DERIVED MYCOTOXIN IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

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Background: Deregulation of NOTCH2 signalling is critically involved in CLL leukemogenesis. We have recently shown that CLL cells are less sensitive to γ-secretase inhibitors (GSI) indicative for the expression of constitutively active NOTCH2 forms which do not depend on γ-secretase for processing and function. In contrast, targeting CLL cells with the Aspergillum derived NOTCH2 transactivation inhibitor gliotoxin efficiently induced apoptosis in the leukemic cells by a mechanism which appeared to involve the induction of NOTCH3.
Aims: To gain deeper insight into the function of NOTCH3 in CLL cells, we analysed NOTCH3 expression in relation to spontaneous and gliotoxin induced apoptosis and tested the effect of *NOTCH3* inhibition on CLL cell viability.
Methods: The *NOTCH3* gene was inhibited by GSI (RO4929097) or by siRNA mediated gene silencing. Apoptosis was calculated as the sum of PI-/AnxV+ (early apoptosis) and PI+/AnxV+ (late apoptosis) cells. The expression of NOTCH and apoptosis related genes was monitored by RT-PCR and FACS analysis.
Results: Results showed that inhibition of NOTCH2 signaling by gliotoxin led to the concomitant induction of NOTCH3 signalling as indicated by the induced co-expression of *NOTCH3* and its ligands *JAG2* (in unstimulated CLL cells) or *DLL1* (in PMA activated CLL cells) at the mRNA level. Gliotoxin induced a tumour suppressive NOTCH3 signalling signature as demonstrated by the induction of the apoptosis regulating NOTCH3 target genes *HEY1*, *NR4A1*, and *CDKN1A* (p21) in the pre-apoptotic phase. In contrast, the anti-apoptotic genes *NOTCH2*, *FCER2* (CD23), and *AKT1* were inhibited by gliotoxin. We then tested the effect of gliotoxin and GSI on induction of apoptosis. Gliotoxin induced apoptosis in CLL cells (mean %±SD: 85±10% *versus* 13±5%), while GSI (RO4929097) mediated NOTCH3 inhibition was associated with a decrease in spontaneous (7±3% *versus* 13±5%) and gliotoxin induced apoptosis (51±7% *versus* 85±10%) in the leukemic cells. To directly confirm that NOTCH3 has a pro-apoptotic role in CLL, we silenced NOTCH3 by RNA interference. After 3 days in culture, *NOTCH3* siRNA decreased the apoptotic effect of gliotoxin leading to a 3.5-fold increase in the percentage of living CLL cells. Inhibition of NOTCH3 by siRNA was also associated with an increased surface expression of CD23, a downstream target of NOTCH2. These data show that the inhibition of NOTCH3 may lead to the upregulation of NOTCH2/CD23 axis and resistance to apoptosis. This reciprocal regulation of NOTCH2 and NOTCH3 together with their opposite effects on cell viability strongly suggests that these two NOTCH receptors have different roles in the regulation of the viability of CLL cells. Interestingly, NOTCH3 mRNA could be detected in patients with early stage of the disease (Rai 0-II) but not in Rai III-IV. Thus, in CLL cells, constitutive active NOTCH2 signalling might suppress pro-apoptotic NOTCH3 signalling enabling the expansion of the malignant clone particularly in advanced stages of the disease.
Summary/Conclusions: In summary, these novel data indicate that selective targeting oncogenic NOTCH2 might recover a pro-apoptotic NOTCH3 activity which should be considered in the design of therapies aimed to target the NOTCH pathway in CLL cells. Moreover, this data points for the first time to a tumour suppressive function for NOTCH3 in CLL.

Chronic lymphocytic leukemia and related disorders - Clinical

E1051

SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA: A POPULATION-BASED STUDY INCLUDING 13,034 PATIENTS DIAGNOSED IN 1982-2013 IN SWEDEN

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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries with a median age between 65-74 years at diagnosis. In clinical trials on CLL treatment, elderly patients with comorbidities have been heavily underrepresented and therefore population-based studies on survival are important to estimate effects of newer treatment on survival. Few observational studies on survival in CLL have been conducted and they have not been consistent in showing improved survival.
Aims: To evaluate the effect of the introduction of new drugs on survival in CLL patients, we conducted a population-based study in CLL patients diagnosed in Sweden from 1982 until 2013, with follow-up through 2014.
Methods: Information on CLL patients diagnosed between January 1, 1982 and December 31, 2013 was retrieved from the well-established Swedish Cancer Registry. The registry has been validated with regards to CLL and has a diagnostic accuracy of more than 90%. Relative survival ratios (RSR) and excess hazard ratios (EHR) were used for estimating the impact on patient survival.
Results: Between January 1, 1982 and December 31, 2013, a total of 13,034 CLL patients were reported to the Swedish Cancer Registry. Age was a significant predictor of survival reflected in reflected in EHRs of 1.48 (95% confidence interval (CI) 1.21-1.81), 2.69 (2.22-3.27) and 5.37 (4.33-6.66) for age groups 51-65, 66-80, and >81 years, respectively, compared to the reference interval of<50 years. Similar results were obtained when comparing 1-, 5- and 10-year survival between age groups. Males had a significantly increased excess risk of death compared to females (EHR=2.12, 95%, CI 1.93-2.34) and worse 1-, 5-, 10- and 15-year RSR. A significantly increased excess risk of death was observed in CLL patients diagnosed in the calendar period 1993-2002 compared to 2003-2013 (EHR=1.36; 95% CI 1.22-1.51). Furthermore, a significantly improved 5- and 10-year RSR was observed comparing CLL patients diagnosed 2003-2013 (5-year RSR 0.81, 95% CI 0.79-0.82 and 10-year RSR 0.61, 0.58-0.65) to 1993-2002 (0.75, 0.73-0.77 and 0.54, 0.51-0.56, respectively. When stratified by age groups, the excess risk of death was significantly higher in 1993-2002 compared to 2003-2013 in age groups<50, 51-65 and 66-80 years old, with EHR 2.87 (95% CI 1.52-5.42), 1.56 (1.26-1.93) and 1.25 (1.08-2.13), respectively. However, it was non-significant in patients 81 years and older (EHR=1.23, 95% CI 0.96-1.58). In age groups<50 and 51-65 years the 5-year survival was significantly better when calendar period 1993-2002 was compared to 2003-2013 (Figure). The 5-year RSR in age group<50 years old was 0.84 (95% CI 0.79-0.90) in 1993-2002 *vs* 0.99 (95% CI 0.97-1.01) in 2003-2013 and in the age group 51-65 years the RSR was 0.84 (0.81-0.86) in 1993-2003 *vs* 0.91 (0.89-0.93) in 2003-2013. The difference in 5-year RSR was non-significant in older age groups.

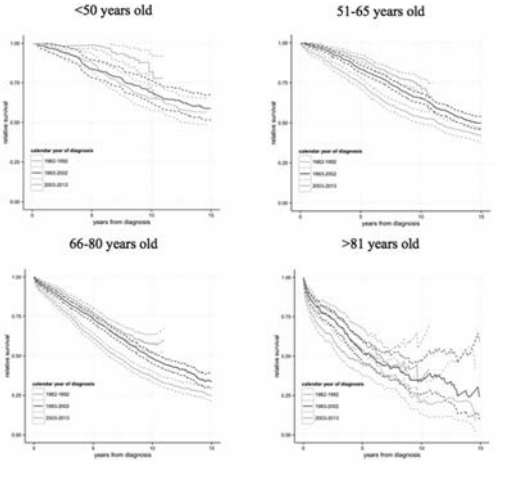

Figure 1.

Summary/Conclusions: We have in this large population-based study, including more than 13,000 patients with CLL, shown improvement in survival of CLL patients in the last decade and the difference is most apparent in younger

patients. Younger patients are in general healthier and less likely to have comorbidities. Based on our results we conclude that risk stratification is likely to benefit older patients for better selection of those patients that can receive intensive treatment. Furthermore, a more significant improvement in overall survival in elderly patients might be obtained with the introduction of a more targeted therapy with less side effects.

E1052

IDELALISIB IN COMBINATION WITH BENDAMUSTINE/RITUXIMAB IMPROVES OVERALL SURVIVAL IN PATIENTS WITH RELAPSED/REFRACTORY CLL: INTERIM RESULTS OF A PHASE 3 RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED STUDY

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Background: Patients (pts) with relapsed/refractory CLL and adverse prognostic features (e.g., del17p/*TP53*mut) respond poorly to standard treatment. Here we present results of a pre-specified subgroup analysis of OS and ORR from an ongoing randomized double-blind placebo-controlled study (ClinicalTrials.gov ID: NCT01569295).

Aims: To demonstrate an improvement in OS and ORR in pts on idelalisib (IDELA) in combination with bendamustine/rituximab *vs* bendamustine/rituximab alone in pts with relapsed/refractory CLL and in a pre-specified pt population with adverse risk features.

Methods: Between June 2012 and August 2014, 416 pts with relapsed/refractory CLL from 180 centers in North America, Australia, New Zealand, and Europe were enrolled in this ongoing study. After informed consent, eligible pts were randomized 1:1 to idelalisib 150 mg BID (207 pts) or matching placebo (209 pts). All pts received bendamustine 70 mg/m^2 on days 1 & 2 and rituximab 375 mg/m^2 day 1 of cycle 1 and 500 mg/m^2 D1 cycles 2 to 6. A cycle of the regimen was Q28 days. Study treatment continued until disease progression, death, intolerable toxicity, or withdrawn consent. Stratification performed by an independent laboratory was by del17p, *TP53* mutations, or mutated *IGHV*, and clinically by refractory (progression<6 months from completion of prior therapy) or relapsed (progression at least 6 months from completion of prior therapy) disease. Median follow-up at the time of this report was 12 months. The primary endpoint of PFS defined by standard criteria was adjudicated by an IRC. Overall survival and ORR were secondary endpoints. Crossover was not permitted at the time of CLL disease progression.

Results: Median overall survival was not reached in either arm; there was a statistically significant improvement in overall survival, after the pre-specified multiplicity adjustment, in the idelalisib arm (HR=0.55; 95% CI: 0.36, 0.86; P=0.008 [stratified]). As of 15 June 2015, there were 34 deaths in the idelalisib arm and 51 deaths in the placebo arm. An improvement in ORR was observed on the idelalisib *vs* placebo arm in all adverse-risk categories evaluated (Table).

Table 1.

Prognostic Feature	IDELA + BR	Placebo + BR	IDELA + BR	Placebo + BR
All pts				
n	207	209	-	-
OS (mo)	NR	NR	-	-
OS HR (95% CI)	0.55 (0.36, 0.86)		-	-
PFS (mo)	23.1	11.1	-	-
PFS HR	0.33, p < 0.001		-	-
ORR (%)	68	45	-	-
Del11q	**Yes**		**No**	
n	78	77	125	129
OS (mo)	NR	NR	NR	22
HR (95% CI)	0.68 (0.32, 1.42)		0.55 (0.32, 0.95)	
ORR (%)	69	38	68	50
Bulky disease (>5 cm)	**Yes**		**No**	
n	140	136	67	73
OS (mo)	NR	21	27	NR
HR (95% CI)	0.51 (0.30, 0.87)		0.82 (0.39, 1.73)	
ORR (%)	69	46	66	44
Del17p or *TP53*mut	**Yes**		**No**	
n	69	68	138	141
OS (mo)	26.8	15.2	NR	NR
HR (95% CI)	0.60 (0.34, 1.06)		0.50 (0.25, 1.00)	
ORR (%)	59	25	73	55

Summary/Conclusions: Idelalisib in combination with bendamustine and rituximab is superior to bendamustine and rituximab alone, reducing the risk of death and disease progression, and increasing progression-free and overall survival. Overall response rate was also increased on the idelalisib arm. These results were consistent across pts with adverse-risk features. The safety profile was consistent with prior reported studies. This trial provides further evidence for the improved outcomes for idelalisib-based therapy in pts with relapsed/refractory CLL. This regimen represents an important new treatment option for the management of relapsed/refractory CLL, further establishing the role of idelalisib in this setting.

E1053

PRELIMINARY RESULTS OF A PHASE I/IB STUDY OF IBRUTINIB IN COMBINATION WITH TGR-1202 IN PATIENTS WITH RELAPSED/REFRACTORY CLL OR MCL

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Background: The oral BTK inhibitor ibrutinib is effective and well-tolerated for patients (pts) with relapsed/refractory (R/R) CLL and MCL; however, the rate of complete remission (CR) is low, and response duration is limited in MCL and in CLL with del(17p) or complex cytogenetics. TGR-1202 is a 2nd generation oral PI3K-delta specific inhibitor with promising efficacy and tolerability. We hypothesized that dual BCR pathway blockade would be tolerable and efficacious.

Aims: The primary aim of this open-label, phase I/Ib investigator-initiated multicenter trial is to determine the RP2D (recommended phase 2 dose) and the safety/tolerability of TGR-1202 plus ibrutinib in pts with R/R CLL or MCL. Secondary aims are to assess efficacy including response rates, duration of response, and PFS/OS.

Methods: Pts received continuous simultaneous daily oral dosing of ibrutinib (420 mg CLL, 560 mg MCL) and TGR-1202, starting at 400 mg daily and escalating in a standard 3+3 design from 600 to 800 mg. The DLT observation period was the first 28 day cycle, with CLL and MCL pts evaluated in separate cohorts. Pts continue until progression or unacceptable toxicity. Eligibility criteria include: ≥1 prior therapy, requiring treatment by IW-CLL criteria, ECOG PS ≤2, and adequate hematologic and organ function. Prior BTK or PI3K inhibitors were allowed. CTCAE v4 and IW-CLL criteria were used to evaluate toxicity and efficacy, with response evaluations after 2 mo., every 3 mo. up to 1 year, and every 6 mo. thereafter.

Results: As of 25 Feb 2016, 20 pts were enrolled, 10 CLL, 10 MCL. The median age at enrollment was 66 yrs. (range 48-83). The median number of prior therapies was 2 for CLL (range 1-6, including 2 with prior ibrutinib and 2 with prior PI3Ki) and 3 for MCL (range 2-5, including 2 with prior ibrutinib). Two CLL pts had del(17p), 2 had del (11q), and 7/10 (70%) had unmutated *IGHV*. The phase I portion in both diseases is now complete. Hematologic toxicities in CLL: neutropenia (37.5%, all gr 3-4), thrombocytopenia (25%, all gr 1), and anemia (37.5%, all gr 1/2). All grade non-hematologic toxicities occurring in >25% of CLL pts: diarrhea and nausea (37.5% each, all gr 1). There were no DLTs, and SAEs included gr 3 atrial fibrillation, gr 3 lipase elevation, and gr 3 CNS infection. The RP2D of TGR-1202 was 800 mg. Hematologic toxicities in MCL: neutropenia (37.5%, 12.5% gr 4), thrombocytopenia (50%, 12.5% gr 3), and anemia (37.5%, 12.5% gr 3). All grade non-hematologic toxicities occurring in >25% of MCL pts: diarrhea (75% [62.5% gr 1, 12.5% gr 2]), fatigue (50%, all gr 1/2), and 37.5% each for nausea (all gr 1/2) and transaminitis, dizziness, hypocalcemia (all gr 1). There were no DLTs, and SAEs included gr 3 hypophosphatemia in 2 pts and 1 pt with gr 3 amylase / gr 4 lipase elevation. The RP2D of TGR-1202 was 800 mg. There were no gr 3/4 bleeding events in either cohort. 6/9 (68%) of CLL pts achieved response, including 1 CR and 5 PR or PR-L. 4/7 (57%) of MCL pts who have reached the first response evaluation achieved response (all PR).

Summary/Conclusions: We report to our knowledge the first clinical data on combined delta-PI3K and BTK inhibition in B cell malignancies. TGR-1202 plus ibrutinib is well-tolerated in pts with R/R CLL and MCL, with no DLTs observed to date. The RP2D of TGR-1202 for both CLL and MCL was 800 mg daily. Preliminary efficacy data suggest a high response rate in both diseases, and phase Ib expansion cohorts at 800mg are now accruing in this ongoing study (NCT 02268851).

E1054

CLINICAL EPIDEMIOLOGY OF CLL AND CYTOGENETIC ABNORMALITIES: ASSOCIATION OF UNIQUE EPIDEMIOLOGIC EXPOSURES WITH 17P OR 11Q DELETION IN NEWLY-DIAGNOSED CLL

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Background: Clinical epidemiologic exposures associated with risk of developing CLL have been described in large case-control studies, including allergy, some autoimmune diseases, obesity, medical and family history, and some occupational exposures including rural/farm habitat. However, it is not known whether specific exposures are associated with a unique disease phenotype, and specifically whether risk factors for development of CLL are themselves associated with adverse cytogenetic profile by FISH, namely del(11q) and del(17p).
Aims: Primary Aim: To determine whether common clinical epidemiologic exposures associated with development of CLL are associated with a unique cytogenetic profile as determined by FISH at the time of diagnosis, and specifically which CLL risk factors are associated with adverse FISH profile [del(11q) and del(17p), either alone or in combination with other abnormalities]
Methods: We used cases from the Mayo Clinic Case-Control Study of Lymphoma, annotated with molecular markers from the Mayo Clinic CLL database to evaluate associations of medical history, lifestyle, family history, and farming history in a case-only study of the risk 17p or 11q deletion in CLL patients. Newly diagnosed CLL patients were enrolled between 2002 and 2012, and weight was patient-reported from 2 years prior to diagnosis. Unconditional logistic regression was used to estimate Odds Ratios (ORs) and 95% Confidence Intervals (CI) for risk adverse FISH profile (11q or 17p deletion). A significance level of 5% was used to determine statistical significance.
Results: We included n=683 patients, of whom n=100 had 11q or 17p deletions. Results of the analysis are noted in the Table.
We observed a significant association of rheumatoid arthritis with adverse FISH profile (11q or 17p deletion) at diagnosis (OR=2.49, 95% CI 1.06-5.86, p=0.037). In contrast, we observed a significant inverse relationship of any Allergy (Food, Plant, Animal, Insect, Dust, Mold) (OR=0.59, 95% CI 0.36-0.96, p=0.035) and of Atopy (OR 0.63, 95% CI 0.40-0.99, p=0.045) with adverse FISH profile. Other common exposures including obesity, smoking, and farm exposures were not associated with high risk FISH profile. Similarly, family history was also not associated with an adverse FISH profile, consistent with earlier reports.

Table 1.

Variable	Level	n.	Odds Ratio	95% CI	P-value
Age	Per 1 year increase		1.01	(0.99,1.03)	0.349
Gender	Male	445	REF		
	Female	238	0.96	(0.61,1.50)	0.848
BMI Category (kg/m²)	18.50-24.99	198	REF		
	25-29.99	266	0.74	(0.43,1.27)	0.271
	>=30	197	1.21	(0.71,2.06)	0.484
Smoking	Never	345	REF		
	Current	38	1.11	(0.44,2.78)	0.830
	Former	297	1.03	(0.66,1.59)	0.908
Alcohol	No	78	REF		
	Yes	604	0.68	(0.37,1.25)	0.211
*Allergy	No	456	REF		
	Yes	227	0.59	(0.36,0.96)	0.035
Asthma	No	612	REF		
	Yes	71	0.72	(0.33,1.55)	0.398
Eczema	No	612	REF		
	Yes	46	0.86	(0.35,2.08)	0.736
Atopy	No	416	REF		
	Yes	267	0.63	(0.40,0.99)	0.045
Contact Dermatitis	No	630	REF		
	Yes	25	1.46	(0.54,3.99)	0.459
Blood Transfusion	No	595	REF		
	Yes	86	0.57	(0.27,1.22)	0.145
Diabetes Mellitus	No	608	REF		
	Yes	63	0.83	(0.38,1.79)	0.629
Hypertension	No	376	REF		
	Yes	287	0.94	(0.61,1.45)	0.778
Rheumatoid Arthritis	No	629	REF		
	Yes	27	2.49	(1.06,5.86)	0.037
Infectious Mononucleosis	No	599	REF		
	Yes	60	0.75	(0.33,1.70)	0.485
Depression (on Therapy)	No	589	REF		
	Yes	78	1.15	(0.61,2.18)	0.660
Family History of Hematologic Malignancy	No	578	REF		
	Yes	97	0.80	(0.42,1.52)	0.491
Composite Total Sun Exposure (hours/week)	Per 1 hour/week increase		1.00	(0.96,1.04)	0.970
Lived on a Farm	No	404	REF		
	Yes	279	1.01	(0.65,1.55)	0.974

* Allergy: Food, Plant, Animal, Dust, Insect, Mold

Summary/Conclusions: Some clinical epidemiologic risk factors (allergy, atopy and rheumatoid arthritis) associated with the development of CLL are themselves significantly associated with specific disease-related cytogenetic abnormalities as determined by FISH profile at diagnosis. This suggests that some CLL risk factors may be associated with a unique clinical and genetic phenotype, which may influence disease biology and prognosis after diagnosis. Specific exposures that appear to mediate disease risk through alternate pathways of immune stimulation (i.e. allergy and atopy *vs* rheumatoid arthritis) are associated with a significantly different cytogenetic risk profile at diagnosis, suggesting that they likely have unique mechanisms of leukemogenesis. Further studies are ongoing to determine the impact of CLL epidemiologic risk factors on clinical outcome in association with other clinical and biological prognostic factors at diagnosis.

E1055

ASSESSING TIME TO FIRST TREATMENT IN EARLY CLL: A COMPARATIVE PERFORMANCE ANALYSIS OF FIVE PROGNOSTIC MODELS WITH INCLUSION OF CLL-IPI

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Background: Recent research in CLL focused on the development of prognostic scores which assemble in a same model different prognostic markers. However, analyses comparing the performances of these models are lacking.
Aims: In a prospective patient cohort we evaluated the capability of four recently proposed prognostic models in CLL (*i.e.*, 2007 MDACC score, GCLLSG score, 2011 MDACC score,O-CLL1 score) to predict time to first treatment (TTFT). Results were compared with those obtained using the CLL-IPI.
Methods: Newly diagnosed Binet stage A patients (n=337) from several Italian Institutions prospectively enrolled into the O-CLL1 protocol (clinicaltrial.gov identifier: 115 NCT00917540) form the basis of this analysis.
Results: The Harrell's c values which indicate the discriminatory power of the models (higher is better) were better for 2011 MDACC score (c-index, 0.71) and the O-CLL1 score (c-index,0.75) which were generated looking at TTFT as endpoint and combine clinical, biological and genetic markers. The same applied with GCLLSG (c-index,0.70) score and CLL-IPI (c-index, 0.70) even though they were originally developed with overall survival as main end pont. Finally, a c-statistic below 0.70 which is the threshold necessary to have value at the individual patient level was observed with 2007 MDACC score (c-index, 0.65) which was designed to assess overall survival as end point and did not incorporate major genetic factors. A global view of performances of different scores was also provided by the area under the receiver operating characteristic (ROC) curve figures considered an alternative measure of discrimination (Figure 1). Furthermore, the time-dependent ROC analysis which is an extension of ROC curves demonstrated that for early time points (<24 months) scores provided limited ability in distinguishing patients with different TTFT. In contrast, for late time points (≥24 months after diagnosis), the predictive accuracy of TTFT was higher for models including both clinical and genetic variables (i.e., O-CLL1, CLL-IPI,2011 MDACC score, GCLLSG score) in comparison with models which included only clinical variables (i.e., 2007 MDACC score). Finally, values of Aikaike information criterion (AIC) which indicates the goodness of the fit (lower is better) were better for the O-CLL1 score (AIC, 810) and worse for 2007 MDACC score (AIC, 851). In between there were the GCLLSG score (AIC, 837), the CLL-IPI (AIC, 841) and the 2011 MDACC score (AIC, 844), respectively.

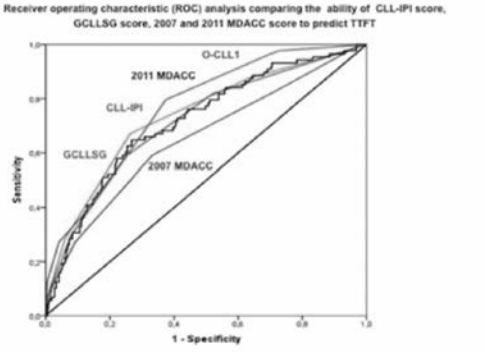

Figure 1.

Summary/Conclusions: Although different with respect to either the endpoints for which they were generated (*i.e.*, OS *versus* TTFT) or the prognostic factors considered (*i.e.*, only clinical *versus* clinical and genetic) all models used in this validation analysis worked in predicting TTFT. Results of AIC and Harrell's c value demonstrated that the predictive accuracy of TTFT was higher for models including both clinical and genetic variables (i.e., CLL-01, CLL-IPI,2011 MDACC score, GCLLSG score).

E1056

BCL6 ABNORMALITIES ON FLUORESCENCE IN SITU HYBRIDIZATION ARE ASSOCIATED WITH EARLY AGE AT DIAGNOSIS, KARYOTYPIC COMPLEXITY, AND AGGRESSIVE DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The clinical course of CLL is variable but can be predicted in part by recurrent cytogenetic abnormalities. BCL6 functions as a transcriptional repressor during germinal center formation, and alteration of 3q27 has been well characterized in some B cell malignancies. However, the incidence and significance of BCL6 abnormalities in CLL is unknown.
Aims: We aimed to determine the incidence and clinical significance of BCL6 abnormalities in CLL by analyzing a large database of CLL patients. We then aimed to analyze subsets of patients enrolled in clinical trials to determine the signficance of primary BCL6 abnormalities compared to acquired BCL6 abnormalities.
Methods: Between 10/2007 and 6/2014, 1811 patients (pts) with CLL at our institution underwent FISH which included a probe for 3q27. Clinical and molecular data were reviewed for pts with rearrangements or extra copies of BCL6. Data were also obtained from pts participating in 4 sequential clinical trials of ibrutinib, and characteristics of pts with and without pre-treatment BCL6 abnormalities were compared.
Results: 152 pts (8.4%) were found to have an abnormality of BCL6 at any time during their clinical course. Clinical and cytogenetic characteristics are summarized below. BCL6 abnormalities were present prior to first therapy in 28 pts, suggesting a primary abnormality. These pts had an early age at diagnosis (62.5 years), short time from diagnosis to first treatment (median 23.6 months), and relatively low 5-year survival (estimated at 62.9%). Pts with BCL6 abnormalities were likely to have complex karyotype and other high risk abnormalities including MYC rearrangement/copy gains and del(17p). Comparing pts with and without BCL6 abnormalities undergoing similar therapy, those with BCL6 abnormalities were more likely to have complex karyotype (p=0.0007), MYC abnormalities (p<0.0001), and del(17p) (p<0.0001).

Table 1.

	All BCL6+ pts (n = 152)	BCL6+ before first therapy (n = 28)
Median age at diagnosis	58.0 years	62.5 years
Median time from diagnosis to first therapy	15.5 months	23.6 months
Karyotype complexity (≥ 3)	71.3%	64.3%
Concurrent FISH abnormalities:		
MYC	49.0%	33.3%
Del(17p)	57.9%	42.9%

Summary/Conclusions: BCL6 abnormalities are associated with other high risk markers in CLL, and these pts have early age at diagnosis, short time to first therapy, and relatively short survival. These data suggest that BCL6 FISH abnormalities may be an important biomarker in CLL. Work to further characterize the biologic basis for these findings is ongoing.

E1057

VERY YOUNG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA HAVE LOWER INCIDENCE OF ADVERSE CYTOGENETICS AND IMPROVED OVERALL SURVIVAL: 10-YEAR EXPERIENCE FROM MEMORIAL SLOAN KETTERING CANCER CENTER

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Background: Young pts, arbitrarily defined as ≤ age 40, comprise a very small fraction of patients with CLL. Risk factors and outcomes in this young age group are not well described.
Aims: To compare biologic disease factors and clinical outcomes between patients age ≤40 with those >40.
Methods: Young pts were arbitrarily defined as ≤40 at diagnosis. Pts from the MSKCC CLL database diagnosed between 1/1/2005 and12/31/2015 were included. Two control CLL pts age >40 were matched to each patient ≤40 and served as controls. Matching was based on diagnosis year (+/- 2 yrs) and the time from diagnosis to the first MSKCC visit (+/- 2 yrs). Patient and disease characteristics were compared using Fisher's exact test. Cox regression analysis accounting for the matched pairs was used to estimate overall survival, time-to-first and –second treatment, and duration of first response. Survivor curves were estimated using Kaplan-Meier methods. The National Cancer Institute Surveillance, Epidemiology and End Results (SEER) database was used to compare the overall survival of pts ≤40 years with those >40 diagnosed between 1990-2005. Statistical analysis was performed using the R statistical package. The project was approved by the MSKCC institutional review board.
Results: 71/3455 (2.1%) pts in the MSKCC database were < age 40. Characteristics of the two groups are shown in Table 1. Significant differences included: initial WBC, presence of B symptoms, B2M and adverse cytogenetics. Second malignancies occurred more frequently in pts >40. There were 6 cases of Richter's transformation (RT) to DLBCL in each group, one case of histologically 'accelerated CLL' in each group and two cases of transformation to Hodgkin lymphoma in the group >40. DLBCL with TP53 mutation/del17p was seen in 1/6 pts ≤40 and 3/6 in >40 group. DLBCL was the initial indication for CLL therapy in 5/6 young pts who had RT, 3 of whom presented concurrently with CLL and RT. No pt >age 40 presented with CLL and DLBCL. There was no difference in the incidence of autoimmune complications in the two groups and no difference in the incidence of hematologic or solid organ malignancy in 1st or 2nd degree family members. The median follow-up of the MSKCC pts ≤40 was 54 months (1-313) and those >40 was 48 months (0-271). 48% of pts in each group required treatment. The time to first therapy and duration of response following 1st and 2nd line therapy were similar (Table 1). Six pts ≤40 (8.5%) and 2 pts >40 (1.4%) underwent allo-BMT (p=0.018). There were 4 deaths in pts ≤40 (5.6%) and 18 deaths in pts >40 (12.7%). Seven of the 22 deaths were unrelated to CLL (Table 1); and 6 of these were due to secondary malignancies. The estimated OS at 8 years was 0.97% in the group<40 and 86% in the group >40 (p=0.053) (Fig 1A). Lastly, in order to compare our results with a larger database, we evaluated young pts in the SEER database. There were 29,822 CLL pts of whom 279 were ≤40 (0.94%). The median OS in SEER cohort for pts age ≤40 was not reached, and in those age>40 was 6.9 years (95% CI 6.8-7.0),(p<0.001) (Fig 1B).

Age at CLL diagnosis	≤40 years	>40 years	P-value
Total Patients/% men	71 / (55%)	142 / (50%)	
Median age (range)	38 (21-40)	61 (41-86)	
Median diagnostic WBC (range)	17 (5-276)	14.2 (2-136)	0.020
B-symptoms at diagnosis	6/63 (8.7%)	2/132 (1.5%)	0.020
Median B2M (mg/L)	(N=32) 1.7 (1.1-5.5)	(N=56) 2.4 (1.4-9.3)	0.001
CD38+ (N) %	(25/64) 39%	(43/104) 41%	0.770
IGHV mutated (N) %	(18/34) 52.9%	(27/59) 45.8%	0.526
Diagnostic cytogenetics/FISH (N)	60	91	
Normal or +12	33 (59%)	45 (50%)	
Del 13q	14 (25%)	24 (27%)	
Del 11q	8 (14.3%)	11 (12.2%)	
Complex	0	7 (7.8%)	0.042
Del 17p/TP53/Complex karyotype	1 (1.7%)	10 (10.9%)	0.051
Acquired Del 17p/TP53	5 (8%)	7 (9%)	0.899
Family history			
Hematologic malignancy	14 (20%)	25 (18%)	0.711
CLL	3 (4.2%)	4 (2.8%)	0.688
Secondary malignancies	5 (7%)	28 (19.7%)	0.016
AIHA	5 (7%)	12 (8.5%)	0.795
Immune thrombocytopenia	4 (5.6%)	6 (4.3%)	0.735
Therapy and outcome			HR (95% CI, P-value)
Median follow-up (range)	54 (1-331)	48 (1-271)	
Time to first therapy, months	76 (65-NR)	61 (38-NR)	0.92 (0.46-1.48), p=0.81
Duration of first response, months	22 (18-53)	25 (14-34)	0.96 (0.55-1.65), p=0.87
Time to second therapy, months	138 (121-NR)	177 (87-NR)	0.86 (0.32-2.62), p=0.75
8-yr estimated OS, (%)	0.97 (0.79-0.94)	0.86(0.79-0.94)	0.34 (0.11-1.02), p=0.053
CLL related deaths/total deaths	3/4	12/18	

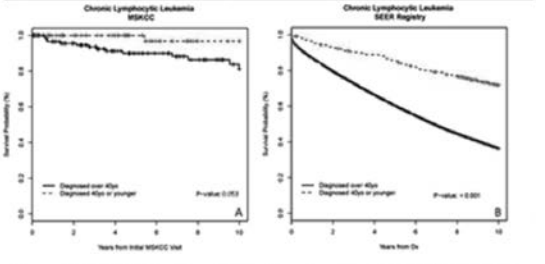

Figure 1 (A) OS of MSKCC cohort calculated from date of first MSKCC visit (2005-2015). (B) OS from date of CLL diagnosis in SEER registry cohort (1990-2005).

Figure 1.

Summary/Conclusions: A very small fraction of pts with CLL are diagnosed before the age of 40. These data suggest: a lower incidence of adverse cytogenetics at diagnosis, equivalent treatment response, and a higher incidence of RT that was not associated with del17p. MSKCC and SEER data suggest that young pts with CLL do not have an inferior survival compared pts >age 40.

E1058

Abstract withdrawn.

E1059

CD49D EXPRESSION IS ASSOCIATED WITH DEVELOPMENT OF LYMPHADENOPATHY IN PATIENTS WITH MONOCLONAL B-CELL LYMPHOCYTOSIS (MBL) AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: CLL clinical presentation is very heterogeneous, including lymphadenopathy, organomegaly, and cytopenias. The biologic factors which determine these differences in clinical manifestation are poorly understood. CD49d is a surface integrin expressed on CLL lymphocytes that facilitates interactions between CLL B-cells and stromal cells in the microenvironment. Although case series suggest high expression of CD49d in CLL patients may be associated with presentation with lymphadenopathy, these series are small and nearly all the information to date is cross-sectional.
Aims: We used the Mayo Clinic CLL database to prospectively evaluate the association between CD49d expression and subsequent development of lymphadenopathy in a cohort of patients with newly diagnosed MBL and CLL Rai stage 0.
Methods: All patients seen at our center between 08/2001 and 12/2015 and

who had pre-treatment CD49d testing available within 1 year of the date of diagnosis were included in the study. CD49d was considered positive if ≥30% of cells expressed CD49d (JCO 32:897). Time to development of lymphadenopathy was defined as time from diagnosis to appearance of palpable lymph nodes; in the absence of development of lymphadenopathy, patients were censored at date of first therapy or last follow-up. The Kaplan-Meier plots display time to development of lymphadenopathy.
Results: The study included 720 individuals, 333 with MBL and 387 with Rai stage 0 CLL. Among these patients, CD49d was positive in 34.5% of those with MBL and in 19.4% of patients with CLL Rai stage 0 (p<0.001). The median% of clonal B-cells expressing CD49d at diagnosis was 12% (interquartile range [IQR] 5%>59%) in individuals with MBL and 3.7% (IQR, 2%>14%) in patients with Rai stage 0 (p<0.001). Fourty-four (15%) patients with MBL and 82 (21%) patients with CLL Rai 0 subsequently developed palpable lymphadenopathy during the course of follow-up (median follow-up= 3.4 years). CD49d positivity at baseline was also associated with shorter time to development of lymphadenopathy both in patients with MBL (p=0.048) and CLL Rai 0 (p<0.001; Figure 1).

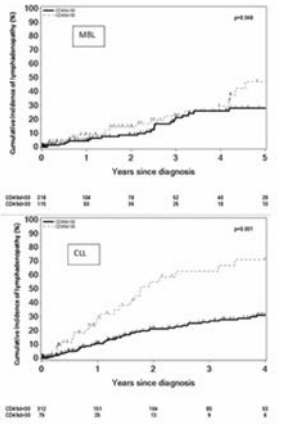

Figure 1.

Summary/Conclusions: CD49d expression identifies a cohort of MBL and Rai 0 CLL patients whose clinical course may be dominated by development of lymphadenopathy as opposed to bone marrow infiltration and cytopenias.

E1060

IMPACT OF THE APOPTOTIC REGULATOR DRAK2 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: In chronic lymphocytic leukaemia (CLL), the impaired balance between the pro-apoptotic and anti-apoptotic stimuli is involved in chemorefractoriness and clinical outcome. The low proliferation rate indicates that one of the primary mechanisms during disease development may be apoptotic failure. The apoptotic pathways and CLL proliferation is controlled by a variety of regulator molecules including various protein kinases. DRAK2 belongs to the Death-associated protein kinase (DAPK) family sharing 52% homology with DAP kinase 1 (DAPK1), a heritable predisposing factor in CLL. DRAK2 is expressed exclusively in lymphoid tissue while *drak2*$^{-/-}$ mice showed five-fold decrease in spleen germinal center compared to their wild-type littermates resulting from increased B-cell apoptosis. Further according to recent studies DRAK2 has been shown to control cell proliferation through its interaction with TβR1 thereby downregulating the smad2/3 mediated signalling.
Aims: The aim of this investigation is to understand the impact of one of the major apoptotic regulators, Death-Associated Protein Kinase-Related 2 (DRAK2) in CLL and its relationship to two distinct cellular networks a) involving TGF-β and b) Zap70-RhoH mediated signalling pathway in CLL
Methods: In our study, we have analyzed 102 CLL samples using TaqMan quantitative real-time PCR (qPCR) and calculated the relative expression by the $2^{-\Delta\Delta Ct}$ method. A ROC curve was generated to identify the optimal diagnostic cut-off. *DRAK2* expression was then split at this cut-off and the resulting dichotomous variable was included in a survival analysis.
Results: The *DRAK2* mRNA expression ranges from 0.16 to 9.26. Interestingly, *DRAK2* low-expressing patients showed significantly shorter survival than *DRAK2* high-expressing patients (p=0.003). The low expression of *DRAK2* is also identified as an adverse prognostic factor within the 13q deleted CLL patients. When we studied the relationship between DRAK2 and critical prognostic factors such as ZAP70 and its interaction partner RhoH, an atypical GTPase which influences the development of CLL, mRNA level of RhoH had no impact on overall survival in our cohort but discovered positive correlation (Pearson's r=0.36, p=0.00002) between DRAK2 and RhoH mRNA expression levels. Further based on the recent literature, which suggests negative feedback loop regulation between DRAK2 and Transforming growth factor β Receptor I, controlling TGF-β/Smads signalling in solid tumours, we measured the *TGFBR1* mRNA expression and found a positive correlation between *DRAK2* and *TGFBR1* expression (Pearson's r=0.446, p=0.000003).
Summary/Conclusions: Together, our data show for the first time an association between *DRAK2* expression and CLL poor prognosis and suggest the possibility of novel TGF-β/DRAK2 apoptotic signalling regulation in CLL. Moreover Zap70-RhoH mediated regulatory mechanism of DRAK2 remains rather complex that needs to be further investigated.

E1061

THE PROGNOSTIC SIGNIFICANCE OF PROLYMPHOCYTES IN CLL AND CORRELATION WITH MOLECULAR MARKERS IN THE LRF CLL4 TRIAL

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Background: An increase in the percentage and number of circulating prolymphocytes in CLL has been associated with strong expression of surface immunoglobulin (SmIg), trisomy 12 and a poor outcome (Melo *et al*, Br J Haematol 1986;63:377 & 1987;65:23; Que *et al*, Blood 1993;82:571).
Aims: This study re-examines the biological and clinical significance of increased peripheral blood prolymphocytes in patients entered into the randomised UK LRF CLL4 trial. It also investigates the associations between increased prolymphocytes and a comprehensive array of biomarkers.
Methods: 777 previously untreated patients were randomised between 1999 and 2004 to receive either chlorambucil or fludarabine, alone or in combination with cyclophosphamide. Median follow-up for overall survival (OS) was 11.8 (range 10.2-15.9) years. The proportion of prolymphocytes was assessed at trial entry in 508 patients.
Results: 270 patients (53%) had <5% prolymphocytes, 167 (33%) had 5-9%, 60 (12%) had 10-14% and 11 (2%) had =>15% prolymphocytes. We confirmed the association of increased prolymphocytes (=>10%) with strong SmIg expression and trisomy 12. In multivariate analysis =>10% prolymphocytes was independently associated with *NOTCH1* mutations, absence of 13q deletion, high CD38 expression and unmutated *IGHV* genes:

Table 1.

Variable	Odds ratio	95% Confidence Limits		p
NOTCH1 mutation	3.88	1.46	10.30	0.006
Absence of 13q deletion	4.41	1.82	10.69	0.001
Positive CD38 expression	6.48	1.44	29.25	0.02
Unmutated *IGHV* genes	5.02	1.39	18.17	0.01

Increased prolymphocytes (=>10%) were associated with a shorter progression-free survival (HR 1.50 [95%CI: 1.16-1.93], p=0.002) and OS (HR 1.99 [95%CI 1.53-2.59], p<0.0001; Figure 1). An absolute prolymphocyte count >=15x10^9/L was also associated with longer OS (HR 1.53 [95%CI 1.15-2.04], p=0.004). =>10% prolymphocytes was an independent predictor of OS when the multivariate model included the significant variables age, disease stage, 11q and 13q deletion and *TP53* del/mut together with any one of the following, each included separately: *IGHV* mutational status, B2M, CD38, Zap70 or *CLLU1* expression, mutations on *NOTCH1* or *SF3B1,* or telomere length. When all the significant variables were included together,% prolymphocytes was not an independent predictor of OS. Deaths due to Richter's syndrome were more common amongst patients who had =>10% prolymphocytes at trial entry (9/71, 13%) *vs* those with<10% prolymphocytes (8/437, 2%; p<0.0001).

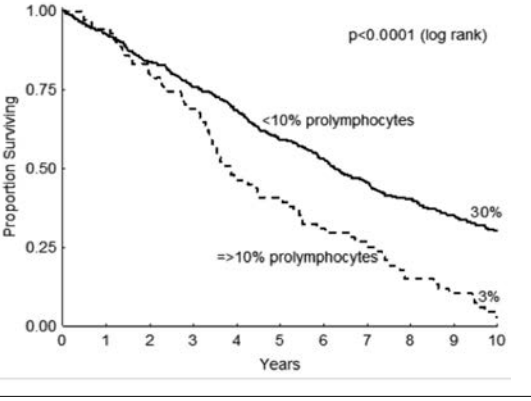

Figure 1. Overall survival by <10% *vs* =>10% prolymphocytes.

Summary/Conclusions: Our data support the routine examination of blood films in CLL and suggest that a finding of an increased proportion of prolymphocytes may be a trigger for a further evaluation of clinical and laboratory features of progressive disease.

E1062

ITALIAN VALIDATION OF THE INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS (CLL-IPI) AND COMPARISON WITH MDACC PROGNOSTIC INDEX: ANALYSIS OF 620 CASES

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Background: In the last two decades, a plethora of clinical, serological and biological markers have been identified that are significantly associated with the prognosis of chronic lymphocytic leukemia (CLL) patients. Recent research has focused on the development of scoring systems capable of integrating the major prognostic parameters. A recent international collaboration proposed an international prognostic index (CLL-IPI) built on clinical, serological, and biological parameters (TP53 deletion and/or mutation, IGHV mutational status, β2M, clinical stage, and age) universally applicable to previously untreated CLL patients to predict overall survival (OS).
Aims: We performed a validation of the CLL-IPI in an independent series of Italian patients. Furthermore, we compared this tool with the prognostic index proposed by the MDACC CLL group in 2007.
Methods: Databases from 4 Italian centers including roughly 3000 newly diagnosed CLL patients were used to evaluate the validity and reproducibility of the CLL-IPI. Baseline data regarding age, Binet stage, *IGHV* mutational status, β2M and *TP53* status, using only del17p, were available for 620 cases. The parameters for the calculation of the MDACC score were also available in these patients. The CLL-IPI and the MDACC score were calculated using the methods proposed. The accuracy of the prognostic models was assessed by the Harrell C index (an index of discrimination), the explained variation in mortality (an index combining calibration and discrimination), and the Akaike information criterion (AIC, an index comparing two non-nested prognostic models). The lower the AIC, the higher the prognostic accuracy of a predictive model.
Results: The median age of the 620 patients was 65 years (range 27-92) with 55.1% males. The majority of patients had Binet stage A (81.9%) and 366 cases (58.7%) had Rai stage 0. All 5 parameters of the CLL-IPI were found to be independently associated with survival in this study cohort (age >65 years: HR 5.48, P<0.0001; Binet B/C stage: HR 2.17, P<0.0001; β2M>3.5 mg/L: HR 1.99, P=0.001; *IGHV* unmutated: HR 1.82, P=0.002; *TP53* deleted: HR 2.84, P<0.0001). According to the CLL-IPI, 57.1% of patients were classified as low-, 23.3% as intermediate-, 14.9% as high-, and 4.7% as very high-risk. The 5-year OS probabilities were: 93.5% for low-risk, 83.2% for intermediate-risk, 68.6% for high-risk, and 35.1% for very high-risk cases (P<0.0001; Harrell C index=73%; P<0.001). The prognostic index risk category remained a predictor of survival when analysis was limited to Rai stage 0 (P<0.0001). Subsequently, we compared the CLL-IPI with the MDACC prognostic index score in our cohort. The Harrell C index of the MDACC score was 70% (P<0.001), similar to the CLL-IPI (73%). The AIC showed the superiority of the CLL-IPI compared to the MDACC score in predicting OS (CLL-IPI, AIC=1372.007 *versus* MDACC score, AIC=1383.364). Accordingly, the explained variation in mortality provided by the CLL-IPI was 29% (P<0.001), a figure higher than that due to the MDACC score (26%, P<0.001), indicating that the CLL-IPI had a higher prognostic accuracy for mortality compared to that of MDACC score.
Summary/Conclusions: Our results confirm the validity of the CLL-IPI to predict survival among patients with previously untreated CLL. Moreover, we have demonstrated that the CLL-IPI which combines clinical and serological data with biological parameters has a higher accuracy for predicting prognosis of CLL patients than the MDACC score, a model built only on clinical and laboratory markers.

E1063

IDELALISIB PLUS AN ANTI-CD20 ANTIBODY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WHO ARE HBV CORE ANTIBODY POSITIVE: SIMILAR PATTERNS OF LIVER TEST ABNORMALITIES

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Background: Increased hepatitis B virus (HBV) reactivation risk has been reported in patients (pts) undergoing treatment with anti-CD20 therapies, including rituximab (R) and ofatumumab (O).[1] Reactivation can also occur after HBV surface antigen (HBsAg) loss, especially in immuno-compromised pts.[2,3] A high rate of reactivation has been reported in such pts receiving an anti-CD20 antibody+chemotherapy.[4] The incidence of transaminase elevation in CLL pts who are core antibody positive (anti-HBc+)/ HBsAg-/HBV PCR- and receiving anti-CD20+kinase inhibitors including idelalisib (IDELA) has not been reported.
Aims: The objective of this analysis was to characterize the patterns of changes in liver tests in pts with CLL and anti-HBc+/ HBsAg-/HBV PCR- and receiving IDELA+anti-CD20.
Methods: The patterns of changes in liver tests were analyzed among 477 pts with relapsed/refractory (R/R) CLL treated with IDELA+R *vs* placebo+R (NCT01539512) or IDELA+O *vs* O (NCT01659021). Pts with anti-HBc+/HBsAg- were enrolled only if they were HBV PCR- by quantitative PCR (qPCR). Pts who were anti-HBc+/HBsAg- at screening were monitored for potential HBV reactivation (manifested as detectable HBV DNA by qPCR).
Results: During the study period beginning May 2012, 283 pts received IDELA+anti-CD20 therapy (110 IDELA+R; 173 IDELA+O) and 194 pts received anti-CD20 monotherapy (108 R; 86 O). Of 477 pts analyzed, 390 pts were anti-HBc-/HBsAg- (86 IDELA+R; 152 IDELA+O; 79 R; 73 O); 85 pts were anti-HBc+/HBsAg-/HBV PCR- (24 IDELA+R; 21 IDELA+O; 28 R; 12 O); 2 pts had missing data (1 R; 1 O). Of the 85 pts who were anti-HBc+, 3 had a prior history of HBV infection and 41 (22/45 on IDELA+anti-CD20 and 19/40 on anti-CD20) had received IV immunoglobulin (IVIG) before screening for treatment of hypogammaglobulinemia; 5 pts on IDELA+anti-CD20 and 3 on anti-CD20 received anti-HBV prophylaxis on-study. In the subgroup analysis, no significant difference in liver test abnormalities was detected between anti-HBc+/HBsAg- and anti-HBc-/HBsAg- subgroups in pts receiving IDELA+anti-CD20 or anti-CD20 monotherapy (table).

Table 1.

	IDELA+anti-CD20		anti-CD20	
	anti-HBc+ (n=45)	anti-HBc- (n=238)	anti-HBc+ (n=40)	anti-HBc- (n=152)
Grade ≥3 ALT/AST+-, n (%)	7 (15.6)	25 (10.5)	1 (2.5)	1 (0.7)
P value	0.449		0.379	
Med Time to Onset, wks	6.9	8.1	-	-
Grade ≥3 total bilirubin+-, n (%)	1 (2.2)	3 (1.3)	0 (0)	1 (0.7)
Grade ≥3 GGT+-, n (%)	1 (2.2)	13 (5.5)	3 (7.5)	1 (0.7)
Drug discontinuation due to hepatic-related TEAE, n (%)	0 (0)	8 (3.4)	0 (0)	0 (0)

Summary/Conclusions: Prior exposure to Hep B does not appear to be a risk factor associated with liver test abnormalities in pts treated with IDELA+anti-CD20.

References

1. Mitka M. JAMA. 2013;310(16):1664;
2. Hoofnagle JH. Hepatology. 2009;49(5 Suppl):S156-65;
3. Mikulska M, *et al.* Clin Microbiol Infect. 2014;20:O694-701;
4. Seto WK, *et al.* J Clin Oncol. 2014;32(33):3736-43.

E1064

SECONDARY CANCERS, MAJOR INFECTIONS AND AUTOIMMUNE DISEASES OCCUR IN DIFFERENT SUBSETS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL), the most common leukemia in western country, is still an incurable disease and the most relevant complications are major infections (MI), second cancers (SC) and autoimmune diseases (AD).
Aims: The aim of this study was to define the prevalence, risk factors and the evaluation of the impact of MI, SC and AD on the survival of CLL patients
Methods: We retrospectively analyzed clinical and biological data from 795 patients referred to Hematology and Clinical Immunology Unit of Padua University hospital. MI, were defined as those infective events that required in patients management or the use of intravenous antibiotics. SC comprised all other malignancies; while low and high-grade dysplasia were excluded. AD included all autoimmune and immune-mediate diseases. FISH analysis

(n=598), CD38 expression (n=656), IGHV mutations (n=507) and TP53 abnormalities (n=527, account for TP53 deletion and mutation) were evaluated according to international guidelines. Statistical analyses were performed with Fisher exact test, Chi-square test, Log-rank test, Kaplan-Meier method and Cox model. Overall survival was calculated from the date of diagnosis to death (event) or last available follow-up (censored).
Results: With a median follow-up of almost 100 months, thirty-four percent of patients developed at least one complication among MI, SC and AD with 95% of them being occurred after CLL diagnosis (Figure 1A). Ninety-eight patients experienced 138 MI, among which pneumonia (65%), septic shock (13%) and deep tissue abscess (10%) were the most common. 120 patients reported 187 SC; skin cancer (32%), colon cancer (16%) and other-hematological malignancies (14%) resulted to be the most common SC. Eighty patients were diagnosed with 103 AD, the most common of which resulted to be hematological (41%), rheumatologic (17%) and thyroid-related diseases (16%). We observed that only 0.9% of the cohort developed all the complications and that 1.4% experienced both SC and AD, 1.8% both MI and AD, and 3.9% both MI and SC during the follow-up period (Figure 1B). These data suggest a low probability to develop two or three complications and, as shown in Figure 1C, they seem to occur in a mutually exclusively manner. MI were found with a higher prevalence in males, in previously treated patients and in those with high-risk cytogenetic by FISH and TP53 abnormalities. SC resulted to be more common in male, in advance Rai and Binet stages at diagnosis, in previously treated patients, in high-risk FISH and TP53 abnormalities. AD were more common in female, in advance stage disease and in patients with 11q. No associations were found between specific treatments. The estimated median time to SC and MI development were 20 and 21 years, respectively; while it was not reached for AD. After 20 years from the diagnosis SC, MI and AD occurred in 48%, 42% and 29% of patients, respectively. *Kaplan-Meyer analysis* showed that patients who developed a MI had the worst prognosis whereas those with AD had the best; in fact medians overall survival were 14.14 and 17.16 years form MI and SC groups, while it was not reached for AD and all others patients not included in the three groups. These data were confirmed in multivariate analyses; MI and SC have a negative impact on the overall survival among with TP53 abnormalities and IGHV mutations (HR 2.04, 1.50, 2.25 and 3.70, respectively).

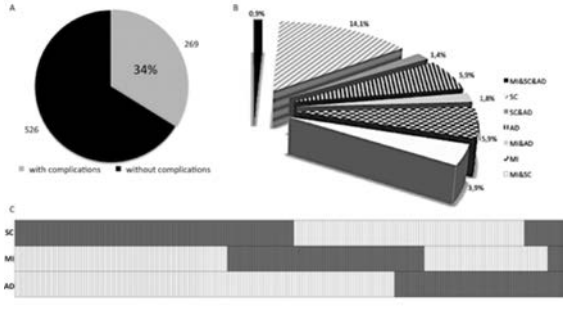

Figure 1.

Summary/Conclusions: We herein provide evidence for the existence of clinical subsets with different predispositions to develop major infections or secondary cancers or autoimmune diseases, with significant impact of the survival of CLL patients.

E1065

HYPOGAMMAGLOBULINEMIA IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICOBIOLOGICAL ASSOCIATIONS

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Background: A frequent feature of chronic lymphocytic leukemia (CLL) is progressive hypogammaglobulinemia that can affect one or more immunoglobulin (IG) subclasses.
Aims: To report the incidence of hypogammaglobulinemia in a large series of patients with CLL and explore associations with various clinical and biological markers.
Methods: Study group included 502 CLL patients with the following characteristics: median age: 65 years; males/females: 326/176; Binet stage A: 330/417 (79%), unmutated IGHV genes (U-CLL): 177/502 cases (35.2%); CD38 expression: 68/390 cases (17.4%); clonotypic IG of the MD or G isotype: 295 (78%) and 53 (14%) cases, respectively; isolated del(13q): 73/208 (35%); trisomy 12: 21/207 (10.1%); del(11q): 21/210 (10%); del(17p): 13/216 (6%); *NOTCH1* del7544-45/p.P2514Rfs*4: 12/259 (4.6%).Within our cohort, we identified cases belonging to one of three different, major subsets with stereotyped B-cell receptor IG (BcR IG), namely: (1) subset #1 (clan I IGHV genes/IGKV1(D)-39): U-CLL, clinically aggressive, n=12; (2) subset #2 (IGHV3-21/IGLV3-21), mixed IGHV mutational status, noted clinical aggressiveness, n=6; and, (3) subset #4, mutated IGHV4-34/IGKV2-30 BcR IG, clinically indolent, n=14.
Results: At diagnosis, decreased IG serum levels in at least one subclass were identified in 283/502 patients (56.3%), as follows: (i) decreased IgM, 224/502 cases (44.6%); (ii) decreased IgG, 105/502 cases (21%); (iii) decreased IgA, 131/502 cases (26%). In 54/502 cases (10.7%), all serum IG subclasses were decreased. Among cases with hypogammaglobulinemia, 114 (40.2%) and 31 (11%) exhibited isolated IgM and IgA subclass deficiency, respectively; isolated IgG decrease, was relatively rare (13 cases, 4.6%). Isolated IgA deficiency was significantly ($p<0.05$) more frequent amongst females, patients with advanced clinical stage (Binet B/C, Rai III/IV) and those with *NOTCH1* mutations ($p=0.01$) compared to isolated IgM deficiency. Notably, all stereotyped subset #2 cases showed low levels of at least one serum subclass, while in 2/6 such cases, all three IG subclasses (IgA, IgM, IgG) were affected. No statistically significant differences were identified between patients with normal serum IG levels *versus* those with hypogammaglobulinemia regarding age, gender, disease burden at diagnosis, IGHV gene mutational status, CD38 expression, cytogenetic aberrations, *NOTCH1* mutations and the incidence of a second malignancy. With a median follow up of 5 years, 192/413 cases [46.5%] received treatment. Patients with hypogammaglobulinemia exhibited increased need for treatment compared to those with normal serum IGs (124/234 *vs* 68/179 respectively, $p=0.002$). IgG and IgA deficiency were correlated with shorter time-to-first-treatment (TTFT) while none of the decreased IG subclasses impacted on overall survival (OS). In multivariate analysis for TTFT, IgG and IgA deficiency did not retain independent significance, with advanced clinical stage, U-CLL, del(11q) and del(17p) remaining as independent adverse factors.
Summary/Conclusions: In conclusion, abnormalities of serum IGs at diagnosis are detected in CLL patients with heterogeneous clinicobiological profiles, including different disease burden at diagnosis, cytogenetic aberrations and IGHV gene mutational status. However, certain observations reported herein, in particular the high incidence of hypogammaglobulinemia in subset #2 and the association of *NOTCH1* mutations with IgA subclass deficiency, open novel possibilities for unraveling the implicated pathophysiological mechanisms.

E1066

THE ASSOCIATION OF DYSLIPIDEMIA WITH CHRONIC LYMPHOCYTIC LEUKEMIA: A POPULATION-BASED STUDY

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Background: Metabolic syndrome (MetS) is a risk factor for the development of cancer. In addition, pre-clinical data suggest that chronic lymphocytic leukemia (CLL) cells are dependent on adipocytes and fatty acids for growth and that aberrant lipid metabolism is an important pathogenic mechanism in CLL.
Aims: Our objective was to determine whether patients with CLL have a higher incidence of MetS prior to their CLL diagnosis compared to those without CLL and to determine the impact of lipid-lowering medications on survival.
Methods: We conducted a population-based retrospective cohort study in Ontario, Canada using administrative databases of adults ≥66 years old to compare the prevalence of MetS and its components (diabetes, dyslipidemia, hypertension) before a diagnosis of CLL compared to age and sex-matched controls without CLL. Logistic regression was used to study the association between MetS and its components to CLL. The Kaplan-Meier method and Cox Regression were used to investigate survival.
Results: We identified 2124 persons with CLL and 7935 controls from January 1, 2000 to December 31, 2005 with follow-up until death or March 31, 2014. The median follow-up was 10.7 years (95% CI, 10.5 to 10.9). Overall, the mean age was 75.6 years and 42.1% were female, 20.2% had diabetes, 35.8% had hypertension, 17.6% had dyslipidemia and 28.0% received lipid-lowering medications prior to a diagnosis of CLL. In multivariable analysis, dyslipidemia (OR 1.34, 95% CI 1.17 to 1.54) and hypertension (OR 1.20, 95% CI 1.07 to 1.34) were associated with the development of CLL, whereas MetS and diabetes alone were not. On univariable survival analysis, dyslipidemia in CLL patients was associated with a significantly worse overall survival, however, not on multivariable analysis (HR 1.06, 95% CI 0.91 to 1.23). In comparison, lipid-lowering medication use at any time was associated with a significantly improved overall survival on univariable (see Figure) and multivariable analysis (HR 0.55, 95% CI 0.49 to 0.63) as was female sex (HR 0.66, 95% CI 0.59 to 0.75) and fewer comorbidities (HR 0.72, 95% CI 0.59 to 0.89). Advanced age (HR 1.08, 95%

CI 1.06 to 1.09) and diabetes (HR 1.31, 95% CI 1.14 to 1.50) were associated with a significantly worse overall survival.

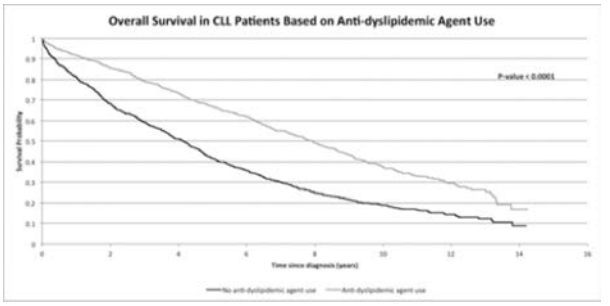

Figure 1.

Summary/Conclusions: We demonstrate an association between dyslipidemia and the development of CLL, supporting pre-clinical data. Furthermore, the relationship is independent of metabolic syndrome and diabetes. Lipid-lowering medications appear to confer a survival advantage in CLL. Prospective studies are needed to confirm these results and test their potential application to intervention strategies.

E1067

PATTERNS OF IDELALISIB TREATMENT-EMERGENT LYMPHOCYTOSIS IN PATIENTS WITH CLL OR SLL

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Background: Lymphocytosis is a known effect of B-cell receptor (BCR) targeted therapies used to treat CLL and SLL, including idelalisib (IDELA). IDELA is a selective oral PI3Kδ inhibitor which inhibits multiple signaling pathways, including those downstream of the B-cell receptor, CXCR4, and CXCR5. Per updated response criteria (Hallek M, el al. *Blood*. 2012;119:5348; Cheson B, et al. J *Clin Oncol*. 2012;30:2820-2822.), drug-induced lymphocytosis alone should not interfere with the time of designation of a partial response (PR) and is not considered progressive disease (PD) in the absence of other signs or symptoms of progression.
Aims: This post hoc analysis characterized the patterns of lymphocytosis observed in patients (pts) with CLL/SLL treated with IDELA-based regimens, and evaluated the effect of lymphocytosis on safety and efficacy.
Methods: Pts from 4 studies (2 phase 1, 1 phase 2 and 1 phase 3) who received IDELA dosed at 100 or 150 mg BID were included in this analysis. Pts were grouped by disease status (treatment-naïve *vs* relapsed/refractory) and treatment regimen (IDELA-monotherapy *vs* IDELA in combination with chemotherapy, an anti-CD20 mAb [anti-CD20], or both. Chemotherapeutic agents included chlorambucil, bendamustine, or fludarabine. Anti-CD20 mAbs included rituximab or ofatumumab. Absolute lymphocyte counts (ALC) were measured throughout each study to calculate peak ALC, time to peak ALC, and time to 50% reduction from baseline ALC. To evaluate the effect of lymphocytosis on safety, grade ≥3 treatment-emergent adverse events (TEAEs) and TEAEs of interest (leukostasis, blood viscosity abnormalities, central nervous system hemorrhage, and disseminated intravascular coagulation) were summarized in pts with ALC >200 K/μl at any time during IDELA treatment. To evaluate the effect of lymphocytosis on efficacy, median progression-free survival (PFS) was analyzed in pts receiving IDELA monotherapy for relapsed/refractory CLL (phase 1 study) and treatment-naive CLL/SLL (phase 2 study) stratified by ALC reduction from baseline (≥50% [yes or no]).
Results: The analysis population included 352 pts (105 treatment-naïve; 247 relapsed/refractory), 63 on IDELA monotherapy and 289 on IDELA combination regimens. 68% of pts experienced a post-baseline increase in ALC, including 83% of pts on IDELA monotherapy and 50% of pts on combinations. Time to peak was 2-4 weeks across groups. 73% of pts achieved a 50% reduction from baseline ALC, including 33% of pts on IDELA monotherapy and 82% of pts on combinations. Median time to 50% reduction from baseline ALC was longest for pts with relapsed/refractory CLL/SLL on IDELA monotherapy (15 wks) and shortest for pts with relapsed/refractory CLL on IDELA+chemotherapy+anti-CD20 (2 wks). The safety analysis included 31/352 pts (9%) with ALC >200 K/μl at any time on IDELA treatment, with a maximum reported ALC of 447 K/μl. Of these 31 pts, Grade 3/4 TEAEs were reported in 8 (26%) during the time when ALC >200 K/μl. No pt with signs or symptoms of hyperleukocytosis was identified. The efficacy analysis included 63 pts (41 treatment-naïve; 22 relapsed/refractory) on IDELA-monotherapy. The median PFS was similar for pts with or without a ≥50% reduction from baseline ALC (Table).

Table 1. PFS by achievement of ≥50% reduction from baseline ALC.

	≥50% reduction from Baseline ALC	<50% reduction from Baseline ALC	Log-rank P value
Treatment-naive CLL/SLL on IDELA-monotherapy (n=41)			
n (%)	17 (41)	24 (59)	
Median PFS (95% CI), mo	NR (8.3, NR)	NR (11.8, NR)	0.6326
Relapsed/refractory CLL/SLL on IDELA-monotherapy (n=22)			
n (%)	4 (18)	18 (82)	
Median PFS (95% CI), mo	9.7 (5.0, NR)	9.1 (2.6, NR)	0.9119

Summary/Conclusions: Addition of anti-CD20 and/or chemotherapy to IDELA abrogated the transient lymphocytosis seen with IDELA-monotherapy, regardless of disease status. Consistent with the updated response criteria, lymphocytosis does not appear to impact the efficacy of IDELA in this pt population. Lymphocytosis also did not predispose pts to severe or unexpected AEs.

E1068

RESULTS OF PROSPECTIVE OBSERVATIONAL TRIAL OF POLISH ADULT LEUKEMIA GROUP (PALG) ON IBRUTINIB COMPASSIONATE USE IN RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) IN POLAND

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Background: Ibrutinib is a selective and covalent inhibitor of Bruton's tyrosine kinase approved for chronic lymphocytic leukemia (CLL). Outcome and burden of adverse events of specific therapy reported in clinical trials may differ from routine clinical practice due to selection bias and different monitoring.
Aims: The aim of this observational study of Polish Adult Leukemia Study Group (PALG) was to prospectively assess the efficacy and toxicity of ibrutinib monotherapy in relapsed/refractory CLL patients treated within the ibrutinib compassionate use named patient program (NPP) in Poland.
Methods: Patients were eligible for ibrutinib compassionate use NPP if they met all following criteria a) relapsed or refractory CLL, b) active CLL/SLL requiring treatment, c) a minimum of 1 prior line of chemotherapy. Data on treatment outcome and complications were anonymously collected using electronic CRFs.
Results: This report is based on data obtained for 163 patients (158 with CLL and 5 with SLL) treated in 15 hematology centers participating in the observational study of PALG. There were 91 male and 72 female patients with median age 63 (range 40-84). Fifty-two patients (32.5%) had advanced disease (III or IV Rai stage) at diagnosis. Median number of chemotherapy lines prior to ibrutinib was 3 (range 1-10). FISH cytogenetics at inclusion to NPP was conducted in 96 (60%) patients, and it revealed del17p in 30 patients (19%). The median follow-up time was 9.2 months (range 0.4-22.2 months). Overall response rate (complete remissions, partial remissions or partial remissions with lymphocytosis) was 73%. Median progression free survival and overall survival (OS) had

not been reached with 24 deaths recorded during the time of observation. The estimated probability of 12-months OS was 0.83 (95%CI: 0.75-0.88) that appears inferior to 0.90 reported in RESONATE clinical trial (Byrd et al, NEJM 2014). Among parameters analyzed as potential prognostic factors, the advanced clinical stage (Rai III and IV) was associated with inferior OS, with estimated 12-months survival of 0.72 (95%CI: 0.577-0.826) (Fig 1A). Interestingly, no negative influence of del17p regarding OS was detected (Fig 1B). Regarding ibrutinib toxicity grade 3 or 4 side effects occurred in 43 patients (27%). Infections of any grade were diagnosed in 57 patients (36%), while atrial fibrillation of any grade was seen in 14 patients (8.8%). Thirty-three patients (20.2%) required ibrutinib dose reductions, mainly due to infections or hematological toxicity. Interestingly, in RESONATE trial with similar follow up time (median 9.4 months) ibrutinib dose reduction was reported only for 4% of patients.

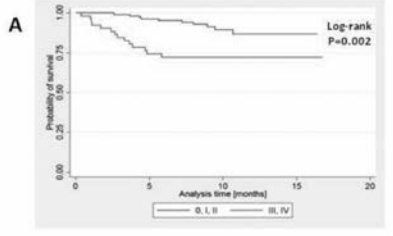

Figure 1. The Kaplan-Meyer plot for the estimated probability of 12-months survival.
A. The advanced clinical stages (Rai III and IV; red line) was associated with inferior estimated 12-month survival (compared to early clinical stages (Rai 0, I and II; blue line; 0.86).
B. No significant difference between patients with revealed del17p (blue line), patients without del17p (red line) and those without performed FISH analysis (green line).

Figure 1.

Summary/Conclusions: This large analysis of refractory/relapsed CLL patients treated with ibrutinib monotherapy in real-world clinical practice shows satisfactory treatment results, however, patients' outcome appears inferior as compared to that observed in the clinical trial. This difference could be partially due to relatively frequent ibrutinib dose reductions and treatment interruptions observed in this patient cohort. Furthermore, our report confirms efficacy of ibrutinib in relapsed/refractory CLL patients with del17p, but also shows that advanced CLL stage at diagnosis retains its prognostic value.

E1069

SF3B1 MUTATIONS, IGHV MUTATION STATUS AND ABNORMALITIES DETECTED BY GENOMIC ARRAYS DETERMINE PROGNOSIS IN CLL WITH NORMAL KARYOTYPE

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Background: Normal karyotype (NK) CLL accounts for 15-20% of all CLL cases and confers *per se* an intermediate risk. Our group reported a higher *SF3B1* mutation (mut) rate compared to other cytogenetic subgroups and only one third of patients showed unmutated *IGHV* status (*IGHV*u). Furthermore, we previously demonstrated that array-based comparative genomic hybridization (aCGH) technology could detect cryptic abnormalities in 19% of NK CLL.
Aims: To further characterize CLL with normal karyotype and identify prognostic parameters.
Methods: We selected 164 patients with NK and CLL cells percentage >15% (16% of total CLL cohort). Chromosome banding analysis yielded normal karyotypes. FISH analyses, performed with probes for 17p13 (*TP53*), 13q14 (D13S25, D13S319, *DLEU*), 11q22 (*ATM*), centromere region of chromosome 12, t(11;14)(q13;q32)(*IGH-CCND1*), 6q21 and *IGH* rearrangements, did not reveal any abnormalities. aCGH was performed with SurePrint G3 ISCA CGH+SNP Microarray (Agilent, Waldbronn, Germany). *IGHV* status, mutational analysis by DNA sequencing for *SF3B1*, *NOTCH1* and *TP53* and information on time to treatment (TTT) were available for all patients. White blood cell (WBC) count was available for 153/164 patients.

Results: Median follow-up was 5.6 years and 10-year OS was 75%. Median age was 63 years (range, 34 to 83) and 93/164 (54%) were male. Median WBC count was 36,500/mL (range, 3,300 to 463,000). Cases with CLL cells ≤5000/μL had nodal presentation. By flow cytometry, median% CLL cells was 52% (range, 16% to 94%). Cut-off for CD38 and ZAP70 positivity was 30% and 20%, respectively. 34 patients (20%) showed CD38 positivity and 61 (36%) ZAP70 positivity. By aCGH, we detected 68 copy number abnormalities (CNA) in 37 patients (22%). Majority (24/37; 65%) had 1 CNA, 9 patients (24%) showed 2 CNA and 4 (11%) more than 2 CNA. *IGHV*u was present in 52/164 (32%) cases. *SF3B1* was mutated in 26/164 (16%), *NOTCH1* in 12 (7%) and *TP53* in 7(4%). Univariate Cox analysis for TTT showed adverse impact for: WBC (HR:1.05 per 10,000/μL increase; 95%CI 1.02-1.09; p=0.001);% CLL cells (HR:1.3 per 10% increase; 95%CI 1.16-1.5; p<0.001); CD38 positivity (HR:2.7; 95%CI 1.5-5; p=0.001); ≥2 CNA (HR:3.6; 95%CI 1.6-8.1; p=0.002); *SF3B1mut* (HR:2.3; 95%CI 1.2-4.5 p=0.013); *IGHVu* (HR:4.8; 95%CI 2.6-8.7; p<0.001). Detection of any CNA, ZAP70 positivity, *TP53*mut and *NOTCH1*mut did not relate with TTT. In multivariate analysis,% CLL cells (HR:1.2 per 10% increase; 95%CI 1.04-1.4; p=0.015), ≥2 CNA (HR:2.9; 95%CI 1.2-7.012; p=0.015), *SF3B1*mut (HR:2.3; 95%CI 1.16-4.5; p=0.016) and *IGHV*u (HR:4.3; 95%CI 2.1-8.4; p<0.001) retained their prognostic value. Therefore, we clustered patients into 3 groups (figure 1; table 1). Patients without risk factors (#0) showed the best outcome (median TTT not reached); patients with 1 risk factor (#1) had a median TTT of 6.5 years; patients with at least two risk factors (#2) showed a median TTT of 6 months (p<0.001).

Table 1. Cluster groups according to prognostic factors.

Cluster	number of cases		Prognostic factors	median TTT
#0	92		no	Not reached
#1	55	15	*SF3B1*mut	6.6 yrs
		35	*IGHV*u	
		5	CNA≥2	
#2	17	9	*SF3B1*mut AND *IGHV*u	6 mo
		6	*IGHV*u AND CNA≥2	
		2	*IGHV*u AND *SF3B1*mut AND CNA≥2	

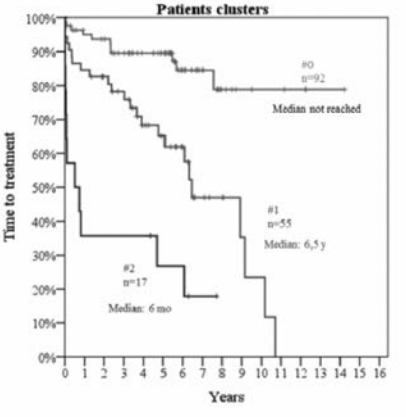

Figure 1.

Summary/Conclusions: Through the evaluation of *IGHV* mutational status, *SF3B1* and aCGH, we identified CLL patients with normal karyotype at the highest risk for need of treatment.

E1070

A SYSTEMATIC LITERATURE REVIEW OF RANDOMIZED CONTROLLED TRIALS FOR THE TREATMENT OF RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Treatment of Chronic Lymphocytic Leukemia (CLL) by chemotherapy is not curative, and patients receive multiple chemotherapy lines in an attempt to manage their disease along the years. Due to cumulative toxicity or resistance, treatment options following first-line therapy quickly become limited and new treatment options are needed. Idelalisib is a novel oral inhibitor of PK13K delta for the treatment of relapsed/refractory (R/R) CLL. This study reviewed randomized controlled trials (RCTs) of idelalisib and treatment options in the management of R/R CLL.
Aims: To review the efficacy and safety of idelalisib relative to other commonly used treatments for R/R CLL.
Methods: A systematic literature review was conducted to identify articles evaluating idelalisib (Id), ibrutinib (Ib), alemtuzumab (A), fludarabine (F), rituximab (R),

ofatumumab (O), bendamustine (B), cyclophosphamide (C), methylprednisolone (Mp), chlorambucil (Ch), lenalidomide (L), doxorubicin (D), vincristine (V), prednisone (P), CHOP, and CVP as single agents or in combination, in adult patients with R/R CLL. Searches were conducted in the following literature databases: EMBASE, MEDLINE, MEDLINE In-Process and CENTRAL. Conference abstracts from ESMO, ASCO, EHA and ASH 2012–2015 were also searched. Screening was carried out independently by two reviewers. English language RCTs reporting overall, complete or partial response, stable or progressive disease, overall survival (OS), progression-free survival (PFS) or safety were included.

Results: Of 2222 potential studies, 29 full papers were screened, eight of which were included in the final review. Nineteen conference abstracts were also included. Overall, data from 14 unique RCTs were included in the final analysis (Table 1). Three RCTs included Id; in combination with R, or O, or BR. The other 11 studies identified are outlined in table 1. Only eight studies reported baseline genetic characteristics. The included studies were heterogeneous with regards to CLL stage, cytogenetics, and prior lines of therapy; all of which are recognised as treatment modifiers. The median duration of progression-free survival (PFS) between treatment arms was available for nine studies and ranged from 5.5 months (R) to 30.6 months (FCR). Across all studies, the greatest difference in the median duration of PFS between treatment arms was 13 months (IdR *vs* R; hazard ratio= 0.15; 95% CI 0.09-0.24). The Median duration of PFS in treatment arms that contained Id ranged from 16.3 months (IdO) to 23.1 months (IdBR). Median OS was reported in 11 studies and ranged from 16.7 months (O) to not reached.

Table 1.

Table 1. Key efficacy outcomes

Study ID*	Treatment	N assessed	Assessed by	ORR N	ORR %	PFS (months) Median	PFS (months) LCI	PFS (months) UCI	OS (months) Median	OS (months) LCI	OS (months) UCI
Robak 2010	FC	276	IA	276	58	20.6	-	-	52	-	-
	FCR	276		276	69.9	30.6	-	-	NR	-	-
	FC	276	IRC	276	49	21.9	-	-	-	-	-
	FCR	276		276	61	27	-	-	-	-	-
Leblond EHA 2012	BR	18	-	18	89	-	-	-	-	-	-
	CR	23	-	23	83	-	-	-	-	-	-
Furman 2014	IdR	110	IRC	110	83.6	19.4	12.3	NR	NR	NR	NR
	R	110		110	15.5	6.5	4	7.3	20.8	14.8	NR
	IdR	110	IA	-	-	NR	11.1	NR	-	-	-
	R	110		-	-	5.5	3.7	7.3	-	-	-
Niederle 2013	B	49	IA	49	76	20.1	-	-	43.8	-	-
	F	43		43	62	14.8	-	-	41	-	-
Elter 2011	FA	168	IRC	168	82	23.7	19.2	28.4	NR	NR	NR
	F	167		167	75	16.5	12.5	21.2	52.9	40.9	NR
Montserrat 1985	CVP	18	IA	18	28	-	-	-	-	-	-
	ChP	17		17	35	-	-	-	-	-	-
Byrd 2014	Ib	195	IRC	195	42.6	NR	-	-	NR	-	-
	O	196		196	4.1	8.1	-	-	NR	-	-
	Ib	195	IA	195	85	NR	-	-	-	-	-
	O	196		196	25	8.1	-	-	-	-	-
Awan 2014	FCR+Lu	207	-	207	71	24.6	23.6	30.8	NR	-	-
	FCR	183	-	183	72	23.9	18.6	27.3	NR	-	-
Robak EHA 2015 (a)	IdO	174	IRC	174	75.3	16.3	-	-	20.9	-	-
	O	87		87	18.4	8	-	-	19.4	-	-
Robak EHA 2015 (b)	OFC	183	IRC	-	84	28.9	22.8	35.9	56.4	44.2	NR
	FC	182		-	68	18.8	14.4	25.8	45.8	37.3	NR
Cramer EHA 2015	IbBR	379	IRC	-	82.7	NR	-	-	NR	-	-
	BR	199		-	67.8	13.3	-	-	NR	-	-
	IbBR	289	IA	-	86.2	-	-	-	-	-	-
	BR	289		-	68.9	-	-	-	-	-	-
Ghia ASH 2015	O	24	-	-	8	6.4	2.3	10.2	16.7	2.3	20.2
	Di	20	-	-	40	14.9	11.3	23	21.2	16.6	NR
Zelenetz ASH 2015	IdBR	207	IRC	207	68	23.1	-	-	NR	-	-
	BR	209		209	45	11.1	-	-	NR	-	-

NR: not reached; "-": not reported; IRC: independent review committee; IA: investigator assessed; Lu: lumiliximab; Di: Dinaciclib; *One study (Gladstone ASCO 2014) only reported safety outcomes

Summary/Conclusions: This systematic literature review suggests that idelalisib (in combination with BR, O and R) is an acceptable alternative treatment compared to interventions currently used in clinical practice for R/R CLL patients.

E1071

PREVALENCE OF AGE-RELATED CLONAL HEMATOPOIESIS (ARCH) AND LOW FREQUENCY MUTATIONS IN CLL & MONOCLONAL B LYMPHOCYTOSIS: A MAYO CLINIC EXOME SEQUENCING COHORT STUDY

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Background: ARCH refers to somatically acquired single-nucleotide variants (SNV's) and small indels in DNA involving hematologic (and non-hematologic) genes (most commonly *DNMT3A, TET2, ASXL1 & PPM1D*) detected by whole-exome sequencing (WES) in peripheral blood in unaffected individuals. The incidence of ARCH increases with age and is asociated with an 11-fold increase in the risk of hematologic malignancy including leukemia. However, the contribution of ARCH to the development of CLL, the hematologic malignancy with highest prevalence, is unknown. Furthermore, the frequency and clinical impact of ARCH-associated and other non-ARCH low frequency mutations is unknown in CLL. We therefore sought to understand the contibution and frequency of ARCH and other low frequency mutations in a cohort of patients with CLL and monoclonal B-lymphocytosis (MBL, an established precursor state with increased risk of CLL development) who have undergone WES.

Aims: To determine the prevalence of ARCH and non-ARCH low frequency mutations and their association with clinical risk factors in a cohort of unaffected (UA) and affected individuals with CLL & MBL using peripheral blood WES.

Methods: Bioinformatic analysis was performed using raw data (BAM and FASTQ files) from WES performed in a cohort of 445 patients, comprised of CLL (n=160), MBL (n=73), and unaffected individuals (UA, n=212). Samples were prepared using the Agilent V2 Exome Capture Kit, and in some cases prepared using the Agilent V4 Exome Capture Kit. WES alignment was performed with Novoalign within GGPS and recalibrated and realigned with GATK. Pileup files were obtained from the recalibrated and realigned BAM files, and identification of clonal and sub-clonal variants performed using DV-Boosting method and Q value of 0.05 as cut off. Consistent with previous evaluation of ARCH, "non-functional" variants were filtered out (synonymous, UTR, intron, etc.). In addition, variants with an Alternative Allele Frequeny (AAF) ≥0.05 in European or Caucasian populations of HapMap, 1000G, and ESP6500 were filtered out as "polymorphisms" and not included as mutations. Remaining variants were categorized as "disruptive" and "non-synonymous" (NS) variants in accordance with previous publications. Disruptive SNV's included those with Start/Stop codon and at splice sites. Because of the inclusion of "affected" individuals in this analysis who may have subclinical clonal disease variants, we defined the "non-inherited" variants more conservatively as those with<40% of the AAF.

Results: The frequency of detectable mutations is noted in Figure. In almost all cases the AAF was ≤10%. Based on previous population-based studies, we anticipated a rate of disruptive (Disrupt) and NS ARCH mutations for the cohort of 445 patients (noted in Table). We did not detect ARCH mutations in any CLL or UA patient, and in total detected *DNMT3A* and *ASXL1* mutations each in 2 MBL patients. We detected non-ARCH low AAF disruptive somatic mutations in other genes in 12 CLL and 11 MBL patients, respectively, including in 4 Tier 1 cancer genes (*JAK2, FGFR3, MLH1 & STK11*) occurring in 3 patients each with CLL and MBL. No mutations were detected in UA population in this analysis. To determine if low AAF mutations were associated with any unique CLL clinical characteristics, we compared frequencies among the subset of CLL patients. Among those with available Rai stage, CLL patients with a detectable low AAF mutation appeared to be more likely to have advanced (stage III/IV) disease at diagnosis (25%) compared with those without a mutations (2.3%) (p=0.03), but there was no difference in early stage disease (Stage 0: 75% *vs* 67.7%; and Stage I/II: 0% *vs* 31.1%, respectively) (p=0.13). There was no difference in age at diagnosis or gender.

Table 1.

	Anticipated Mutations		Observed Mutations					
Gene			CLL		MBL		UA	
	Disrupt	NS	Disrupt	NS	Disrupt	NS	Disrupt	NS
DNMT3A	1	2	0	4	2	8	0	9
TET2	0.8	0	0	12	0	10	0	9
ASXL1	0.6	0	0	12	2	3	0	15
PPM1D	0.4	0	0	0	0	0	0	0

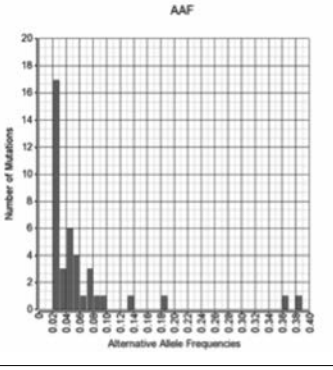

Figure 1.

Summary/Conclusions: ARCH appears to be a very infrequent event in CLL, and therefore is unlikely to constitute a major risk factor for CLL development. Low AAF non-ARCH mutations are detectable in some patients with CLL (7.5% in this analysis), including in some Tier 1 cancer genes, and may be associated with advanced stage disease at diagnosis, although this is based on a limited number of observations and will require prospective confirmation. These results suggest an important role for Deep Sequencing in advanced CLL focusing on Tier 1 cancer genes.

E1072

PERFORMANCE STATUS AND COMORBIDITY AFFECT SURVIVAL IN ELDERLY PATIENTS WITH CLL AFTER LOW DOSE ALEMTUZUMAB COMBINED WITH CHEMOTHERAPY: RESULTS FROM THE HOVON68 TRIAL

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Background: In CLL, chemo-immunotherapy is the standard of care. Until recently fludarabine and cyclophosphamide (FC) has been considered the chemotherapy of choice in fit patients irrespective of age. However, ageing results in a decline in organ function and immune competence resulting in higher susceptibility to the toxicity of chemotherapy, while FC+Rituximab resulted in more infections when compared to Bendamustine+Rituximab, also in the elderly (Eichhorst et al. 2014). Furthermore, in the HOVON68 CLL trial, patients above 65 years of age had no survival benefit from the addition of low-dose alemtuzumab to FC in contrast to younger patients (Geisler et al. 2014).
Aims: Here we address the issue of why elderly patients with CLL did not benefit from the addition of low-dose alemtuzumab to FC, using a 5 year update of the HOVON68 trial.
Methods: 272 patients from the HOVON68 trial were included in this study. All data were collected from the HOVON database, updated as of February 2015, to reach a median follow-up period of 61 months. Baseline characteristics, causes of death, comorbidities, organ related adverse events, infections, and lymphocyte counts during treatment and the follow-up period were extracted as variables. The statistical analyses included survival- and competing risk analysis using univariate and multivariate cox regression performed in STATA version 13.1. P-values were two-sided and considered as statistically significant if below 0.05.

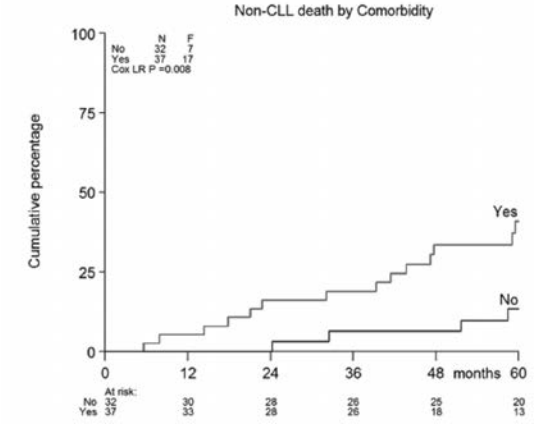

Figure 1.

Results: The addition of alemtuzumab to FC (FCA) improved overall survival from a median of 77 months to median not yet reached (log rank P=0.049) in the 5 year update of the HOVON68 trial. In addition, we confirmed that elderly patients aged 65 to 75 of age had no improvement in OS (p=0.38). Overall 40% (110/272) of patients died in the follow-up period. Excluding deaths related to allogeneic transplant (n=10), 44% (44/100) died from CLL-related causes. The elderly patients who received FCA had close to a significantly higher non-CLL related death rate compared to all others (p=0.05). Infections accounted for almost half (46.4%) of all non-CLL related causes of death. However, among FCA treated patients, non-CLL related deaths in the elderly were primarily due to secondary cancer or cardiovascular disease (10/14, 71%). At trial entry, most of the comorbidity in the elderly was cardiovascular (23/37, 62%) including hypertension, atrial fibrillation, history of transient cerebral ischemia, or ischemic heart disease. The comorbidities were balanced between the treatment arms.
In the elderly, performance status (PS)>0, del(17p), β2microglobulin≥3.5mg/dL, opportunistic infection, and comorbidity>0 (Figure 1) were significantly associated with non-CLL related mortality by univariate Cox regression using competing risk analysis. By multivariate analysis of factors found to be significant on univariate analysis, only PS>0 (HR 5.25 [1.88-14.63], p=0.002), del(17p) (HR 4.47 [1.35-14.81], p=0.014), and comorbidity (HR 5.4 [1.66-15.33], p=0.004) remained independently significant. No interactions were found between the three significant variables with treatment arm, or between PS and comorbidity.
Summary/Conclusions: FCA induced more non-CLL and non-infection related deaths in elderly patients compared to younger patients with biological high risk CLL in the HOVON68 trial. Both PS and comorbidity had a significant impact on non-CLL related mortality in the elderly after treatment with FC or FCA.

E1073

OUR APPROACH TO HAIRY CELL LEUKEMIA (HCL): 31 YEARS OF EXPERIENCE

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Background: HCL is a rare chronic lymphoid malignancy generates from mature B cells. The frequency is 2% in all leukemia and diagnosed mostly in middle ages. Patients response to purine analogues treatment.
Aims: In this study our aim is to present our HCL patients with demographic features, treatment modalities and responses.
Methods: 38 HCL patients who were diagnosed and treated at Ankara University Department of Hematology between 1984 and 2015 were retrospectively evaluated. Ki-square test and student t test were used in comparison. P<0.05 was considered statistically significant.
Results: Median age of patients were 38 (range, 31-79). 29 of patients (76%) were male. The average leucocyte, hemoglobin, platelet, lactate dehydrogenase (LDH) levels and the spleen size at diagnosis were as follows: 5,1 x 10^9/L (range, 1,5-20), 12,4 g/dl (range, 9-17,5), 118x10^9/L (range,15-300), 179 IU (range,107-394), 155 mm (range,110-290). BRAF mutation could be evaluated in one patient and revealed as positive. 8 patients (21%) underwent splenectomy prior to chemotherapy. All patients received cladribine (0.1mg/kg, 7 days) as a first line treatment; 8 patients (21%) had two courses for remission. 11 patients (30%) had treatment related febrile neutropenia, 2 patients (0.05%) had transient skin rash after infusion. 7 patients (18%) had a relapse and 1 patients received pentostatin (4mg/m^2, 1 dose every 2 weeks, 6 months), 6 patients were retreated with the first line treatment and achieved complete response. The overall survival was 67 months, respectively. Leucocyte, hemoglobin, platelet levels were lower, LDH levels were higher and spleen size was increased in patients with relapse however no statistical difference were detected.The patients with splenectomy had improved survival compared to patients without splenectomy (107 *vs* 292 months, P=0.04) (Figure).

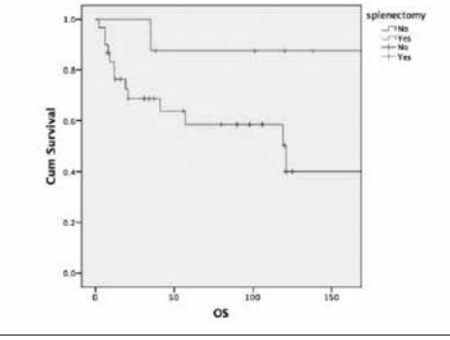

Figure 1. Splenectomy had increased overall survival.

Summary/Conclusions: We detected 18% relapse rate in our series and all patients were followed in remisson after second line purine analogue treatment. Splenectomy has improved overall survival and possibly still has a role to play in HCL therapy. Generally, HCL is a chronic disease with a favorable prognosis.

E1074

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION HAS PROGNOSTIC VALUE IN CHRONIC LYMPHOCYTIC LEUKEMIA BEYOND STANDARD FLUORESCENCE IN SITU HYBRIDIZATION

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Background: Chronic lymphocytic leukemia (CLL) has significant clinical heterogeneity. Fluorescence in situ hybridization (FISH) is an integral component

of current risk stratification models, with poor prognosis associated with deletions of 11q and 17p and good prognosis with deletions at 13q. In addition to identification of clinically significant clonal aberrations by FISH, array comparative genomic hybridization (aCGH) offers a high resolution assessment of genetic imbalance.
Aims: To demonstrate prognostically significant features using aCGH beyond that shown by FISH alone.
Methods: Consent for genomic investigation on excess material was given by 91 patients (67 diagnostic, 19 post-treatment, 5 relapse) from 5 referral centres. Diagnosis was confirmed by standard morphology, immunophenotype and FISH and/or karyotype. FISH was performed using commercial probes (Abbott Molecular, UK and Cytocell, UK). aCGH was performed on DNA extracted from either peripheral blood, bone marrow aspirate, stored frozen cells or post-culture fixed chromosome preparations using a standard 8 x 60K Agilent array platform (SurePrint G3 Human CGH Microarray Kit, G4450A). Additional FISH analysis was performed to address questions raised by the microarray results when necessary. Clinical data was collected retrospectively.
Results: Of 91 patients (males 73.4%, females 26.6%), data regarding treatment was available for 67 (73.6%). Of these, 54 (80.5%) required chemotherapy treatment. Of those treated, median time to treatment (TTT) was 34 months (range: 0-264 months). Median time from diagnosis to end of follow-up in the untreated group was 74 months (range: 30-432 months). The known prognostic markers assessed demonstrated: diagnostic lymphocyte count >30 x10^9/l (73.3%); CD38 positive (48.3%); ZAP70 positive (46.5%); IGHV unmutated (43.9%); β-2 microglobulin elevated (40%). Elevated lymphocyte count and ZAP70 positivity were statistically significant between treated and untreated groups with p values of 0.023 and 0.0043, respectively. The other markers showed trends towards a difference between treated and untreated groups, but did not have a p value<0.05. There was a strong inverse relationship (see Figure 1) between TTT and total genomic aberrations (TGA) observed in the treated group (p=0.0014). Using a cut-off of 2Mb, aCGH was able to delineate between small deletions (<2Mb) and large deletions (>2Mb) at chromosome 13q14.1. This difference cannot be delineated by standard FISH probes. Interestingly, of the untreated group, 7/13 (53.8%) had a small deletion, while 0/13 had a large deletion detected, compared with the treated group where 10/54 (18.5%) had a small deletion, while 16/54 (29.6%) had a large deletion. The difference here, where large deletions are seen in only the treated group, suggests the large deletions are associated with more aggressive disease. Another interesting observation was that by using aCGH, deletions involving Mitogen-Activated Protein Kinase Kinase 4 (MAP2K4) found at chromosome 17p, which has not yet been described in CLL, was observed in 5 patients, all in the treated group. Three of the 5 were associated with Tumor Protein P53 (TP53) found by FISH.

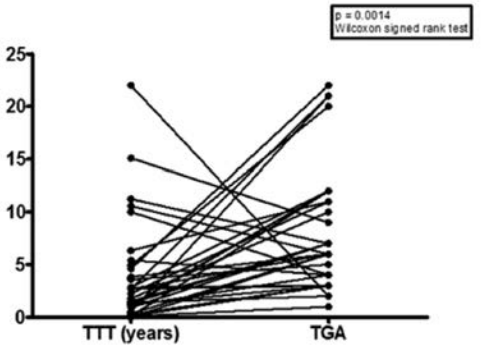

Figure 1. Relationship between Total Genomic Aberrations (TGA) and Time to Treatment (TTT).

Summary/Conclusions: The correlation between aCGH and clinical data demonstrates the utility of aCGH beyond that of standard FISH. Increased TGA is significantly associated with shorter TTT from diagnosis. Detecting the difference between a deletion of 13q14.1 involving >2Mb (large) compared with<2Mb (small) by aCGH, which cannot be differentiated by standard FISH probes, may be useful in predicting patients that will need to be treated. MAP2K4 may be an important marker in CLL in predicting patients that may need treatment, but will need further investigation.

E1075

RISK FACTORS AND PROFILE OF INFECTIONS ON IBRUTINIB THERAPY OUTSIDE CLINICAL TRIALS: A SINGLE CENTER EXPERIENCE OF 68 PATIENTS

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Background: Ibrutinib is a first in class oral inhibitor of Bruton's Tyrosin Kinase, a protein critical for the B-cell receptor signaling cascade, but with significant activity also against NK, T cell, and macrophage signaling pathway downstream other receptors. High response rates in relapse/refractory chronic lymphoid leukemia (CLL), mantle cell lymphoma (MCL) and Waldenström's disease have granted ibrutinib a global market approval in these lymphomas. In the context of various clinical trials, investigators reported 24% of grade ≥3 infections, more frequently bacterial.
Aims: In this study, we aimed to assess in "real life" the profile of infections related to this targeted therapy, and identify risk factors.
Methods: Between March 01, 2014 and January 15, 2016, we included all patients starting ibrutinib (and exposed for >1 month), referred to the Toulouse University Hospital Cancer Center (IUC-Toulouse Oncopole).
Results: We enrolled 68 patients with a median age of 69.7 years, 76.5% of the patients with CLL and 23.5% with MCL. Forty-three infectious adverse events (IAE) occurred in 30/68 patients (44.1%), including 18/43 (41,9%) grade 1-2, 21/43 (48,8%) grade 3-4 and 4/43 (9,3%) grade 5. Median time to IAE onset was 2,9±1,7 months, 33/43 (76,7%) being observed the first 6 months of therapy, suggesting recovery of the normal immune system with CLL improvement. Among 43 IEA, 23 were of unknown origin (treated with wide spectrum antibiotics, most therefore of bacterial origin), and 20 IAE were documented: 13 bacterial (including 2 Campylobacter *jejunii* and 3 Pseudomonas *aeruginosa*), 5 fungal (2 invasive aspergillosis, 1 mucormycosis, 1 pneumocystosis and 1 disseminated cryptococcosis) and 2 viral (hepatitis E virus reactivations). In accordance with published results from clinical trials (Maddocks *et al.*, JAMA Oncol 2015), PFS was significantly shorter in those patients with onset of any grade IAE during the first 3 months of therapy (median PFS 8mo *vs* NR, p=0.02), or with onset of severe IAE grade ≥3 (median PFS 10mo *vs* NR, p=0.02). The baseline characteristics of the patients were well balanced between the two study groups (infected and non infected) regarding comorbidities, history of previous infections, number of previous lines of therapy (median of 3), or primary prophylaxis (cotrimoxazole+/-valaciclovir: 40% of patients with IAE *vs* 50% of patients without). There was no clinical or biological parameter before commencing ibrutinib correlated to IAE risk (median neutrophil and lymphocyte count, CD4/8 T lymphocyte count and gammaglobulin levels). However, peak lymphocytosis induced by ibrutinib at month 1 and 2 was correlated to IAE risk (median 126843/ml *vs* 69409/ml after 1 month, 100238/ml *vs* 63485/ml after 2 months, Mann-Whitney test p<0,05 for both).
Summary/Conclusions: In this real-life practice series, 44% of patients have developed IAE of which 50% were severe (grade ≥3) including 13% fungal infection. As compared to clinical trials, we confirmed the significant correlation of early-onset IAE with PFS, and that IEA risk decreases with time (even after 3 months), without disappearing over time (10% IAE occuring >6 months). Interestingly, and consistent with an improvement of normal immune responses linked to the desinfiltration of lymph nodes/spleen/bone marrow, ibrutinib-induced lymphocytosis was associated with a lower incidence of infection.

E1076

IMPACT OF ANTI-CD20 ANTIBODIES ON THE INCIDENCE OF SECONDARY CANCERS IN PATIENTS TREATED FOR CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: An increased incidence of second primary malignancies (SPM) is described in chronic lymphocytic leukemia (CLL). Current hypothesis to explain this phenomenon mainly relies on immunosuppression conferred by both CLL itself and CLL treatment. The imputability of fludarabine has been long recognized but the risk associated with monoclonal antibodies, especially rituximab recently shown to improve overall survival in CLL when combined with fludarabine and cyclophosphamide (FC), remains unknown.
Aims: The objective of this study was to investigate the impact of anti-CD20 antibodies on the incidence of secondary cancers in patiens treated for chronic lymphocytic leukemia.
Methods: We conducted a retrospective study of the incidence of secondary cancers in 1255 CLL patients diagnosed since 1980 in the University Hospital of Lille to better characterize the possible risk of SPM associated with rituximab.
Results: Of 1255 patients, 651 (52%) received therapy including rituximab (38%), FC (26.7%), F alone (22.4%), chlorambucil (27.5%), alemtuzumab (15.5%) and bendamustine (9.3%). Rituximab was given either in combination with FC (27.5%), other chemotherapy (2.6%) or as a single-agent (3.5%). There was no significant difference in terms of age (58 *versus* 62 years), gender, Binet stage, cytogenetic abnormalities and number of regimen received between patients treated with or without rituximab. Median follow-up was 6 years for all patients (range 2-23), 4.8 years (range, 2-8) since initiation of therapy for patients treated without rituximab and 3.6 years (range, 0.2-11) for patients who received rituximab. Median overall survival (OS) was 18 years for patients treated with R-chemotherapy *versus* 11 years for patients treated with chemotherapy alone (p<0,001). Of 1255 patients, 21.5% were diagnosed with SPM. The incidence of SPM was 17.1% in patients who did not receive treatment compared to 10% in those treated. Among treated patients, the incidence

of SPM was significantly higher (19% v 2%) in patients who received rituximab (p<0.001). SPM incidence was increased after R-FC (24.4%), FC (10.5%) compared to other regimen (p<0,001). Most frequent SPM were skin (25%)-and urologic cancers (23%). Median onset of SPM was 5 years (range, 2-20) without rituximab and 2 years (range, 1-7) with rituximab.
Summary/Conclusions: In this large single center retrospective study, we found an earlier and significantly increased incidence of SPM, mainly skin cancers, in CLL patients treated with R-chemotherapy compared to those given chemotherapy alone. It remains to be determined whether this increased SPM incidence is a due to a direct toxicity of rituximab or merely a collateral consequence of improved OS observed after rituximab.

E1077

BACH2 AND BCL6 COOPERATIVELY FUNCTION AS TUMOUR SUPPRESSORS IN CLL

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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults in the Western World. Aberrant germinal center (GC) reactions could be a triggering factor in CLL, potentially contributing to its pathogenesis. The transcriptional repressors BCL6 (B-Cell CLL/Lymphoma 6) and BACH2 (BTB and CNC Homology 1, Basic Leucine Zipper Transcription Factor 2) are crucial regulators of GC B-cell fate. The double heterozygous $Bcl6^{-/+}$ $Bach2^{-/+}$ mice exhibit profound reduction in GC formation in response to T-cell dependent antigen immunization. In GC B cells, the stability of BACH2 protein is regulated by BCL6 and 30% of BACH2 DNA binding sites overlap with BCL6 binding sites. Both proteins are upregulated and interaction between BACH2 and BCL6 represses transcription of PRDM1 (PR Domain Containing 1, With ZNF Domain), a key driver of plasma cell differentiation.
Aims: The aim of the study is to determine the role of these two critical GC B-cells regulators, BCL6 and BACH2 in CLL and investigate whether there is a functional collaboration between them in CLL using a patient sample cohort comprising 102 patients.
Methods: We have analyzed 102 CLL samples using TaqMan quantitative real-time PCR (qPCR) and calculated the relative expression by the $2^{-\Delta\Delta Ct}$ method. A ROC curve was generated to identify the optimal diagnostic cut-off. The expression was then split at this cut-off and the resulting dichotomous variable was included in a survival analysis.
Results: We observed different levels of *BCL6* and *BACH2* expression throughout the CLL cases analysed. Median RQ (Relative Quantity) was 32.47 (range 4.04-153.29) for *BCL6* and 232.62 (range 16.50-1132.30) for *BACH2*. Interestingly, CLL cases expressing low levels of *BCL6* and *BACH2* mRNA had significantly shorter overall survival (OS) than high *BCL6* and *BACH2*-expressing samples. Moreover, the low expression of *BACH2* and *BCL6* specifically decreased OS of CLL patients with mutated IgV_H and 11q and 13q deletions. *BACH2* mRNA expression negatively correlates to the expression of CD38 (Pearson Correlation coefficient=-.418, p=.011), a poor prognostic factor in CLL. Finally, we identified a positive correlation between BCL6 and BACH2 expression (Pearson correlation coefficient =.335, p=.001), suggesting that these two factors could act synergistically in CLL. We also assessed the impact of *BACH2* expression in combination with other variables that are known to be relevant for CLL prognosis (age and 17p deletion) and we identified a prognostic index (PI=2.6). According to this model, patients with a PI<= 2.6 have a shorter OS compared to patients with a PI >2.6 (p=0.004).
Summary/Conclusions: Taken together, our study shows the prognostic role of BCL6 and BACH2 in CLL and the possibility of increased B cell receptor signalling due to the lack of Bcl6 and BACH2.

E1078

IMPORTANCE OF PHARMACIST CONSULTATION TO PREDICT EARLY TOXICITIES WITH IBRUTINIB AND IDELALISIB: A SURVEY FROM 119 PATIENTS TREATED IN A SINGLE INSTITUTION

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Background: Ibrutinib (IB) and idelalisib (ID) have changed treatment paradigms in the management of R/R B-cell malignancies but their safety profile in the real-world setting is ill-defined.
Aims: Here, we report on the impact of drug-drug interactions (DDI) on early toxicity in a series of 119 patients (28 prospectively, 91 retrospectively evaluated) with CLL, mantle cell (MCL) and follicular lymphoma (FL), treated outside the context of clinical trials.
Methods: Consecutive IB/ID pts who initiated therapy off-protocol at Institut Universitaire du Cancer (IUC) Toulouse-Oncopole from Apr2014 to Dec2015 were included (given they had been exposed to drug at least 1mo). A medication review for potential DDI was performed by a clinical pharmacist on all files. Baseline characteristics, concomitant medications (COMED), and toxicities were recorded. Toxicity at month 1 (TOXM1) was defined by any grade 3-4, or grade 2 but leading to dose reductions due to impact on quality of life (always on patients' demand).
Results: 67 IB pts (52 CLL, 12 MCL) and 52 ID pts (39 CLL, 13 FL) were assessed (all with a median of 3 previous lines of therapy). *Ibrutinib*: median age was 68.6y, with a median number of 3.6 COMED (range 0-14, 30% with COMED>5, defining polypharmacy). Fifty-five% were taking substrates of CYP3A4/Pgp, 21% inhibitors of Pgp, 16% and 25% inhibitors of CYP2D6 and 3A4, respectively. No dose reduction was allowed at the initiation of therapy. TOXM1 was reported in 22/67 pts (32.8%), with only 5/22 grade 3-4 toxicities, and 17/22 grade 2 impacting QoL (mostly cramps, diarrhea, dyspepsia, rash). TOXM1 was not correlated to type of lymphoma, previous therapies, gender, age, COMED>5, but correlated statistically to Pgp inhibitors (52.4% *vs* 23.9%, p=0.02) or CYP3A4 inhibitors (57% *vs* 26.4%, p=0.03) concomitant drug intake, as could be expected from pharmacokinetics profile of ibrutinib. PFS was not impacted by dose reduction. *Idelalisib*: median age was 74y, with a median number of 4.2 COMED (range 0-12, 41% with COMED>5). Sixty-five% were taking substrates of CYP3A4/Pgp, 36% inhibitors of Pgp, 0% and 25% inhibitors of CYP2D6 and 3A4, respectively. TOXM1 was reported in 15/52 pts (28.8%), but due exclusively to grade 3-4 events: neutropenia (23%), mucositis (4%), diarrhea/transaminitis (2% each). TOXM1 was not correlated to type of lymphoma, age, number or type of COMED, but to female gender (50% *vs* 14%, p=0.01). Aldehyde oxydase activity (the main pathway of ID metabolism) is poorly defined in humans, but reduced in female rats. When looking at the first 3 mo of ID therapy, women had an increased frequency of neutropenic (39% *vs* 14.44%) and diarrhea (16.7% *vs* 2.9%) events of any grade, but less transaminitis events(5.5% *vs* 9%), as compared to men. TOXM1 led to dose reductions after a month of ID in 44.5% and 11.2% of female/male pts, respectively (impacting PFS). At 6mo follow-up, 38.5% of patients were still on therapy (most discontinuations due to grade 3-4 AE).
Summary/Conclusions: DDI were found to impact early toxicities with IB but not with ID, emphasizing the urgent need of better PK/toxicity evaluation for both drugs in real-life practice. Such prospective evaluation is just starting in our institution (protocol PK-E3i) to better monitor plasma levels (and adequately adapt drug dosage), and better support patients' compliance in the long-term.

E1079

IBRUTINIB FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): IMPACT OF THE YOU&I™ PATIENT SUPPORT PROGRAM ON TREATMENT ADHERENCE

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Background: Oral therapies for cancer can present several advantages over intravenous drugs, including greater flexibility and convenience for the patient and reduced healthcare costs. However, for treatment to be effective, a high degree of adherence must be maintained. Prior studies have shown a range of non-adherence to long-term oral anticancer therapy, with adherence rates varying considerably with patient characteristics. Consequences of low adherence can include increased healthcare costs and inferior patient outcomes, including decreased time to relapse and decreased survival. The YOU&i™ Patient Support Program (PSP) supports patients with reimbursement services and provides patients and their physicians with a comprehensive nurse-coaching program in an effort to encourage treatment adherence.
Aims: To examine patient satisfaction and adherence to ibrutinib within the YOU&i™ PSP.
Methods: Using evidence-based literature reviews and global/local market research, barriers to treatment adherence were identified. These included a lack of disease/treatment education, high financial burden, negative psychosocial/motivational factors, poor medication routine-building, and suboptimal patient:physician communication. Each patient's risk for non-adherence was calculated using the Morisky Medication Adherence Scale© score and the total number of barriers identified at initial assessment, and the frequency of nurse-coaching calls was adjusted accordingly. Patients were categorized as "adherent" if they had ≥85% compliance (obtained refills within 4 days of expected refill date) and as "discontinued" if ibrutinib was not dispensed for ≥3 months, if the patient was confirmed as discontinued prior to ibrutinib not being dispensed for ≥3 months, or if the patient was confirmed as deceased. All other patients were categorized as "partially adherent". Patient questionnaires were used to gauge satisfaction with the YOU&i™ PSP.
Results: As of January 19, 2016, a total of 903 CLL patients were enrolled in the PSP; 87% of patients opted in for nurse coaching. Most patients (58%) had received 1-2 prior lines of therapy. At 9 months from treatment initiation, 81% of patients who received nurse coaching *vs* 59% of those who did not were categorized as "adherent" (excludes patients who discontinued due to disease progression or death). Similarly, only 7% of those who received nurse

coaching *vs* 23% of those who did not were discontinued from ibrutinib for reasons other than disease progression or death. In the overall PSP population, 17% discontinued the program; among the patients discontinuing, the most common reason was death (49%). Overall, 91% of patients reported that they were satisfied/very satisfied with the PSP, with 87% likely to recommend the program. Furthermore, 78% reported that the PSP was very helpful in supporting their adherence to ibrutinib.
Summary/Conclusions: Although there is an inherent potential for self-selection bias, these results from the YOU&i™ PSP suggest that nurse coaching may contribute to improved patient adherence to oral anticancer treatment. In the YOU&i™ PSP, nurse coaching appeared to also be associated with decreased treatment discontinuation. This program, or similar supportive programs, may be helpful in assisting patients in maintaining adherence to treatment with ibrutinib.

E1080

INCIDENCE OF AND RISK FACTORS FOR MAJOR HEMORRHAGE IN US VETERANS ADMINISTRATION PATIENTS WITH NEWLY DIAGNOSED CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Patients with chronic lymphocytic leukemia (CLL) have an increased risk for major hemorrhage (MH) compared to the age- and gender-matched general population (Gifkins et al, Blood, 2015). However, the specific risk factors associated with bleeding among patients with CLL are not well established.
Aims: Our objective was to estimate the incidence of MH among patients newly diagnosed with CLL receiving care at US Veterans Administration (VA) healthcare facilities and to identify risk factors associated with MH in these patients.
Methods: Patients diagnosed with CLL from 1999 through 2013 were identified using ICD-9 codes from VA electronic medical records. Those with<6 months of VA care before CLL diagnosis were excluded from analysis to capture a newly diagnosed patient cohort. Follow-up was from initial CLL diagnosis until death, 12/31/2013, or MH onset, whichever occurred first. We defined an MH event as a diagnosis code of bleeding in a critical area or organ or bleeding that was treated with blood transfusion within 7 days. We calculated the incidence rate of MH after CLL diagnosis. Potential risk factors included demographic characteristics and selected medical history based on ICD-9 codes within 6 months before the first CLL diagnostic code. We performed univariate comparisons and a backward stepwise Cox proportional hazards regression analysis.
Results: Of the 24,581 veterans with newly diagnosed CLL, 24,166 (98.3%) were male, 20,464 (83.3%) were white; median age at diagnosis was 72.0 years; 2,013 (8.2%) experienced MH after CLL diagnosis, with a median time of 2.6 years between diagnosis and MH event. The incidence of MH was 16.4 per 1,000 person-years (95% CI, 15.7-17.1/1000). Statistically significant (P<0.05) risk factors for MH were (HR; 95% CI) history of MH (4.0; 3.4-4.7), anemia (2.6; 2.0-3.4), stroke (2.0; 1.2-3.5), male (2.0; 1.3-3.0), African American (1.7 *vs* white race; 1.5-2.0), uncontrolled hypertension (1.7; 1.3-2.2), atrial fibrillation (1.5; 1.2-1.7), CAD (1.3; 1.1-1.4), and age >75 years (1.1 *vs*<65 years; 1.0-1.3).
Summary/Conclusions: The incidence of MH in newly diagnosed CLL patients was 16.4 per 1,000 person-years. Risk factors most strongly associated with MH after CLL diagnosis are history of MH, anemia, stroke, and being male.

E1081

MALE GENDER IS ASSOCIATED WITH AN IMPAIRED OVERALL AND PROGRESSION FREE SURVIVAL IN PATIENTS WITH PROGNOSTIC LOW RISK CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Recently the new International Prognostic Index for Chronic Lymphocytic Leukemia (CLL) patients (CLL-IPI) was developed (Bahlo J, et al: The International Prognostic Index For Patients With Chronic Lymphocytic Leukaemia (CLL-IPI). ICML, Lugano, 2015). The index does not include gender as a weighted factor. However, previous analyses of Danish CLL patients indicate gender specific variations in Overall Survival (da Cunha-Bang C, et al: Improved Survival for Patients with CLL in the Era of Combination Chemoimmunotherapy - a Danish Population Based Study. Blood 126:1740-1740, 2015).
Aims: To analyze gender-specific outcome for patients following diagnosis with CLL for different CLL-IPI risk groups based on the Danish CLL-database.
Methods: All patients diagnosed with CLL in Denmark were registered and prospectively followed in the Danish National CLL database (2008-2015). The present analysis included all patients for whom all five factors included in the CLL-IPI score were registered (del(17p/TP53, IGHV mutation status, beta-2-microglobulin, Binet/Rai stage, age). Overall survival (OS), treatment free survival (TFS) and progression free survival (PFS) were calculated using Kaplan Meier and multivariate Cox regression models adjusted for relevant confounders.
Results: In total, 1541 patients were included. Analyzing the entire population, no difference in OS was found based on gender. However, analysis of the 1314 (778 (59%) male, 536 (41%) female) patients from the low and intermediate risk CLL-IPI score groups showed an impaired OS ((Hazard ratio (HR (95%CI)) for death; 1.7 (1.2-2.3) P=0.002)) for male *versus* female patients. No variation in TFS was found. However, following treatment male patients had a significantly impaired PFS (HR for progression; 3.0 (1.5-6.2) P=0.003)) *versus* female patients.
Summary/Conclusions: Male CLL patients in low and intermediate CLL-IPI risk groups have a higher risk of dying than their female counterparts. The increased risk of dying from non-CLL related causes for male patients may explain this difference, which is overruled by the increased risk of dying from CLL among high and very high CLL-IPI risk groups. Following CLL treatment the gender disadvantage is apparently accentuated. Focus on reducing pretreatment risk factors for male CLL patients may improve survival in this population. Further analyses of cause of death following treatment in male CLL patients are warranted.

E1082

BENDAMUSTINE PLUS RITUXIMAB IN 65 YEAR AND ABOVE UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background: Frontline Chemoimmunotherapy with Bendamustine and Rituximab (BR) in Previously Untreated and Physically Fit Patients (pts) with Advanced Chronic Lymphocytic Leukemia (CLL) has been compared against the standard Fludarabine, Cyclophosphamide, and Rituximab (FCR) and suggested as an option for elderly pts *(Eichhorst el al ASH 2014).* There is a lack of data regarding BR as CLL front line in routine clinical practice.
Aims: The aim of this study is to evaluate the toxic profile and effectiveness of BR as front line in 65 yr and above CLL patients in daily practice and to identify risk factors.
Methods: We performed the analysis of the 65yr and above patients included in the *MDA-LLC-2015-02* study, which consisted of a retrospective study CLL patients from 12 Spanish sites treated with BR as front line (B 90 mg/m2 days 1 and 2 every 28 days and R 375 mg/m2 on day 1 of the first cycle and 500 mg/m2 on day 1 of the other cycles). We evaluate effectives as IWCLL, toxicity as *CTCAE v4.0 of NCI,* overall survival *(OS)* and progression free survival *(PFS)* (Kaplan-Meier). This study has been approved by the Spanish Medicines Agency *AEMPS.*
Results: Sixty two (male 46 pt/female 16 pt) out of 85 pt included in MDA-LLC-2015-02 study were 65 or older, median age 72 (65-84). Risk factors as follows, ECOG≥2: 6 pt, high LDH: 25 pt, high β2 microglobulin: 50 pt, Binet C: 22 pt, Creatinine >1.3 mg/dL: 8 pt. Toxicity: A total of 316 cycles were administered, median 6 per patient, grade 4 neutropenia: 9pt, hospitalization do adverse events 13 pt, all febrile neutropenia. No cases of treatment related mortality. Efficacy: ORR 90.2%, with a median follow up 36 months 19 pt required second line therapy and 10 pt died, 2 because of progression. Median OS 56 months and median PFS 45 months (3 year OS 85.5%). ECOG≥2 (p0.002, p0.008) and grade 4 neutropenia (p0.017, p0.006) predict OS and PFS. High LDH, high β2 microglobulin, Binet C, Creatinine >1.3 mg/dL, trisomy 12, del11q, del13q did not predict OS neither PFS.
Summary/Conclusions: This results confirms BR as an effective (ORR 90.2% and PFS 45 months) and safe (no cases of treatment related mortality) as first lines for 65 year old and above CLL patients.

LB2252

PHASE 1 FINAL RESULTS AND PHASE 2A DOSE SELECTION FOR CERDULATINIB (PRT062070) A DUAL SYK/JAK INHIBITOR IN PATIENTS WITH RELAPSED/REFRACTORY B CELL MALIGNANCIES

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Background: Subsets of B cell lymphomas demonstrate a reliance on B-cell antigen receptor (BCR) and/or cytokine JAK/STAT signaling for survival. SYK is upstream of BTK, PI3Kδ, and PLCγ2 on the BCR pathway, making it a potential therapeutic target. Additional survival mechanisms are mediated by cytokine-induced JAK/STAT pathways, derived from the tumor or tumor infiltrating leukocytes, which leads to upregulation of BCL2 family members. In pre-clinical tumor models, cerdulatinib induces apoptosis in a genetically diverse panel of cell lines and primary B cell tumors in the 1 to 2 μM range.
Aims: The primary aim of the study was to establish the maximum tolerated dose of cerdulatinib.
Methods: This is a 3+3 dose escalation study with 28-day cycles. Once daily (QD; up to 100 mg) and twice daily (BID; up to 45 mg) dosing was evaluated. Pharmacokinetics (PK), pharmacodynamics (PD), and safety were monitored, as well as an initial assessment of efficacy. Clinical response was assessed by standard criteria. Potency and specificity for SYK and JAK pathway inhibition were measured in whole blood assays by monitoring signaling responses following ligation of the BCR and receptors for IL2, IL4, IL6, and GM-CSF. Serum markers of inflammation were also measured.
Results: 43 patients with CLL/SLL or B-cell NHL were enrolled on the completed phase 1. Median age is 67 years (range 23-85) and median prior therapies is 3 (range 1-8). Treatment related AEs ≥ grade 3 occurring in 2 or more patients with daily dosing were: fatigue (n=5), anemia and neutropenia (n=3 each), and abdominal pain, neutrophil count decrease, and pneumonia (n=2 each). A number of these events occurred in the setting of progressive disease. The highest overall exposure was achieved at the 45 mg BID dose, in which 2 DLTs occurred: grade 3 pancreatitis and grade 3 fatigue. Enrollment to this dose was discontinued, and the phase 2a dose was selected. In review of the 40-100 mg QD doses, the average SS C_{min} and C_{max} concentrations plateaued at 0.70±0.20 and 1.38±0.23 μM, respectively. QD dosing of 40-100 mg resulted in 50 to 100% (SS C_{min} to C_{max}) inhibition of SYK and JAK signaling in peripheral blood, and significant inhibition of serum markers of inflammation. The extent of inhibition of SYK and JAK signaling as well as inhibition of serum markers of inflammation significantly correlated with tumor response. While the PK is suitable for QD dosing with a $t_{1/2}$ of 12-16 hours and a 2:1 peak-trough ratio, the pH dependent low solubility of cerdulatinib limited dissolution, and physiologic modeling suggested that BID dosing would increase overall exposure. This was accomplished with the 45 mg BID dose, where complete inhibition of SYK and JAK at SS C_{min} (~1.5 μM) in peripheral blood assays was observed, consistent with an approximate doubling in exposure. Partial responses in phase 1 were observed in 5 heavily pretreated patients with CLL, FL, and transformed DLBCL at doses ranging from 30-65 mg QD. Two PRs were observed in the 45 mg BID dose group, one in a patient with FL and another with CLL. Responses typically occurred after 2 cycles of treatment. Multiple patients have demonstrated nodal reductions and maintained clinical benefit for over a year. Based on the PK/AE profile, there appeared to be higher grades of adverse events at SS C_{min} of 1.25 μM or greater. PK modeling indicated a dose of 35 mg BID would yield a SS C_{min} of 1.02 μM and a SS C_{ave} of 1.16 μM, which is predicted to be tolerable, efficacious, and achieve complete inhibition of SYK and JAK in peripheral blood. 35 mg BID was therefore selected as the phase 2a dose.
Summary/Conclusion: Cerdulatinib demonstrates clinical activity in heavily pretreated patients with CLL/B-cell NHL and is generally well tolerated. BID dosing has overcome the previous plateau in exposure and has enhanced PD effects. A phase 2a study is now open for CLL/SLL, FL/other indolent NHL and aggressive B-cell NHL at a dose of 35 mg BID.

Chronic myeloid leukemia - Biology

E1083

ABERRANT MECHANISMS OF TELOMERE MAINTENANCE IN CHRONIC MYELOID LEUKEMIA - THE POTENTIAL ROLE OF POT1

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Background: As in most cancers, a reduction in the telomere length is one of the features of chronic myeloid leukaemia (CML) and telomere shortening is correlated with disease progression from the chronic phase (CML-CP) to the blastic phase (CML-BP). Targeted therapy in CML with tyrosine kinase inhibitors (TKIs), such as imatinib, gives long-lasting remission in the majority of patients. However, significant proportion of patients may develop resistance and progress to advanced phases with limited therapeutic options. Majority of studies are focused on monitoring the telomere length during the progression of the disease. However the precise role of telomere-associated proteins, including shelterin complex in *BCR/ABL1*-mediated genomic instability in CML progression and resistance to TKIs have not been fully elucidated.
Aims: The aim of our study was to determine the role of the members of the shelterin complex in aberrant telomere maintenance mechanisms in CML progression with special focus on *POT1*.
Methods: We employed human *BCR-ABL1*-positive cells (K562), and *BCR-ABL1*-negative cells (HL60), as well as CD34+ primary cells isolated from peripheral blood leukocytes of CML patients at various stages of the disease (chronic phase, chronic phase TKI-resistant and blastic phase). Blood samples were taken after informed consent. The components of telomere complex were studied at the mRNA level (RT-qPCR) and protein level (Western blotting). The activity of telomerase was measured using ELISA. The mean TRF length was measured using the TeloTAGGG telomere length assay and individual telomere length using primed in-situ labelling technique. Global DNA damage was assessed using comet assay. ROS and RNS were analysed using fluorogenic probes.
Results: Initially, we confirmed with Southern blotting that the telomere shortening was positively correlated with CML progression (CML-CP in comparison to CML-BP). However, in samples from CD34+ CML-CP TKI-resistant patients in comparison to CML-CP patients, an increase in telomere length was observed. Dynamic changes in telomere length were neither associated with expression of TERT/TERC nor with enzymatic activity of telomerase in the course of the disease. Therefore, we decided to examine the potential role of shelterin complex in telomere maintenance in disease progression looking at the expression pattern of selected members of telomeric complex. We found that the expression of *POT1* was significantly upregulated in CML-BP as compared to CML-CP (Fig.1). Moreover, expression of *POT1* was positively correlated with *BCR-ABL1* expression. To verify the hypothesis that telomeres are elongated in TKI-resistant cells, imatinib-resistant K562 cells were used. We showed that telomeres in the resistant cells were significantly longer which was associated with differential expression of shelterin complex members, including *POT1*. These findings suggest that actual telomere length in disease progression may change in biphasic mode and may include initial telomere lengthening (possibly by alternative telomere lengthening mechanisms) in TKI-resistant cells. We also found that the changes in telomere *length* in TKI-resistant cells were accompanied by the genomic changes at chromosome level and oxidative/nitrosative stress

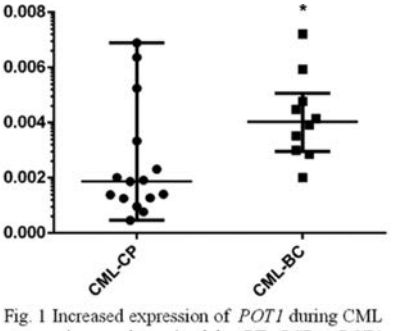

Fig. 1 Increased expression of *POT1* during CML progression as determined by RT-qPCR. *POT1* expression in samples from 15 CML-CP patients at diagnosis and 10 CML-BC patients. (*)P<0.05 (significance was tested using a non-parametric Mann-Whitney test).

Figure 1.

Summary/Conclusions: In conclusion, we postulate that abnormal expression of members of the shelterin complex, such as *POT1,* may be responsible for aberrant telomere maintenance mechanisms in CML cells and may play role in genomic instability associated with CML progression as well as the clonal selection and resistance to TKIs.

E1084

MONOCYTIC MYELOID DERIVED SUPPRESSOR CELLS (M-MDSC) AS PROGNOSTIC FACTOR IN DASATINIB TREATED PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Recently, we and another group demonstrated that Myeloid suppressor cells play an important role of immune escape in chronic myeloid leukemia (CML) patients.
Aims: Investigating the effect of the tyrosine kinase inhibitors (TKI) therapy on MDSC and possible correlation with clinical response.
Methods: CML patients at diagnosis (n=30) and during TKI treatment (n=43) were enrolled in this study. Eighteen patients were treated with imatinib (IM), 13 with nilotinib (NIL) and 12 with dasatinib (DAS). MDSCs were also analyzed in peripheral blood of 20 healthy donors (HD). Granulocytic MDSCs (G-MDSCs) were identified as CD11b+CD33+CD14-HLADR- cells while monocytic MDSCs (M-MDSCs) as CD14+HLADR by cytofluorimetric analysis. Their immunosuppressive activity was tested through incubation with autologous CFSE+T cells. Exosomes were isolated from CML serum at diagnosis (n=5) by sequential ultracentrifugation.
Results: G-MDSCs and M-MDSCs percentages in CML patients were greater than HD (respectively 82.5±9.6% *vs* 56.2±5.4% and 33.6±19% *vs* 5.9±4%, p<0.0001). Both isolated subpopulations showed expression of BCR/ABL and were able to inhibit T cells proliferation in comparison to positive control (from 48±7.6% to 25±5% for G-MDSC, p=0.0057 and 16.7±0.6% for M-MDSC, p<0.0001). No suppressive effect was observed in co-cultures with G-MDSC and M-MDSC obtained from HD. In addition, M-MDSC percentage correlated with BCR/ABL transcript levels in patients at diagnosis (r=0.579, p=0.0004). Evaluating the effect of TKI therapy on MDSC levels, we found that both IM, NIL and DAS induced a significant reduction of G-MDSC percentage at 6 months (from 82.5±9.6% to 55±17.3% after IM, to 60.9±9% after NIL and to 48.7±13% after DAS, p<0.0001) and 12 months (61.2±9.7% after IM, 61.4±6.7% after NIL and 33.4±14% after DAS, p<0.0001) of treatment. The levels of M-MDSCs significantly decreased only after DAS therapy (from 33.6±19% to 6.8±12.6% at 6 months, p=0.014 and 11.4±12.3% at 12 months, p=0.008). M-MDSC reduction was also present but did not reach statistical significance after IM treatment (22.2±24.5% and 22.3±21.7% respectively at 6 and 12 months) and after NIL therapy (21±19.9% and 17.4±16.3% at 6 and 12 months) with a great variability among patients. Subsequently, correlation of MDSC with clinical response to TKI therapy was investigated. We found that in DAS, but not in IM or NIL treated patients, a correlation between percentage of Major Molecular Response (MMR) and number of persistent M-MDSCs was found. A significant difference was calculated comparing M-MDSC levels in the MMR group (n=6) *versus* no MMR (n=6) at 12 months (p=0.008). To evaluate if leukemic cells are able to expand MDSC releasing soluble factors or exosomes, we incubated monocytes obtained from HD with sera or exosomes from CML patients at diagnosis or healthy subjects. M-MDSCs percentage significantly increased only in conditions with CML serum (29±13%; p=0.0006) or exosomes (8±2.8%; p=0.01). No effect was observed on G-MDSC percentage.
Summary/Conclusions: Therapy with TKI reduces the percentage of MDSCs and levels of the monocytic subset correlates with MMR value in patients treated with dasatinib, suggesting their importance in clinical investigation as prognostic factor. Moreover, our data suggest the possible development in CML patients of a circuit primed by tumor cells that, through the release of soluble factors and exosomes, are able to expand M-MDSCs, creating an immunotolerant environment that results in T cell anergy and facilitates tumor growth.

E1085

THE USE OF THE EAC PRIMERS IN DIGITAL PCR FOR MONITORING MINIMAL RESIDUAL DISEASE IN CML RESULTS IN A SHIFT BETWEEN MOLECULAR RESPONSE GROUPS.

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Background: Digital PCR (dPCR) generates an absolute read out that is largely robust to variations in PCR efficiency and should reduce the requirement for standardisation by laboratory-specific conversion factors.
Aims: The aim of this study was to compare the results of dPCR to qPCR (quantitative PCR) in blinded samples from two independent laboratories with respect to the observed rates of molecular response (MR) in CML patients (pts) having undergone 18 months of nilotinib treatment in the ENEST1st trial (ClinicalTrials.gov:NCT01061177).
Methods: A total of 230 cDNA samples from CML pts with e13 or e14/a2 BCR-ABL fusion genes who were treated within the ENEST1st trial between 2012 and 2013 were analysed in Leipzig (L, n=75) or Mannheim (M, n=155) with qPCR. BCR-ABL levels were determined relative to those of ABL and standardization was achieved using plasmid DNA. Both labs are accredited by the European Treatment and Outcome Study (EUTOS) collaboration. The cDNA samples were blinded for the qPCR results and re-analysed in L with a duplex dPCR using a QX200 Droplet Digital PCR System (BIO-RAD). In line with the manufacturer's recommendations, duplicate samples yielding a minimum of 3 positive droplets from the 12-19.000 routinely analysed droplets were scored as positive. Depth of MR was scored using the EUTOS definitions used in the ENEST1st trial.
Results: A previous comparison between dPCR and qPCR showed significant differences in the distribution of the molecular response (MR) categories (p=0.02) with more residual disease (RD) measured by dPCR. In detail, significantly fewer patients achieved deep molecular responses (MR4 to MR5) by dPCR (p=0.035). Of the 230 samples analysed by the two methods, 58% (n=134) were classified in the same MR category, while 11% (n=25) skipped to classes with less RD and 31% (n=71) moved to classes with more RD by dPCR, mostly over a range of 1-2 classes. ABL copies in non-concordant samples were median 1.1 fold higher by dPCR than by qPCR, while BCR-ABL transcripts were 3 fold higher by dPCR in 56/71 samples analysed, resulting in significantly more patients having more RD by dPCR. Experimental tests of the sensitivity between 0.25 and 25 BCR-ABL copies showed a LOQ (limit of quantitation) of 3 positive droplets in duplicates (cut-off 3). Interestingly, application of the laboratory-specific qPCR conversion factor to the dPCR data (cut-off 3) independently reduced the difference in distribution categories between the qPCR and dPCR to below significance (Table 1).

Table 1. Distribution of MR classes.

MR class dPCR versus qPCR	dPCR with Cut-off 3 for positivity	dPCR multiplied by CF qPCR
Concordance [%]	58	61
Less RD [%]	11	16
More RD [%]	31	23
X-fold more RD/less RD	2.8	1.4

Summary/Conclusions: The use of M-BCR-ABL primers according to the EAC (Europe Against Cancer) protocol in dPCR with a cut-off of 3 droplets results in a shift of molecular response categories towards more minimal RD by dPCR than by qPCR. This difference is reduced to below significance by application of the laboratory specific qPCR conversion factor to the dPCR data.

E1086

PRE-EXISTING SOMATIC MUTATIONS IN GENES COMMONLY MUTATED IN MYELOID MALIGNANCIES (RUNX1, DNMT3A, ASXL1) MAY CONFER BAD PROGNOSIS IN CHRONIC MYELOID LEUKEMIA

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Background: Chronic Myeloid Leukemia is readily manageable disease for majority of patients. However, a subset of patients either never achieve or lose response to tyrosine kinase inhibitors (TKIs) and progress from chronic (CML-CP) to blastic phase (CML-BP). The genetic basis of this transition remains only partially understood, particularly when loss of TKI sensitivity cannot be attributed solely to *BCR-ABL1* mutations.
Aims: We sought to determine the genetic background of CML progression in patients who experienced TKI treatment failure not associated with *BCR-ABL1* mutations using targeted enrichment and next-generation sequencing strategy.
Methods: Sequential DNA samples were collected from 9 patients who were diagnosed in CML-CP and progressed to CML-BP (8 myeloid, 1 lymphoid). SeqCapEZ Choice (NimbleGen) enrichment was used to capture coding sequences of 952 genes (7Mb) implicated in human cancer (with focus on hematological malignancies), followed by high-throughput sequencing on Illumina HiSeq 1500 (>90% ge20, >100x mean coverage).
Results: In 7 patients we observed variants in *RUNX1*, *DNMT3A*, *ASLX1*, *PTPN11* and *IDH1* genes (table), all of which were considered as damaging and located in previously known hotspots (except for novel *DNMT3A* mutations).

Almost all variants could be detected at CML-CP at varying allele frequency, thus preceding diagnosis of CML progression.

Table 1.

Patient	Mutation(s)	NCBI Reference	Pre-existing in CML-CP?	Allele frequency CML-CP	Allele frequency CML-BP(s)	CML-BP type
1	**RUNX1** R230*	NM_001754.4	Yes	1%	39% | 46% | 18%	lymphoid
2	**IDH1** R132S	NM_005896.3	Yes	8%	52%	myeloid
3	**RUNX1** R166L	NM_001754.4	No	0%	34%	myeloid
	E202_P203ins	NM_001754.4	No	0%	18%	
4	**DNMT3A** Y481*	NM_175629.2	Yes	52%	54%	myeloid
	PTPN11 A72V	NM_002834.3	No	0%	45%	
5	**RUNX1** E316_L317fs	NM_001754.4	Yes	17%	18%	myeloid
6	**DNMT3A** L798F	NM_175629.2	Yes	50%	57%	myeloid
	ASXL1 G643_G644fs	NM_015338.5	Yes	21%	40%	
7	**ASXL1** G626_A627fs	NM_015338.5	Yes	39%	22%	myeloid

Summary/Conclusions: Our results suggest that in rare, refractory CML cases mutations in genes frequently mutated in myeloid neoplasms might cause or contribute to an aggressive CML phenotype. Early detection of such genetic lesions might be important for patients who will not achieve remission with currently available standard treatment regimens and who should be considered as high-risk patients.

E1087

EVOLUTION OF RUNX1 AND ASXL1 MUTATIONS DURING THE PROGRESSION OF CHRONIC MYELOID LEUKEMIA TO MYELOID BLAST PHASE: AN ANALYSIS OF 52 MATCHED PAIRED SAMPLES AT BOTH INITIAL DIAGNOSIS AND BLAST PHASE

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Background: Before imatinib era, most patients with Ph-positive chronic myeloid leukemia (CML) diagnosed at chronic phase (CP) or accelerated phase (AP) will transform to blast phase (BP) compared with a small number of patients receiving imatinib therapy. The biology of CML-BP is still largely unknown. Data from direct comparison of matched samples between both diagnosis and BP was limited.
Aims: We aimed to determine the role of *RUNX1* and *ASXL1* mutations in addition to *BCR-ABL1* kinase domain (*ABL1*-KD) mutations during the progression of CML in a larger cohort of matched paired samples in CP/AP and myeloid BP.
Methods: Bone marrow samples from 52 patients with CML at initial diagnosis (47 CP and 5 AP) and myeloid BP transformation were enrolled. Nineteen patients received imatinib therapy before BP transformation (group A) and the remaining 33 patients had not been treated with imatinib (group B). Semi-nested polymerase chain reaction (PCR) assay followed by denaturing high-performance liquid chromatography and/or direct sequencing was used to detect *ABL1*-KD mutations. *RUNX1* (exons 3-8) and *ASXL1* (exon 12) mutations were analyzed by PCR-based assays and direct sequencing. Patients with *ABL1*-KD, *RUNX1,* or *ASXL1* mutations in myeloid BP were subjected to the analyses of the corresponding genes in the matched diagnosis samples. Pyrosequencing was used to measure the mutant levels.
Results: The median age of patients at the time of CML diagnosis was 45 (16-84) years. The median time from CP/AP to myeloid BP was 26 (1.9-162.9) months. Eight (42.1%, 8/19) of group A patients had *ABL1*-KD mutations at BP compared with none in group B patients (*P*=0.0003). None of the 8 patients carrying *ABL1*-KD mutations at BP had *ABL1*-KD mutations detected in the diagnostic samples. Ten (19.2%, 10/52) patients in myeloid BP had *RUNX1* mutations, but none of them had *RUNX1* mutation in the corresponding diagnostic samples. Only one of the 10 patients with *RUNX1* mutations in myeloid BP had *ABL1*-KD mutations. Two patients (10.5%, 2/19) acquired *RUNX1* mutations at BP in group A compared with 24.2% (8/33) in group B (*P*=0.227). *ASXL1* mutation was detected in 6 (11.8%, 6/51) samples in myeloid BP, of which only one was positive for *ASXL1* mutation in the diagnostic sample which was in AP. All the 5 patients who acquired *ASXL1* mutations did not have *ABL1*-KD mutation in myeloid BP. Acquisition of *ASXL1* mutations in myeloid BP was 0% (0/18) and 18.2% (6/33) in group A and group B, respectively (*P*=0.054). Together, 3 patients had co-existence of *RUNX1* and *ASXL1* mutations at myeloid BP. Emergence of *ABL1*-KD mutations did not significantly affect the acquisition of *RUNX1* or *ASXL1* mutations during myeloid BP transformation.
Summary/Conclusions: *RUNX1, ASXL1 or ABL1*-KD mutations were not detected in CML patients at initial diagnosis except one who presented with AP. Acquisition of *RUNX1* and/or *ASXL1* mutations occurred in 25.5% (13/51) of patients during myeloid BP transformation. *ABL1*-KD mutations only occurred in patients treated with Imatinib, and *ASXL1* mutations were prone to develop in patients not treated with Imatinib. (Grant support: XMRPG360366 and OMRPG3C0021).

E1088

NEXT-GENERATION SEQUENCING OF AMPLIFIED DNA FRAGMENTS WITH LENGTH POLYMORPHISMS ASSOCIATED WITH CHRONIC MYELOID LEUKEMIA REVEALED POLYMORPHISMS IN REPETITIVE ELEMENTS AND CANCER RISK LOCI

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Background: A genetic predisposition and molecular mechanisms of chronic myeloid leukemia development (CML) and of resistance to the treatment with tyrosine kinase inhibitors (TKI) is the matter of the research worldwide. High-throughput techniques such as next generation sequencing (NGS) enable whole-genome analysis per each individual, however, often with limitations in a large volume of obtained data requiring extensive bioinformatics analysis.
Aims: We created an approach to scan the individual CML genomes using the combination of highly reproducible amplified fragment length polymorphism (AFLP) analysis and of NGS in order to identify polymorphic loci associated with CML or response to imatinib (IM).
Methods: AFLP was performed on 65 CML patients treated with IM first line (39 pts with optimal response; 26 pts with IM failure) and 30 healthy donors; for confirmatory analysis next 30 controls were added. The median age of patients was 55 years (range 18-84). AFLP analyses were performed using MseI and EcoRI endonucleases and commercially available kits and protocols. Fragmentation was run on ABI PRISM 3130 sequencer. The lengths of DNA fragments were analyzed with GeneMapper. DNA fragment frequency among CML patients and controls were analyzed by the exact binomial test. Nine selected DNA fragments were further characterized by NGS and the obtained data were evaluated using NextGENe. Polymorphisms in identified DNA fragments were confirmed by Sanger sequencing.
Results: AFLP generated 3912 AFLP markers/DNA fragments with diverse distribution among CML patients and healthy controls. Among them 199 were significantly associated with CML and 5 with IM response. Using NGS, we characterized 7 loci associated with CML and 2 with response to IM. In CAC_ACC_25, we confirmed SNP rs113864098 (C/T) resulting in an introduction of EcoRI restriction site. The locus is on chromosome 1 and is a part of L1MC1 and AluSz repetition. The significantly higher frequency of rs113864098-T allele was found in the patient cohort (60%) in comparison to healthy donors (13%) (P=0.016). SNP rs7906704 (G/T) was confirmed in the CAT_ACC_54 fragment localized on chromosome 10 between NRAP and CASP7 genes resulting in the introduction of MseI restriction site in this locus. The frequency of minor rs7906704-T allele was significantly higher in CML patients (38%; P=0.04) in comparison to healthy controls. This SNP was found to be involved in apoptosis pathway in pancreatic tumorigenesis (Li et al., 2012). Further, we identified DNA fragment CTT_ACA_57, which was significantly frequently present in patients with optimal response to IM (65%) in comparison to patients with IM failure (19%) (P=0.03). The fragment is a part of satellite sequence ALR/Alpha occurring in centromeric regions with the highest frequency on chromosome 5. Wide evidence of small genomic aberrations of ALR/Alpha was found in chronic lymphocytic leukemia (Kim et al., 2010).
Summary/Conclusions: In this work, we applied whole-genome fragmentation analysis to identify DNA polymorphisms associated with CML and response to IM and subsequently confirmed these potential novel DNA markers by genotyping. Up to now, we identified and confirmed 2 SNPs significantly associated with CML and 1 repetitive element polymorphism associated with response to IM. We assume identification of more genomic markers associated with CML and suppose potential use of AFLP/NGS combinational approach as the alternative to whole-genome sequencing.
Supported by Ministry of Health of CZ grant IGA NT11555 and project 00023736.

E1089

DROPLET DIGITAL PCR IMPROVE MINIMAL RESIDUAL DISEASE DETECTION IN >35% OF SAMPLES COMPARING TO RQ-PCR

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Background: The gold standard for BCR-ABL quantification in chronic myeloid leukemia patients (CML) is the real time quantitative polymerase chain reaction (RQ-PCR), this technique has a limited sensibility to MR4.0 or MR4.5 in the majority of laboratories. Several discontinuation studies in chronic phase CML (CP-CML) patients reveal that both, the achievement a deeper response and a long time in this response seem the main factors for good outcomes. The droplet digital PCR (ddPCR) had shows more sensitivity to detect minimal amounts of BCR-ABL transcripts in patients with undetectable BCR-ABL transcripts by RQ-PCR, leading an opportunity to investigation in this field.
Aims: To evaluate the usefulness of the ddPCR in detect the presence of BCR-ABL transcripts in CP-CML patients under TKI therapy, compared with RQ-PCR.
Methods: Between August-2013 to Mar 2015, a total of 112 samples from 34 CP-CML patients under TKI therapy were analyzed. The blood samples were obtained in EDTA for RQ-PCR and in Tempus™ tube for RNA isolation. The RQ-PCR was performed following the current recommendations. The RNA was obtained from peripheral blood using Tempus™ Spin RNA isolation kit according manufacturer recommendations. The analysis by ddPCR for each sample was done using 5 µg of RNA obtained using One-step kit and processed in

Qx100 Droplet Digital PCR, Biorad; using primers and probes described by Gabert et al., 2003, the results were analyzed using QuantaLife Biorad software and expressed in absolute number of BCR-ABL copies and positive droplets. Samples were processed per triplicate (3 wells). Positive samples: were considered when the sum of ≥3 positive droplets in minimum of 24,000 droplets.
Results: From the 112 samples analyzed by RQ-PCR, 69 (61.6%) were negative and 43 (38.4%) were positive. According molecular response the distribution by international scale (MR) and number of transcripts (NT). Table 1. The ddPCR analysis reveals that 65 (58.0%) of the samples were positive, median of positive droplets: 22.5 (3-35,529). Median BCR-ABL NT: 41.5 (5.5-11,129.3). For samples with a negative result by RQ-PCR (n=69), in 44 (63.7%) the analysis by ddPCR confirmed the negativity of the sample, however in 25 (36.2%) there were positivity in 3 or more droplets (median 4, 3-25), with a median NT 9.9 (5.5-44.7) copies. Table 1 exposed also the relation between the MR by RQ-PCR and the positivity by ddPCR. However if the threshold is settled in two positive droplets with a minimum of 3.5 NT, the number of positive samples increase to 38 (55.1%), with a median NT: 8.1 (3.6-44.7). There was a correlation in the number of copies between both techniques, but with a significant increase in the amount of NT copies.

Table 1.

Positive samples (43/112, 38.4%)					
RQ-PCR	No MR	MR3.0	MR4.0	MR4.5	MR5.0
MR	24 (55.8%)	13 (30.2%)	6 (14.%)	0	0
Median NT	118.5(68.9-291.4)	7.5 (4.8-59.1)	3.3 (3.0-1,371)	0	0
Ratio	1.18 (0.68 – 2.9)	0.075 (0.011-0.09)	0.008 (0.003 – 0.008)		
RQ-PCR negative samples (69/112, 61.6%)					
MR		5 (7.2%)	49 (71.1%)	14 (20.3%)	1 (1.4%)
ddPCR + (3 droplets) 36.2%		2 (40%)	17 (34.7%)	5 (35.7%)	0 (0%)
ddPCR – (3 droplets) 62.3%		3 (60%)	32 (65.3%)	8 (57.1%)	1 (100%)

Summary/Conclusions: The ddPCR offer a new way to assess CP-CML patients, in special for patients with MR4.0 or undetectable molecular response by RQ-PCR, and would offer a more secure alternative to select patient for discontinuation trials. In our cohort, at least an improving of 36% on identification of samples with presence of minimal residual disease has been founded. The use of this technique to monitoring and select patients for discontinuation trials is warranted.

E1090

DROPLED DIGITAL PCR MAY HAVE A PROGNOSTIC VALUE FOR PREDICTING RELAPSE AFTER IMATINIB DISCONTINUATION

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Background: Treatment-free remission is a major goal in CML. Nowadays it is possible to safely discontinue imatinib but it is still not clear which patient (pt) will relapse. High sensitivity techniques like droplet digital (dd)PCR may help to discriminate pts who still present a significant amount of disease despite being in MR4 by standard real-time quantitative PCR (RQ-PCR).
Aims: To evaluate the capability of ddPCR to predict relapse after imatinib discontinuation in CML pts with stable MR4 by RQ-PCR.
Methods: Total RNA was extracted at different time-points from 19 pts in stable MR4 for two years before discontinuation of imatinib. ddPCR was carried out in triplicate using 200 ng of cDNA for each replicates and droplets were analyzed by QX100™ droplet reader (Bio-Rad) using the DigiDrop P210 Master Mix Kit (Bioclarma). All results were expressed according to the new recommendations [Cross, Leukemia 2015].The Wilcoxon test was used for the comparison of the total ABL medians; the Agreement Coefficient of the 2 methods was calculated with Cohen's K test; Logistic Regression was used to assess the relationship between the probability of relapse and disease level by RQ-PCR and ddPCR.
Results: A total of 48 samples were retrospectively analyzed by RQ-PCR and ddPCR. All pts discontinued imatinib; 8/19 pts had to restart treatment for loss of MMR/MR4 after a median of 5.3 mos (2.2-8.5). A difference between ddPCR and RQ-PCR median total ABL copies (p<0.001) was found with the following medians (ranges): 46290 (8420-124600) and 93060 (25440-210000) for RQ-PCR and ddPCR respectively. A difference between ddPCR and RQ-PCR median total BCR-ABL1/ABL1% (p<0.001) was found: 0 (0-0.09); and 0.0009 (0-0.015) for RQ-PCR and ddPCR respectively. Sixteen pts had >2 evaluations, 3 had an evaluation between 14 and 18.5 mos before discontinuation. 6 pts had an evaluation at -12 mos (+/-0.81), 10 at -9 mos(+/-1.3), 14 at -6 mos(+/-0.9), 15 at -3 mos(+/-1.2), considering as time "0" the date of discontinuation. At time -12, only 1pt resulted BCR-ABL1 positive by both methods, 1 by ddPCR only and 0 by RQ-PCR only (K0.6, moderate agreement, p 0.13). At time -9, 3 pts resulted BCR-ABL1 positive by both methods, 6 by ddPCR only and none by RQ-PCR only (K=0.1, slight agreement, p 0.34). At time -6, 3 pts resulted BCR-ABL1 positive by both methods, 5 pts by ddPCR only and none by RQ-PCR only, (K=0.3, fair agreement, p 0.08). At time -3, none resulted BCR-ABL1 positive by both methods, 4 pts were positive by ddPCR only and 1 by RQ-PCR only (K=-0.1, no agreement, p 0.62). Over one year of molecular follow-up before discontinuation, for negative values obtained measuring BCR-ABL1 by RQ-PCR the probability of relapse was equal to about 50% (45%, Figure 1), *i.e.* the capability of RQ-PCR of detecting relapse was basically same as random being almost constant for all the positive values of BCR-ABL1. On the contrary the probability of a relapse for negative values of BCR-ABL1 by ddPCR was about 30% and it grew up linearly at increasing level of transcript, achieving a probability of 69% when BCR-ABL1/ABL1% was equal to 0.008%. The difference between RQ-PCR and ddPCR in predicting the probability of relapse was statistically significant (p 0.02).

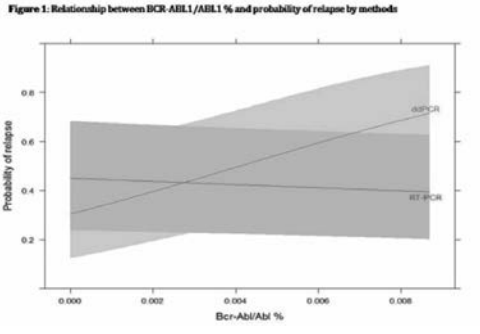

Figure 1.

Summary/Conclusions: Despite the small number of cases, our results confirm that the ddPCR is more sensitive than RQ-PCR for low levels of disease. Molecular follow-up by ddPCR at several time-points before discontinuation may have a prognostic value for predicting relapse, and this is worthy to be tested prospectively and in larger cohorts of pts.

E1091

PROGRAMMED CELL DEATH PROTEIN 1 (PD-1) DOWNREGULATION ON REGULATORY T CELLS DURING TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC MYELOID LEUKEMIA

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Background: Programmed death-1 (PD-1) receptor and its ligands (PD-L1 and PD-L2) are involved in attenuating tumor immunity and facilitating tumor progression. PD-1, PD-L1 and PD-L2 therapeutic blocking agents have been reported to have significant antitumor effects. In chronic myeloid leukemia (CML), the expression of this receptor and its ligands is not fully characterized for the different subsets of cells of the immune system in which their expression is found constitutively or post-induction.
Aims: In this study, we analyzed the expression of PD-1 and its ligands on regulatory T cells (Tregs) in chronic phase CML patients to understand the mechanisms underlying suppressor effects that inhibit the anti-leukemia immune response.
Methods: Peripheral blood samples from chronic phase CML patients (n=50) under Interferon-alpha 2b (IFN-α 2b), imatinib, dasatinb, nilotinib, bosutinib and ponatinib therapy were analyzed by multi-parametric flow cytometry for the characterization of regulatory T cells and surface expression of PD-1. Buffy coats (n=13) from healthy blood donors were used as control. Cytokines and chemokines were evaluated in a 34-plex panel by xMAP technology (Luminex®). Gene expression analysis and miRNA profiling were also performed for these samples.
Results: PD-1 Tregs were found significantly decreased (p<0.0001) in CML patients and down-regulation of this receptor was also observed (p<0.01). Naïve and memory Treg subsets were equally affected. No significant alterations were observed for PD-L1 and PD-L2 ligands. Although TGF-β and IL-10 production were not significantly altered by down-regulation of PD-1 in Tregs, the overall effect of tyrosine kinase inhibitor (TKI) therapy suggests a negative impact in these cells concerning the anti-leukemic immune response.

Summary/Conclusions: Regulatory T cells represent a major population of suppressors in the immune response against leukemia. Down-regulation of PD-1 receptor in Tregs during chronic phase CML reinforces the notion that discontinuation of treatment must be carefully evaluated beforehand, since these suppressor cells could permit the proliferation of existent residual leukemic cells.

E1092

SINGLE NUCLEOTIDE POLYMORPHISMS IN DRUG-RELATED GENES ARE ASSOCIATED WITH RESISTANCE OR TOXICITY TO IMATINIB IN CHRONIC MYELOID LEUKAEMIA

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Background: Imatinib (IM) induces apoptosis in *BCR-ABL1* positive cell lines and is extensively used to treat patients with newly diagnosed Philadelphia chromosome-positive Chronic Myeloid Leukaemia (Ph+CML). However, about 20% of Ph+CML patients fail to achieve optimal response, and a substantial proportion develop intolerance or toxicity. Amplification and overexpression of the *BCR-ABL1* gene, *BCR-ABL1* kinase domain point mutations and genetic variability (as genetic polymorphisms), among others, may explain IM resistance.
Aims: To assess the association between single-nucleotide polymorphisms (SNP) in drug-related genes and IM resistance or toxicity in CML patients.
Methods: In a pilot study with 45 Ph+CML patients treated with IM from three Spanish centers, we tested 1936 SNPs in 225 drug-related genes using the Affymetrix Drug Metabolism Enzymes and Transporters (DMET) microarray. Response criteria were assessed according to ELN2009 or ELN2013 guidelines. Relevant toxicity to IM was defined as the need for switching to another tyrosine kinase inhibitor (TKI). SNPs significantly associated with response or toxicity to IM treatment were validated in an independent group of 105 Ph+CML patients from 8 Spanish centers. Informed consent (according to the Declaration of Helsinki) was obtained from all patients. Validation genotyping was performed using Fluidigm 96.96 Dynamic Array with BioMark HD Systems (Fluidigm Corp., CA). Data were analysed by the BioMark SNP Genotyping Analysis software to obtain genotype calls. Candidate SNPs were primarily evaluated for adequacy of Hardy-Weinber Equilibrium (HWE) using the Chi-square test. For the association study, the SNPassoc package from R was used (PMID: 17267436, www.r-project.com).
Results: In this pilot study we found 76 SNPs to be significantly associated with response or toxicity to IM treatment. In the validation cohort, 5/76 SNPs and 2/76 SNPs were related to IM resistance or toxicity, respectively (*Table 1*). Patients carrying at least one variant allele for *CYP2C8* (C), *UGT2A2* (A), *CYP19A1* (T), *ABCC3* (A) and *CYP2C8* (A) were significantly associated with IM treatment failure. In the same way, AC-AA genotypes in *CYP19A1* and CT-TT genotypes in *CYP11B1* were significantly associated with lower risk of IM-toxicity.

Table 1. Significant SNPs related to IM resistance or toxicity (Log-Additive Model).

dbSNP ID (Gene)	Genotype	Controls N(%)	Cases N(%)	OD (CI95%)	p-value
rs10509681[1] (*CYP2C8*)	T/T C/T C/C	56 (86.2) 8 (12.3) 1 (1.5)	18 (64.3) 9 (32.1) 1 (3.6)	2.76 (1.10-6.97)	0.028
rs4148304[1] (*UGT2A2*)	G/G A/G A/A	49 (75.4) 15 (23.1) 1 (1.5)	16 (57.1) 8 (28.6) 4 (14.3)	2.38 (1.13-5.04)	0.021
rs700519[1] (*CYP19A1*)	C/C C/T T/T	54 (84.4) 9 (14.1) 1 (1.6)	18 (64.3) 6 (21.4) 4 (14.3)	2.71 (1.22-6.01)	0.011
rs11568591[1] (*ABCC3*)	G/G A/G A/A	51 (78.5) 11 (16.9) 3 (4.6)	16 (57.1) 6 (21.4) 6 (21.4)	2.30 (1.18-4.45)	0.012
rs11572080[1] (*CYP2C8*)	G/G A/G A/A	56 (86.2) 8 (12.3) 1 (1.5)	18 (64.3) 9 (32.1) 1 (3.6)	2.76 (1.10-6.97)	0.028
rs4646[2] (*CYP19A1*)	C/C A/C A/A	48 (61.5) 30 (38.5) 0 (0)	22 (81.5) 4 (14.8) 1 (3.7)	0.48 (0.18-1.29)	0.014
rs1134095[2] (*CYP11B1*)	C/C C/T T/T	15 (19.5) 45 (58.4) 17 (22.1)	10 (37.0) 14 (51.9) 3 (11.1)	0.50 (0.25-1.02)	0.049

[1] RESPONSE. Data available in 93 patients (29 failed to achieve IM response)
[2] TOXICITY. Data available in 105 patients (27 developed IM toxicity)

Summary/Conclusions: The SNPs found in the present study allow the identification of CML patients at risk to develop IM resistance or toxicity, suggesting that a second generation TKI should be offered to these patients as first line therapy. Furthermore, our results suggest that SNP analysis may be included in clinical practice as a predictive tool for response and toxicity at diagnosis of CML patients.
Acknowledgements: This study was supported by an unrestricted grant from Novartis.

E1093

DIFFERENT SETS OF GENES WERE ASSOCIATED TO THE MOLECULAR RESPONSE AFTER 3 AND 6 MONTHS OF FIRST-LINE NILOTINIB TREATMENT BY MICROARRAY OF CD34+/LIN- CELLS OF CHRONIC PHASE CML PATIENTS AT DIAGNOSIS

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Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder and the biomolecular mechanisms of CML response to tyrosine kinase inhibitors are not fully defined.
Aims: Thirty chronic phase CML patients were investigated at diagnosis and at 3, 6 and 12 months of first-line nilotinib treatment and optimal response was achieved at 3, 6 and 12 months after nilotinib in all the 30 patients (figure 1). We established cut off values of molecular response (MR) to define groups of patients, and to explore differences of gene expression profiles (GEP) between patients at diagnosis based on the MR achieved after 3, 6 and 12 months of nilotinib, respectively.
Methods: Cut off values were: 1% IS at 3 months of nilotinib, 0.1% IS at 6 months of nilotinib, and 0.01% IS at 12 months of nilotinib. We divided patients into 2 groups based on MR values of each patient at 3 months of nilotinib: group A (n=24) with MR≤1.0% IS and group B (n=6) with MR>1% IS. At 6 months of nilotinib, patients were divided into: group C (n=22) with MR≤0.1% IS and group D (n=8) with MR>0.1% IS. At 12 months of nilotinib, patients were divided into: group E (n=18) with MR≤0.01% IS and group F (n=12) with MR>0.01% IS. GEP on the selected bone marrow (BM) CD34+/lin- cells of 30 patients at diagnosis was performed using Affymetrix-GeneChip-HTA 2.0. RMA was used for preprocessing data. SAMR and GSEA detected differentially expressed genes and pathways associated with the different groups, respectively. MTC was performed using the FDR with a threshold 0.05 for SAMR and 0.25 for GSEA.

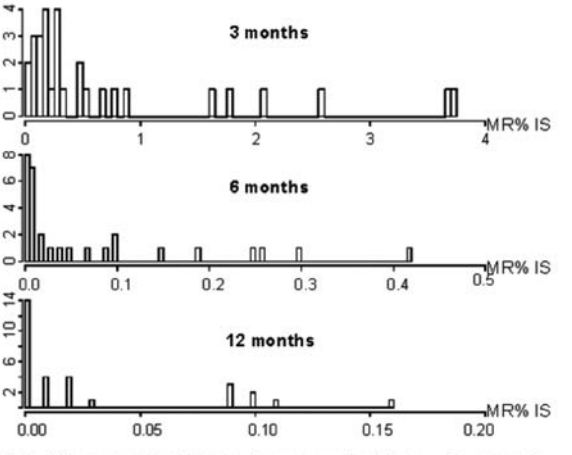

Fig.1 Histograms showing the frequency of patients with a certain Molecular Response (MR% IS) at 3, 6 and 12 months (upper, middle and lower panel, respectively)

Figure 1.

Results: GSEA identified significant differences in the transcriptional signature between group A and group B associated with the MR at 3 months as well as between group C and group D in respect to the MR at 6 months of nilotinib. Based on the MR at 3 months, Reactome-pathways-"P130-cascade" and "GRB2-SOS-linkage-to-MAPK-signaling-for-integrins" were significantly upregulated in group A. *FGA*, *FGB*, *FGG*, *APBB1IP*, I*TGA2B* and *CRK* genes contributed to call the pathways upregulated. From literature data, nilotinib inhibits BCR-ABL1 mediated phosphorilation of CKRL which acts in the CML hematopoietic stem cells in diverse signaling pathways playing an apoptotic role in CML. CRK was overexpressed in patients with a better MR≤1% IS at 3 months of nilotinib. Aminoacid-and-amine-transport-across-the-plasma-membrane-pathway was significantly overexpressed, whereas "lipid metabolism" was significantly downregulated (*AKR1C1*, *ANGPTL3*) in group A compared to group B, respectively. Based on the MR at 6 months, "positive-regulation-of-cytokine secretion" and "interleukin1-secretion" were significantly upregulated in group C compared to group D, involving *PYCARD8, NLRP2, NLRC4, NLRP3* and *CARD8* genes. *PYCARD* and *CARD8* promote caspase-mediated-apoptosis processes involving the recruitment of recognition receptors: *NLRP2, NLRP3* and *NLRC4.*
Summary/Conclusions: The study showed that MAPK-signaling-for-integrins and amine-transport-across-the-plasma-membrane were significantly overexpressed in CML patients with MR≤1.0% IS while lipid metabolism was significantly underexpressed in CML patients with MR≤1.0% IS after 3 months of nilotinib. Positive-regulation-of- cytokine-secretion-and-interleukin1-secretion was significantly overexpressed in CML patients who achieved MR≤0.1 IS after 6 months of nilotinib. In summary, distinct sets of genes involved in apoptosis were differentially expressed in 30 CML patients at diagnosis based on the MR at 3 and at 6 months of first-line nilotinib treatment.

E1094

MATE1 REGULATES CELLULAR UPTAKE AND SENSITIVITY TO IMATINIB IN CML PATIENTS

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Background: Imatinib is highly effective in the treatment of chronic myeloid leukemia (CML). Up to 30% of CML patients treated with Imatinib as a first-line therapy experience treatment failure. The response rate varies within different stages of the disease. Besides other mechanisms of resistance such as mutations in the *Bcr-Abl1* fusion gene, the transport of Imatinib into its target cells has been proposed to be crucial for its effectiveness. As an organic cation, Imatinib has to be actively translocated across the cell membrane. Though some studies suggested a critical role of the organic cation transporter 1 (OCT1) in regulating Imatinib efficacy, other studies could not confirm these findings. Although OCT1 is generally capable to transport Imatinib, it might have minor relevance under clinical conditions. We recently demonstrated that the multidrug and toxin extrusion protein 1 (MATE1) accepts Imatinib as a substrate.
Aims: In this study, we focused on transporters, which are relevant for Imatinib uptake and investigated their expression pattern in PBMCs and if they contribute to Imatinib uptake and expression in samples from CML patients or CML cell lines.
Methods: We used PBMC from 6 healthy volunteers and transporter expression profile was analyzed by qPCR and intracellular Imatinib accumulation was quantified by HPLC-UV using stably transfected HEK cells. To assess Imatinib dependent biologic effects on MATE1 expression, activity of bcr-Abl1 protein was detected by Western blot analysis using anti-phospho ABL and GAPDH antibody. To address the clinical significance of MATE1 on the Imatinib therapy in CML, we evaluated the clonal growth of the immortalized CML cell line K562 cells stably transduced with shRNA against MATE1 in methylcellulose medium supplemented with Imatinib.
Results: The results showed that Imatinib uptake was significantly increased in OCT1, OCT2- and MATE1 expressing HEK-cells. We found that the transport via MATE1 is clinically highly relevant as represented by a remarkably high quotient for therapy regimes. This is supported by qPCR expression of MATE1 in 31 patients with Bcr-Abl1 major transcript (p210) positive CML.The mRNA expression of potential Imatinib transporters in PBMC of healthy volunteers as well as in K562 cells showed that in both cell types OCT1, OCT2 and MATE1 were detected at similar levels comparable to the Imatinib uptake rate into these cells. Inhibitor studies showed that MATE1 plays an important role in Imatinib accumulation. In patients, Imatinib non-responders showed a significantly lower MATE1 expression than Imatinib responders and we found a correlation between MATE1 transporter expression and clinical response to Imatinib treatment, suggesting that MATE1 might enable to predict whether CML patients are likely to respond to Imatinib therapy.
Summary/Conclusions: Thus, we identify MATE1 as the main transporter for the Imatinib dependent therapeutic response of CML patients.

LB2253

TRANSPOSON-MEDIATED GENERATION OF BCR-ABL1-EXPRESSING TRANSGENIC CELL LINES FOR FAST AND UNBIASED SENSITIVITY TESTING OF TYROSINE KINASE INHIBITORS

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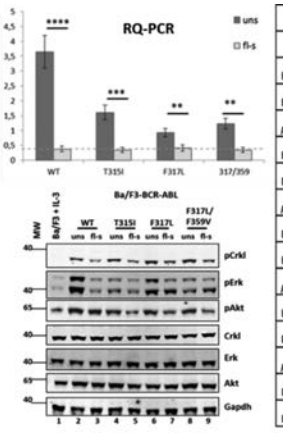
Background: Point mutations in the ABL1 kinase domain are an important mechanism of resistance to tyrosine kinase inhibitors (TKI) in BCR-ABL1-positive and, as recently shown, BCR-ABL1-like leukemias. The cell line Ba/F3 lentivirally transduced with mutant BCR-ABL1 constructs is widely used for *in vitro* sensitivity testing and response prediction to tyrosine kinase inhibitors. The transposon-based *Sleeping Beauty* system presented offers several advantages over lentiviral transduction including the absence of biosafety issues, faster generation of transgenic cell lines, and greater efficacy in introducing large gene constructs. Nevertheless, both methods can mediate multiple insertions in the genome.
Aims: Establish a method for enrichment of virtually pure cell fractions carrying single insertions of the gene construct to permit unbiased *in vitro* testing of drug resistance.
Methods: Transposon-mediated gene transfer, flow soritng, FISH, RQ-PCR, qPCR, immunoblotting, survival assays
Results: We showed that multiple BCR-ABL1 insertions result in elevated IC_{50} levels for individual TKIs, thus overestimating the actual resistance of mutant subclones. The establishment of a flow-sorting-based fractionation of BCR-ABL1-transformed Ba/F3 cells has enabled us to enrich for cells carrying single-site insertions, as demonstrated by FISH analysis and real time PCR. Fractions of unselected Ba/F3 cells not only showed a greater number of BCR-ABL1 hybridization signals, higher expression levels of BCR-ABL1, and increased activation of BCR-ABL1 downstream signaling, but also revealed higher IC_{50} values for the TKIs tested.



IC_{50} values of unselected and flow-sorted Ba/F3-BCR-ABL1 (nM)

	Nilotinib	Dasatinib	Ponatinib
BCR-ABL1^{WT} unselected	25.0 ± 6.0	4.2 ± 1.3	10.0 ± 2.0
BCR-ABL1^{WT} flow-sorted	10.0 ± 3	2.3 ± 1.2	2.1 ± 0.8
p-value	*≤0.001*	*≤0.05*	*≤0.001*
BCR-ABL1^{T315I} unselected	>5000	>5000	30.0 ± 5.0
BCR-ABL1^{T315I} flow-sorted	>5000	>5000	10.0 ± 2.0
p-value	*NA*	*NA*	*≤0.001*
BCR-ABL1^{F317L} unselected	120.0 ± 20.0	20.0 ± 3.0	8.2 ± 2.7
BCR-ABL1^{F317L} flow-sorted	54.0 ± 12.0	8.3 ± 2.6	2.5 ± 1.1
p-value	*≤0.001*	*≤0.001*	*≤0.05*
BCR-ABL1$^{F317L/F359V}$ unselected	2200.0 ± 300.0	35.0 ± 8.0	45.0 ± 10.0
BCR-ABL1$^{F317L/F359V}$ flow-sorted	1000.0 ± 80.0	12.0 ± 4.0	15.0 ± 5.0
p-value	*≤0.001*	*≤0.001*	*≤0.001*
LV BCR-ABL1^{WT} unselected	75 ± 10	4.0 ± 2.0	4.0 ± 2.0
LV BCR-ABL1^{T315I} unselected	>5000	>5000	35 ± 12

Figure 1.

Summary/Conclusions: The generation of multiple gene construct insertions in Ba/F3 cells by lentiviral- or transposon-mediated transfer has not been reported previously as a relevant problem in the context of *in vitro* drug sensitivity testing. Our data demonstrate that targeted flow-sorting of low-fluorescent Ba/F3-BCR-ABL1 cells facilitates enrichment of virtually pure cell fractions carrying single insertions of the gene construct. Employment of this selection step is highly recommended to permit unbiased *in vitro* testing of drug resistance. The clinical relevance of our observations has far reaching consequences beyond Ph+ neoplasia. The rapidly increasing number of reports on BCR-ABL1-like leukemias involving a variety of activated kinases highlights the relevance of TKI-based treatment and *in vitro* prediction of sensitivity to these agents. Appropriate modeling of mutant kinases inserted at single sites into Ba/F3 cells, as presented in this report, is therefore of paramount importance for reliable prediction of treatment responses to kinase inhibitors.

LB2254

THE PROLIFERATIVE ACTIVITY OF THE BONE MARROW CELLS INVESTIGATED *IN VITRO* CELL CULTURE OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH TYROSINE KINASE INHIBITORS

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Background: The mechanisms of CML progression were postulated based on *in vitro* modeling even before they were detected in the clinical practice. CML

progression can be driven by different mechanisms on tissue, cell, chromosomal and molecular level. Recent *in vitro* studies of CML bone marrow cells also suggest that disease progression can be caused by functional changes in pool of more matured hematopoietic progenitor cells rather than in population of HSCs. Such evolution of HPCs during CML progression can be identified by changes in their clonogenic potential during cultivation in semi-solid agar.
Aims: To analyze the changes in functional properties of HSCs and HPCs, such as ability to form cell aggregates during cultivation in CFU assay with further evaluation of cell composition of individually picked-up cell aggregates, with respect of correlation with CML progression in each individual patient.
Methods: Totally 51 samples of bone marrow were analyzed from patients with CML in chronic phase, who were treated with TKI, Imatinib. *CFU-assay:* Mononuclear fraction was separated from bone marrow aspirates by centrifugation over Ficoll-Hypaque gradient. All results were expressed as the mean number obtained from quadruplicate cultures. Clones of > 40 cells were counted as colony forming units granulocyte monocyte (CFU-GM) and those with 5 - 40 cells as cluster forming units (ClFU).
Results: CFU assay indicated that there was a significant ($p < 0.05$) difference in clonogenic potential (number of CFU-GM and ClFU) between groups of patients with different response to the therapy. CFU numbers were lower in group of patients with optimal response to the TKI therapy (mean value of 25.54±4.6) and for patients with TKI treatment failure (26.00±9.70) when compared to CFU numbers for groups of patients before TKI treatment (70.40±22.51) and patients with suboptimal response to TKI treatment (72.37±15.32). Significant decline ($p < 0.05$) was also indicated for mean numbers of ClFU for group of patients with optimal response for TKI (24.05±6.75) when compared to groups of patients with suboptimal response to TKI (49.94±7.89), but also for group of patients with TKI treatment failure (51.17±9.22). The mean number of ClFU for patients before TKI treatment was 41.80±9.78 and was not significantly different from other groups ($p > 0.05$). As to colony to cluster ratio our results showed, that there is a correlation ($\rho = 0.67$) between CCR index and number of bone marrow cells with Ph-chromosome. To have a possibility of identification of cells, composing aggregates obtained in CFU assay, a positive correlation was also found between numbers of colonies, formed during cultivation in CFU assay and level of CD34+ cells in bone marrow ($\rho = 0.46$) and between number of clusters and CD33+ cells of bone marrow ($\rho = 0.76$). To evaluate changes in the differentiation potential of HSCs and HPCs that can be related to pathologic process of CML, we picked-up individual cells aggregates, formed during in vitro CFU assay of bone marrow mononuclear cells of CML patients. Cells, composing those aggregates were analyzed with calculation of maturation index (MI) for each individual sample. It was indicated, that MI correlates with level of bone marrow cells, containing Ph-chromosome. CFU assay is one of the standard methods used to identify functional properties of HSCs and HPCs of bone marrow. Our data indicated alteration of clonogenic activity of bone marrow hemopoietic cells for patients with different response to TKI treatment. Thus, CFU and ClFU numbers were increased in case of examination of bone marrow of patients before TKI treatment, so as for patients with suboptimal response and TKI treatment failure.
Summary/Conclusions: In summary, obtained results suggest that different mechanisms (BCR-ABL dependent and independent) may be involved in CML progression process in the same time. Disease prognosis should be preferably carried out on an individual basis.

Chronic myeloid leukemia - Clinical

E1095

IMPLEMENTING A CERTIFIED PLASMID REFERENCE MATERIAL FOR COPY NUMBER CALIBRATION DEMONSTRATES GOOD REPORTING CORRELATION BETWEEN % BCR-ABL/ABL (IS) AND % BCR-ABL/ABL (COPY NUMBER) IN XPERT® BCR-ABL ULTRA

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Background: Development of a quantitative BCR-ABL monitoring assay with sensitivity down to 4.5 logs below baseline ($MR^{4.5}$) is important for patients being considered for tyrosine kinase inhibitor (TKI) discontinuation, and it is essential to ensure that variability in testing methodologies across laboratories is tightly controlled. Through analytical and clinical evaluations, Xpert BCR-ABL Ultra, an automated cartridge-based real-time qPCR BCR-ABL monitoring assay, has demonstrated sensitivity reaching $MR^{4.5}$, and every production lot of cartridges is assigned a unique conversion factor assuring substantial alignment to the WHO International Scale (*IS*).
Aims: Here we describe the implementation of a certified plasmid reference material (IRMM_ERM-AD623 BCR-ABL Calibrator) for the standardization of BCR-ABL mRNA quantification through copy number calibration to ensure sufficient BCR-ABL and ABL copies are present to support the $MR^{4.5}$ sensitivity claim for Xpert BCR-ABL Ultra.
Methods: To establish the correlation between Cycle threshold (Ct) reporting and copy number for BCR-ABL and ABL, and to understand the assay sensitivity limit in terms of relative copy number, the IRMM plasmid was used in a titration study involving three independent BCR-ABL Ultra assay lots. Based on the titration study, a copy number calibration curve was derived and used to estimate the copy number for BCR-ABL and ABL in analytical studies previously performed for CE registration, including the Linearity/Dynamic range, Analytical LoD and Clinical LoD studies.
Results: The results demonstrate that a linear detection range between $1x10^6$ copies down to 3 copies was achieved with 5 copies being detected in 24 out of 24 replicates and determined as the Limit of Detection (LoD) for both BCR-ABL and ABL. The overall ABL copy number present in clinical samples in each study was at least 5-10 times the required minimal ABL copy number of ≥32,000 to support a claim of $MR^{4.5}$ and ≥100,000 for $MR^{5.0}$. This is explained by the fact that 4ml whole blood is used to prepare the sample lysate and an aliquot of which equivalent to 0.6ml whole blood is then processed completely in a fully automated and integrated GeneXpert system for nucleic acid extraction, target amplification, and quantification, resulting in improved target recovery. The%ratio of the derived BCR-ABL and ABL copy numbers was further calculated and compared to the original assay lot-specific%reporting (*IS*) per WHO calibration, revealing an unbiased correlation between%BCR-ABL/ABL (Copy number) and%BCR-ABL/ABL (*IS*) across the range 100% to 0.001% (*IS*). To validate the assay performance, Xpert BCR-ABL Ultra was further evaluated in field studies compared to three independent laboratory developed tests (LDTs) previously calibrated with the IRMM plasmid using either ABL or GUSB as control genes, demonstrating a correlation of >0.91, covering samples in the range 100% - 0.001% (*IS*).
Summary/Conclusions: These studies, using two different calibration systems (WHO/*IS* and IRMM/Copy number) and two different control genes (ABL and GUSB), carried out in four independent research labs, demonstrate, for the first time, good reporting correlation between%BCR-ABL/ABL (*IS*) and%BCR-ABL and ABL (Copy number) in Xpert BCR-ABL Ultra. Furthermore, these data demonstrate that from a typical clinical specimen, the BCR-ABL Ultra assay captures more than enough ABL copies to support a sensitivity claim of $MR^{4.5}$.

E1096

META-ANALYSIS OF THE RISKS OF ARTERIAL AND VENOUS OCCLUSIVE EVENTS WITH NEW GENERATION BCR-ABL TKIS IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA

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Background: A recent meta-analysis demonstrated that 3 of the new generation BCR-ABL tyrosine kinase inhibitors (TKIs) (i.e. dasatinib, nilotinib and ponatinib) are associated with an enhancement of major molecular response without improving the overall survival at one year in patients with PH+ chronic myeloid leukaemia (CML) compared with imatinib. This study also highlighted their association with an increased risk of vascular occlusive events compared with imatinib but distinction between arterial of venous events was not assessed.
Aims: To perform a meta-analysis on the risk of arterial and venous occlusive events in patients with Ph+ CML treated with new generation BCR-ABL TKIs in randomized clinical trials (RCTs).
Methods: The literature search (data lock point: November 26, 2015) was conducted according to a registered protocol (PROSPERO 2014:CRD420140 14147). All RCTs comparing a new generation BCR-ABL TKI *versus* imatinib in patients with Ph+ CML were included. Two independent investigators were responsible of the screening, the review and the extraction of the data using standard forms. The meta-analysis was performed using a random (REM) and a fixed (FEM) effect model according to the characteristics of the included studies. ORs with 95% CIs were computed using the Peto method. Statistical heterogeneity was quantified using the I^2 value, and publication bias was assessed by funnel plots.
Results: Among the 400 abstracts identified, 12 studies fulfilled the established criteria and were included in the statistical analysis. Overall, 4.47% (99/2,217) of patients developed arterial occlusive events with new generation BCR-ABL TKIs compared with 0.80% (15/1,884) with imatinib (REM OR_{PETO}: 3.46; 95%CI: 2.35 to 5.10). Ponatinib (REM OR_{PETO}: 3.26; 95%CI: 1.12 to 9.50), nilotinib (REM OR_{PETO}: 3.60; 95%CI: 2.21 to 5.86) and dasatinib (REM OR_{PETO}: 3.32; 95%CI: 1.37 to 8.01) are associated with higher risk of arterial occlusive events than imatinib. No significant difference was found with bosutinib. Venous occlusive events occur in only 0.86% (13/2,217) of patients treated with new generation TKIs and in 0.16% (3/1,884) of imatinib-treated patients. Overall, new generation TKIs increase the rate of venous occlusive events (REM OR_{PETO}: 2.85; 95%CI: 1.04 to 7.78) but stratifications by treatment provided nonsignificant results. Funnel plots demonstrate no evidences of publication bias, and the I^2 statistic specifies no heterogeneity among studies. Limitations of this meta-analysis include the absence of a time-to-event analysis and the inconsistent report of cardiovascular events in the literature. However, the use of a clinical trial register aimed to decrease this heterogeneity.
Summary/Conclusions: The increased risk of vascular occlusive events associated with new generation BCR-ABL TKIs is mainly driven by thrombotic events occurring at the arterial side. Additional investigations are required to assess the underlying pathophysiological mechanisms and provide appropriate risk minimization measures.

E1097

REL-PROTOCOL PHILOSOPHI34 CONFIRMS THAT NILOTINIB RAPIDLY INDUCES CD34+/LIN-PH+ CELLS DISAPPEARANCE IN PATIENTS WITH CHRONIC MYELOID LEUKAEMIA (CML) IN CHRONIC PHASE (CP)

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Background: CML is a clonal disorder characterized by the presence of the Philadelphia (Ph) chromosome which encodes for the bcr-abl tyrosine-kinase (TK). Target therapy with the TK inhibitor (TKI) imatinib (IM) has greatly improved outcome in CML; however, only a minority of responsive pts can discontinue treatment without relapsing in the short term and even less pts can be considered free of disease. This data probably relates to persistence of quiescent CD34+Ph+ stem cells (SC) whose persistence has been documented even among pts in optimal response (Bocchia et al, 2008). Compared to IM, faster and deeper responses in CP-CML pts can be achieved with the second generation TKI Nilotinib (NIL); however, only preliminary data are available on NIL potential to eradicate Ph+ SC. Moreover, in vitro studies (Corbin AS, et al 2011; Hamilton A, et al 2012) suggest that CD34+Ph+SC may not be sensitive to TKI-mediated Bcr/Abl inhibition. However, *in vivo* data (Defina et al, 2012) have shown that, compared to IM treated pts, residual leukemic SC are very rarely detected among NIL treated pts in CCyR even after short-term NIL therapy and even if duration of treatment is shorter for NIL compared to IM treated pts (median 39 *vs* 22 mos). At present, no clear evidence of TKI effect on the leukemic SC clearance is available.
Aims: On behalf of the Rete Ematologica Lombarda (REL), the PhilosoPhi34 protocol (EudraCT: 2012-005062-34), an open label, single arm, phase II study, was designed to investigate the efficacy of NIL 300 mg BID in BM CD34+/lin-Ph+cells clearance at 3, 6 (primary objective) and12 months of treatment in newly diagnosed CP-CML pts.
Methods: Enrolled pts' BM cells were collected and stored at diagnosis and at 3,6 and 12 mos of treatment. CD34+/lin- cells were purified using a Diamond CD34 Isolation Kit Miltenyi (97% of purity). FISH analysis of selected unstimulated CD34+/lin- cells was performed according to standard method; considering the low sensitivity of the test, at least 200 nuclei were examined in order to define the test as negative. This phase II study was designed according to the A'Hern single stage design, where, at 6 mos, we assumed a proportion defining a poor response as PP=0.10, and the proportion defining a good response as PG=0.30, PA being the proportion of responders.The study required 41 subjects to decide, with a power of 95%, whether PA ≤0.10 or PA ≥0.30. To have 41 evaluable, 87 pts had to be enrolled, considering 80% of them being fully analysed and 60% of these reaching CCyR (according to ENESTnd data).
Results: Enrolment of 87 pts was completed by June 2015; the database hasn't been locked yet. At present, FISH analysis on CD34+/lin- cells at 6 mos of treatment is evaluable for 68 of 75 pts with a documented CCyR; 7 negative tests were not evaluable since less than 200 nuclei were analysed. Only 5/68 (7,3%) evaluable FISH tested positive. Results at 3 and 12 mos are as follows: 8/58 (13,8%) and 0/46 (0%) evaluable FISH respectively tested positive (7 and 6 negative test excluded respectively). Sokal score did not predict for FISH positive results at any time point.
Summary/Conclusions: These results strongly point to a role for NIL 300 mg BID in fast clearance of BM CD34+/lin-Ph+ cells in a high proportion of treated pts. In view of the potential clinical relevance of our data, the protocol was amended in order to revise our FISH data using a test with higher sensibility and specificity, i.e.: the gDNA PCR. These results might give insight into a possible correlation with depth and stability of molecular response.
We acknowledge all REL Colleagues for their collaboration and Novartis SpA for the partial financial support to the study.

E1098

MEANING OF MOLECULAR RESPONSES FLUCTUATION IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH LONG-TERM IMATINIB

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Background: Prognostic significance of molecular response fluctuation in the long-term outcome during imatinib treatment in chronic phase chronic myeloid leukemia (CP-CML) patients have not been so far reported in details.
Aims: Aim of our study was to correlate, over a median follow-up period of 7 years, how instability of molecular response can affect overall survival (OS) and failure-free survival (FFS).
Methods: A series of 208 patients treated with imatinib first-line was retrospectively analysed. There were 144 males and 94 females, median age was 57.7 years. According to the IS, we considered as MR3 a BCR-ABL/ABL ratio of ≤0.1%, as MR4 a ratio ≤0.01% and as MR4.5 a ratio ≤0.0032%; stable response was defined as a response that persisted over 3 consecutive RQ-PCR tests, whereas fluctuation, was defined as the alternation of a test that reached or was below the threshold ratio according to IS and the consecutive was above the threshold. We considered OS the time elapsed from diagnosis to death for any cause, and FFS the timespan that follows therapy without signs of recurrence (loss of hematologic and/or cytogenetic response, acquisition of clonal cytogenetic abnormalities or mutations).
Results: At a median follow-up of 7 years, overall incidence of MR3 was 64.4%: during the course of treatment, 17 patients (11.6%) had a fluctuation above and then below the cut-off of 0.1%. Of these 17 patients, 13 (76%) lost the response and 3 subsequently developed secondary resistance (17.6%) with an estimated FFS of 82.4% and an OS of 94.6% at 7 years. Considering the threshold of MR3, of the remaining 117 patients that never had a fluctuation and maintained a stable response over time, only 3 (2.5%) subsequently lost the response and developed secondary resistance with an estimated FFS of 93.2% (p=0.02) and OS of 94% (p=ns). All patients were analysed for mutational screening according to Sanger sequencing analysis: only two patients were found to develop a mutation of the ABL1 kinase domain, in particular 1 patient a E255K and 1 patient a M351T. Cumulative incidence of MR4 was 51% and of MR4.5 was 34.6%, obtained after a median time of 3.8 and 5.4 years, respectively. Overall, 7 patients (6.5%) showed a fluctuation of MR4 response and 4 subsequently lost the response. None of these patients showed secondary resistance or acquired mutations in the ABL kinase domain and main reason

for loss of response was confirmed to be low adherence to long-term treatment. Eighteen patients (25%) showed an unstable MR4.5, but none of them developed secondary resistance or progressed to blast phase. Considering the threshold of MR4, fluctuation of molecular burden was associated to FFS of 100% and to OS of 98.2% as compared to patients with stable response that had a FFS of 99.4% and OS of 97.8%, without any statistical significance.
Summary/Conclusions: Our results showed that unstable MR3 was indeed associated to an increased probability to develop resistance, whereas long-term fluctuation of deeper molecular response (MR4-4.5) did not influence the long-term outcome of CML patients treated with imatinib.

E1099

BCR-ABL FUSION TRANSCRIPT B2A2 IS ASSOCIATED WITH A HIGHER RISK OF NON-OPTIMAL EARLY RESPONSE IN CP-CML PATIENTS TREATED WITH STANDARD DOSE IMATINIB: A STUDY FROM GRUPPO TRIVENETO LMC

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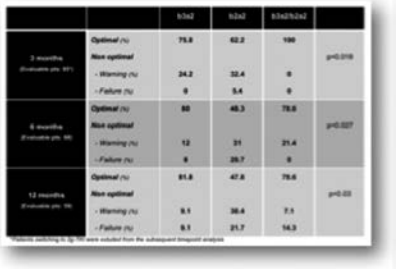
Background: The clinical significance of BCR-ABL transcript type in the tyrosine kinase inhibitors (TKI) era has recently been debated. However, few data concerning the impact of different BCR-ABL transcripts on early response dynamics in chronic phase-chronic myeloid leukemia (CP-CML) patients treated with standard dose imatinib (IM) have been reported.
Aims: To evaluate the probability of optimal response at early timepoints, according to different p210 BCR-ABL1 transcripts, in a real life population of CML-CP patients treated with standard dose front-line imatinib.
Methods: 93 CP-CML patients treated with IM 400 mg daily, diagnosed from 2010 to 2015 were retrospectively analyzed. The type of BCR-ABL1 transcript was determined by multiplex RT-PCR at diagnosis. Molecular monitoring was performed with quantitative RT-PCR and results were expressed in International Scale (IS) values. Optimal, warning and failure categories were analyzed according to the 2013 ELN recommendations. Frequencies were compared by chi-squared test ($\chi 2$) or Fisher's exact test. Progression-free survival (PFS) was calculated from the start of IM to ABP or death. Overall survival (OS) was calculated from the start of IM to death.

Table 1. Response rates at 3, 6 and 12 months according to BCR-ABL trascript type.



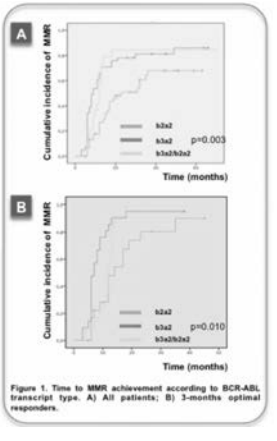


Figure 1. Time to MMR achievement according to BCR-ABL transcript type. A) All patients; B) 3-months optimal responders.

Figure 1.

Results: Patients with b3a2, b2a2, b3a2/b2a2 BCR-ABL transcript were 44.1%, 36.6% and 19.4%, respectively. The cohorts were comparable for age, sex and risk score (both Sokal and Eutos). Forty-three patients (46.2%) moved to second generation TKI (2GTKI), 24 (55.8%) due to primary or acquired cytogenetic or molecular resistance, 19 (44.2%) for intolerance. Rates of optimal *vs* non optimal response at 3 months were different between b3a2 *vs* b2a2 *vs* coexpressed transcript groups (75.8% *vs* 62.2% *vs* 100% respectively, p=0.018). Interestingly, all failures at 3 months were observed in the b2a2 group (Table 1). At 6 months, optimal responses were documented in 80% *vs* 48.3% *vs* 78.6% of b3a2, b2a2 and b3a2/b2a2 group, respectively (p=0.027); 60% of warnings and 75% of failures were associated to b2a2 transcript. At 12 months timepoint, major molecular response **(**MMR) rate was significantly different between transcripts (81.8% *vs* 47.8% *vs* 78.6% respectively, p=0.03). 52% of treatment changes within 12 months, either for resistance or intolerance, were observed in the b2a2 group. Differences in early response did not translate into inferior cumulative incidence of complete cytogenetic response (CCyR) (93.9% *vs* 82.1% *vs* 83.3% respectively, p=0.30) or different time to CCyR achievement. Patients with b3a2 and b3a2/b2a2 transcript had comparable rates of cumulative MMR (81.8% and 88.9%, respectively), superior to b2a2 patients (66.7%, p=0.12) with a significantly faster time to MMR achievement, either on IM treatment or with sequential IM+2GTKI approach (Figure 1A). Notably, among optimal responders at 3 months, MMR was obtained significantly later by patients with b2a2 transcript (Figure 1B). Long term outcomes (PFS, OS) were not affected by transcript type.
Summary/Conclusions: This retrospective analysis suggest a different kinetics of early optimal response achievement based on transcript type, with inferior rates in patients expressing b2a2. However, this difference did not translate into unfavourable clinical outcome. Longer time to MMR among optimal responders presenting with b2a2 could partially account for the negative impact of this transcript type on stable deep molecular response, previously observed by our group.

E1100

CHRONIC MYELOID LEUKEMIA WITH EXTREME THROMBOCYTOCIS AT THE DIAGNOSIS: A NEW SUBSET

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Background: Chronic myeloid leukemia (CML) is the result of t(9;22) balanced translocation and/or presence of BCR-ABL1 fusion gene. Usually CML presents with marked leukocytosis and only in rare cases CML can present at the diagnosis with an isolated and marked thrombocytosis, defined as platelet count >1000x10^9/L. There are no data on the optimal treatment of these subcategories of "theoretically high risk" patients in the era of tyrosine kinase inhibitors (TKIs).
Aims: To identify a specific subset of CML chronic phase patients with extreme thrombocytosis (platelet count >1000x10^9/L) at diagnosis and to assess clinical outcomes: overall survival, progression free survival and cumulative response in terms of CCyR and/or MMR at 12 and 24 months
Methods: Data were collected and evaluated through the analysis of 1591 CML CP patients registered in 16 Italian Haematological Departments from January 2002 to December 2015
Results: The incidence of extreme thrombocytosis at diagnosis was 5,4% (87/1591). Median age was 59 years (range 18-87), F/M 63/24. Median spleen size below the costal margin was 0 cm (range 0-15), and liver size below the costal margin was 0 cm (range 0-3). Median white blood cell count was 31.600x10^6/microl(range 6.340-390.000), median haemoglobin level was 11,8 g/dl (range 7,7-16,3), and median platelet count was 1466x10^9/L. (range 1054-4720). Sokal score was low in 6 patients (6,8%), intermediate in 23 (26,4%) and high in 55 (63,2%). Hasford score was low in 17 patients (22%), intermediate in 22 (28,6%) and high in 38(49,42%) out of 77 evaluable cases. Coagulation tests were available in 59/87 (67,8%), and aPTT was abnormal in only three patients (5%). Bleeding time test was available only in 5 patients and was prolonged in all of them (median value 41 sec1"). Iron assessment was reported in 56 out of 87 patients and was consistent with iron deficiency in 7 patients (12,5%); assessment of the, JAK2V617F mutation was performed in 43 (49,4%) patients. At diagnosis, 9 out of 87 (10.3%) patients showed haemorragic or thrombotic complications, with only 3 of grade >2 according to the NCI-CTC.In order to reduce severe thrombocytosis, 73/87 patients (83%) received hydroxyurea before starting TKI and in two patients thrombocyta-

pheresis was reported[s1]. As first line TKI 63/87 (72,4%) patients received imatinib, 16 (18,4%) dasatinib and 8 (9,2%) nilotinib.Seventy-seven out of 87 patients were evaluable after 12 months of treatment, and 67 of 77 (87%) obtained cCyR or major molecular response (MMR). Sixty-seven patients were evaluable at 24 months of treatment and 51 out of 67 (76,1%) were in cCyR and/or MMR. After a median follow-up of 66 months (range 3-179), 81/87 (93,1%) patients are still alive. Six patients (6,9%) died: two from myocardial infarction, three from concomitant neoplasia, and one from GvHD.Nine (10,3%) patients developed primary resistance to imatinib and they were shifted to the second line. Eight additional patients developed secondary resistance, mainly after imatinib

Summary/Conclusions: The incidence of extreme thrombocytosis in CML chronic phase is rare;this subset of patients shows the following peculiarities: predominance of female sex, high or intermediate risk score with a favourable outcome in terms of both cumulative response rate and survival, low incidence of thrombotic/haemorragic risk[s1]. Due to the inclusion of patients prior to the acquisition of JAK2 V617F in the differential diagnosis of thrombocytosis, only half of the patients were assessed for this mutation

E1101

BCR-ABL1 TRANSCRIPT LEVEL HALVING TIME IS THE ULTIMATE PROGNOSTIC FACTOR FOR ACHIEVING MAJOR AND DEEP MOLECULAR RESPONSE IN CML PATIENTS

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Background: Since 2012, the dynamics of early molecular response to tyrosine kinase inhibitor (TKI) treatment is being considered a very useful prognostic factor for further outcome of chronic myeloid leukemia (CML) patients. Two ways of determining dynamics of response are being used: *BCR-ABL1* transcript level at 3rd month of the therapy and individual transcript reduction rate, expressed as *BCR-ABL1* halving time (HT).
Aims: The goal of this analysis was to determine whether *BCR-ABL1* transcript level at third month or *BCR-ABL1* HT is more useful for predicting the molecular responses. Another aim was to perform the analysis using *GUSb* as a reference gene, which should provide more accurate results for high *BCR-ABL1* transcript level samples.
Methods: The analyzed group consisted of 96 patients diagnosed with CML in Faculty of Hematology and Bone Marrow Transplantation at Poznań Medical University, who were monitored and treated according to ELN recommendations. The *BCR-ABL1* transcript level assessment was performed using an in-house method with *GUSb* as a reference gene. The method is being validated on a regular basis in the reference laboratory in Adelaide, and in 2014 was positively validated for MR4.5 assesment (ELN/EUTOS performance evaluation). Statistical analysis was made using Statistica 10. All analyses were performed only in patients with full clinical documentation, non-degraded pre-treatment sample and at least 3 years of follow up. The Kaplan-Meier analyses of MMR, MR4 and MR4.5 were performed for groups divided by *BCR-ABL1* halving values (5-40 days interval). The frequencies of molecular responses in 12th, 24th and 36th month of therapy were aggregated in tables and the graphs showing MMR, MR4 and MR4.5 in selected time points depending on HT cut-off values were plotted.

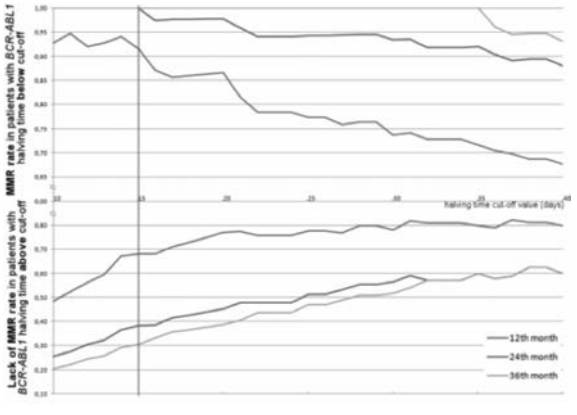

Figure 1.

Results: The *BCR-ABL1* transcript level at 3rd month was proven to have a limited prognostic value. The values greater than 10% and lower than 1% have significant prognostic implications; the interval between 1 and 10% (49% of our patients) does not predict any molecular outcome. On the other hand, the *BCR-ABL1* HT of 15 days was proven to be a great predictor of MMR, MR4 and MR4.5. 91,5% of patients with HT shorter than 15 days achieved MMR by 12th month of treatment (100% by 24th and 36th month). In case of deep molecular responses, 61,1% achieved MR4 after a year of treatment (83,3% by 24th and 94,4% by 36th month) and 33,3% MR4.5 after twelve months of TKI therapy (80,5% by 24th and 83,7% by 36th month). The molecular outcome of patients with *BCR-ABL1* HT greater than 15 days was significantly worse: 31,8%, 61,7% and 69,4% had MMR by 12th, 24th and 36th month of therapy, respectively. Moreover, the frequency of deep molecular responses was also significantly lower: 9,1%, 36,5% and 41,4% for MR4 and 4,5%, 16% and 28,8% for MR4.5, respectively.
Summary/Conclusions: Our results confirmed that the use of *GUSb* as a reference gene allows precise evaluation of transcript level at the beginning of the treatment and probably increases the prognostic value of *BCR-ABL1* HT. The *BCR-ABL1* HT below 15 days is an accurate predictor of molecular response for TKI treated CML patients. The response to the therapy is so good that the surrogate endpoint such as MMR is achieved by vast majority of them, regardless of the initial response dynamics. The *BCR-ABL1* HT greater than 15 days also predicts significantly lower molecular response rates, especially MR4 and MR4.5. It is worth pointing out that the proposed *BCR-ABL1* HT cut-off is applicable to the entire group of patients and allows for early identification of potential non-responders.

E1102

MEASUREMENT OF BCR-ABL1 BY RT-QPCR IN CHRONIC MYELOID LEUKAEMIA: FINDINGS FROM AN INTERNATIONAL EQA PROGRAMME

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Background: *BCR-ABL1* measurement by RT-qPCR is integral to both the diagnosis and monitoring of Chronic Myeloid Leukaemia (CML), and has been central to the remarkable improvement in patient outcomes seen in this disease. As an ISO 17043 accredited international provider of External Quality Assessment (EQA)/Proficiency Testing (PT) in this area, UK NEQAS for Leucocyte Immunophenotyping (UKNEQAS LI) has a unique perspective on the evolving field of *BCR-ABL1* testing in CML by having access to participants' technical data.
Aims: To assess the impact of technical standardisation and the development of the International Scale (IS) on the accuracy of *BCR-ABL1* testing, by reviewing our EQA trial data.
Methods: We analysed all trial data submitted to UKNEQAS LI that could be meaninfully compared between 2007 and 2015 (16 trial issues, 32 samples). In order to normalise the log normal EQA data, and allow the application of parametric statistics, a constant was applied to all participant data before log10 transformation. Robust statistics were used to assess variation, including the exclusion of outliers as per Algorithm A from ISO 5725-5. Whilst variation was calculated using log transformed data, where a meaningful data midpoint was required, medians were used because log transformed means (geometric means) are difficult to interpret and back transforming log transformed means is not recommended as it leads to underestimation.
Results: Comparison of participant results identified considerable variability at high and low levels of disease, including current and proposed therapeutically important levels. Despite rapid adoption of the IS by laboratories, and the fact that we have shown lower standard deviations in our IS data sets when compared to our unconverted data sets, suggestive of decreased variability, a statistically significant difference in variation between our unconverted and IS data sets could not be proven. We found that different methods of converting to the IS are producing consistently different median results within UKNEQAS LI IS data sets.

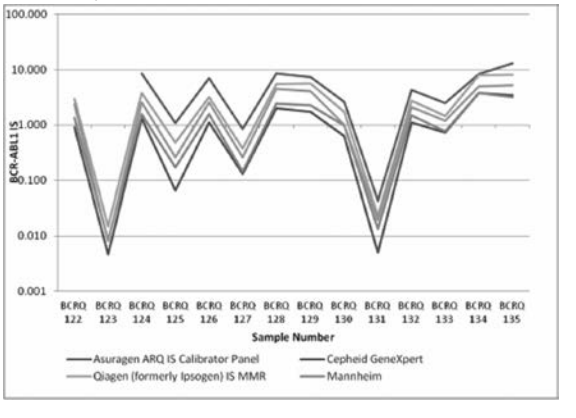

Figure 1.

Summary/Conclusions: To allow better quantification of 'Early' and 'Deep' Molecular Response to fully realise the benefits of improved treatment regimens our data suggests that further refinement of RT-qPCR protocols, or perhaps a

switch to new technologies such as droplet digital PCR, will be required. The finding that different methods of converting to the IS are producing consistently different median results suggests the need for a review of the process of converting to the IS.

E1103

VERY EARLY MOLECULAR RESPONSE AT 1 MONTH CAN PREDICT 12-MONTH MAJOR MOLECULAR RESPONSE IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA TREATED WITH TKIS

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Background: Imatinib (IM) has been the standard of care for chronic phase (CP) chronic myeloid leukemia (CML). Recent studies have demonstrated that BCR-ABL1 transcript levels at 3 and 6-month of front-line therapy is a useful predictor of long-term outcomes. Neelakantan *et al(Blood 2013; 121:2739-42).* found that the prognostic value of the 3-month early molecular response (EMR) could be improved by combining the 3- and 6-month results, but suggested that the 3-month EMR had superior prognostic value than the 6-month EMR so early interventions could be determined based on the 3-month result. However, prognostic value of very early molecular response (VEMR) have not been fully defined.
Aims: In this study, predictive value of BCR-ABL1^{IS} at 1 month after front-line therapy for an achieving MMR at 12 months was evaluated. In addition, the difference of prognostic role of VEMR in IM group and 2G TKI group were compared.
Methods: With a data cut-off date of 20 Feb 2016, among 223 newly diagnosed CP CML patients who received IM (N=127) and 2G TKI (N=96) treatment and had molecular data at 1 month, 98 patients in IM group and 65 patients in 2G TKI group with at least 1 year of follow-up and available RQ-PCR at 12 months were included. Based on receiver operating characteristic (ROC) curve analysis, the predictive cutoffs of BCL-ABL1 transcripts at 1 month for achieving MMR at 12 months in IM group and 2G TKI group were evaluated. All RQ-PCR were tested with at least 4.5-log sensitivity in the central laboratory (Catholic Leukemia Research Institute, The Catholic University of Korea, Seoul, Korea).
Results: A total of 163 patients (101 men and 62 women) were analyzed. With a median age of 44 years (range, 18-81 years), the distribution of low, intermediate, and high Sokal risk scores were 41%, 39%, 20%. 36 (37%) of 98 patients in IM group and 46 (71%) of 65 patients in 2G TKI group achieved MMR at 12 months of front-line treatment. The predictive cutoffs of BCR-ABL1^{IS} level at 1 month for achieving MMR at 12 months were 42.57% (sensitivity 66.67%, specificity 56.45%, AUC 0.585(0.481-0.684), P=0.1617)and 38.41% (sensitivity 84.78%, specificity 89.47%, AUC 0.846(0.734-0.923), P=0.0001)in IM group and 2G TKI group, respectively based on ROC curve analysis. In IM group, 24(47%) of 51 patients with ≤42% and 12(25%) of 47 patients with >42% achieved 12-month MMR respectively (P=0.027). In 2G TKI group, 39(95%) of 41 patients with ≤38% and 7(30%) of 24 patients with >38% achieved 12-month MMR respectively (P<0.001).
Summary/Conclusions: Our results demonstrated that VEMR is a predictor for an achieving 12-month MMR in both IM-group and 2G TKI group. Specially, 38.4% cutoff in 2G TKI group showed higher sensitivity and specificity, compared with those of IM group.

E1104

PONATINIB EVALUATION AND SAFETY IN REAL LIFE CHRONIC PHASE (CP) CML PATIENTS FAILING ≥2 TYROSINE KINASE INHIBITORS (TKI): UPDATE OF THE PEARL OBSERVATIONAL STUDY

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Background: Ponatinib (PON) induces high response rates in heavily pretreated CML patients (pts) failing TKIs. A substantial proportion of pts on PON [17% by 3 years, J. Cortes, Haematologica 2015] experience severe arterial thrombotic events (ATE). The impact in the real-life setting of this recently licensed agent is unknown yet.
Aims: We analyzed the French PON compassionate use program (19/04/2012-30/09/2013) to evaluate outcomes in the real-life setting.
Methods: Multicenter observational retrospective study to examine safety and efficacy of PON in CML failing TKIs, benefiting from the national PON compassionate use program. Data collection followed the regulations of observational studies in France. Pts were analyzed in intention-to-treat. Molecular biology tests were expressed as *BCR-ABL/ABL* (%IS) in all centers. Standard clinical data, cardiovascular risk factors (CVRF), onset of any CV events (before and during PON treatment) and metabolic biological parameters were captured.
Results: 48 patient records were collected in CP, 5 in AP and 6 in BC. We focused our analysis on CP pts only. There were 24 (50%) males, median age of 53 (18-76) years at CML diagnosis and 60 (20-82) years at PON initiation. Sokal scores were high in 15 (31%), intermediate in 17 (35%), low in 6 (12%) and unknown in 10 (22%) pts. Fifteen pts (31%) were treated for hypertension, 2 were diabetic, 11 (23%) had dyslipidemia (all on statins), tobacco abuse was present in 19 (39%) pts (1 unknown) and 21 (44%) pts had some pre-existing cardiovascular risk factors (CVRF) in total prior to PON. Median weight was 66 (50-107) kg, and BMI was 24 (17.85-43) kg/m^2. Fifteen (31%) pts (3 unknown) were on anti-aggregants or anti-coagulants (AAG/C) before PON. Two pts had nilotinib, 4 dasatinib and all other pts had imatinib first-line for a median of 22 (2-132) months, 25 (52%) dasatinib and 20 (42)% nilotinib for 14 (0.5-64) months, as second-line, 4 pts developed a T315I mutation after imatinib only; 29 (60%) had received all 3 TKIs prior to PON. At PON initiation, 11 (23%) harbored a T315I mutation, 9 (19%) other mutation(s), 24 (50%) none (3 not done). The trigger for PON was resistance in 34 (71%) pts, intolerance in 11 (23%) pts and both in 3 (6%) pts. Pts were initiated at a median of 45 (15-45) mg daily after a median of 73.5 (17-217) months of disease duration. The median follow-up was 26.5 (2-42) months since PON initiation, and 102.5 (27-230) months since diagnosis. Median time on PON was 19 (0.13-41.79) months. Eight pts died, 6 of disease progression, 1 of myocardial infarction and 1 of PAOD-related complications. The probability of OS was 81.5 (70.5-94)% at 36 months (Fig. 1a) with no influence of the presence of mutations or reason for PON prescription. Overall, the cumulative incidence of MMR was 55 (35-73)% at 18 months. Seven (14.5%) pts had hematologic AEs imposing transient PON withhold, and 19 (40%) diverse grade 1-2 non-hematologic, non-CV AEs (pancreatic, hepatic, skin toxicities, no grade 3-4). Significant CV AEs (including hypertension) occurred in 29 (60%) pts after a median of 5.8 (0.1-25.4) months of PON (Fig 1b), in 11 pts without CVRF. Eight thrombotic events occurred (3 heart strokes, 2 brain strokes, 1 PAOD, 2 DVT). Of note, 3 thrombotic AEs occurred on AAG/C. Lipids and HbA1c do not seem to be modified on PON. At last follow-up 22 (46%) pts are still on PON.

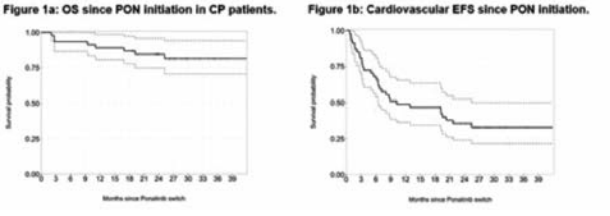

Figure 1.

Summary/Conclusions: In this real-life setting, CP-CML patients resistant or intolerant to previous TKIs, PON still displayed strong efficacy, ATEs represent the main adverse factor observed.

E1105

IMATINIB FIRST-LINE WITH SWITCH TO 2ND GENERATION TYROSINE KINASE INHIBITORS IN CASE OF FAILURE OR TOXICITY: REAL-LIFE DATA FROM A POPULATION-BASED REGISTRY OF BCR-ABL1+CML PATIENTS

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Background: The main academic-and company-sponsored studies of the treatment of CML with TKIs focus on the results achieved with only one TKI, in single-arm trials or in randomized trials comparing two TKIs as first line treatment. After the discontinuation of the study drug, few data on further treatments are collected. In real life and in many countries, for several years imatinib has been used as first-line treatment, but nilotinib and dasatinib have been available and used as second-line therapy.
Aims: To investigate the response and the outcome on first-line imatinib, with switch to 2nd generation tyrosine kinase inhibitors (TKIs) in case of failure or toxicity, in BCR-ABL1+ chronic myeloid leukemia (CML) patients enrolled in a population-based registry (real-life setting).
Methods: From a cohort of 337 consecutive, unselected, newly diagnosed, adult, chronic phase, Ph+, BCR-ABL1+, CML patients who were registered according to population-based criteria in two Italian Regions (Emilia-Romagna and Sicily) between 2008 and 2012, we identified 236 patients who were treated with imatinib 400 mg once daily in first-line. The decision to switch to second-line treatment was up to the Local Investigator (no predefined criteria) Definitions: major molecular response (MMR): BCR-ABL1^{IS} ratio<0.1%; MR4.0: BCR-ABL1^{IS} ratio<0.01% with >10,000 ABL1 copies; failure and suboptimal response: according to 2009 European LeukemiaNet (ELN) criteria; progression: transformation to advanced phases according to ELN criteria; leukemia-related death (LRD): death after progression.
Results: Sokal risk distribution was 29% low, 47% intermediate, and 24% high. Median age was 60 years at diagnosis and 64 years at last analysis, with a median follow up of 4 years. 145 patients (61%) received only imatinib, 57 (24%) were switched to 2nd generation TKIs for failure, and 34 (14%) were switched to 2nd generation TKIs (n=31) or to Hydroxyurea (n=3) for toxicity. The median time to switch was 20 months for failure and 8 months for toxicity. After the switch, the molecular response improved of 1 to 3 logs in 57% of patients. Molecular responses and outcomes are shown in the Table. Eleven patients (5%) progressed to blast phase, and all of them died, but one. The 5-year leukemia-related survival was 95%. The 5-year overall survival was 89%, with 6% of patients dying in major molecular remission or in chronic phase, without any evidence of progression. Noticeably, 48% of living patients had achieved MR 4.0 (BCR-ABL 1 ≤0.01% IS) by 4 years (including first- and second-line treatment).

Table 1.

No. of patients	236
Early molecular response (BCR-ABL1 ≤ 10% at 3 months)	80%
Major molecular response (BCR-ABL 1 ≤ 0.1%) at 1 year	55%
Major molecular response (BCR-ABL 1 ≤ 0.1%) at 2 years	69%
Major molecular response (BCR-ABL 1 ≤ 0.1%) at 4 years	75%
MR 4.0 (BCR-ABL ≤ 0.01%) at 1 year	24%
MR 4.0 (BCR-ABL ≤ 0.01%) at 2 years	40%
MR 4.0 (BCR-ABL ≤ 0.01%) at 4 years	48%
Failure and suboptimal response$^{\Phi}$ (including progression)	23%
Progression to blast phase/death in blast phase$^{\Phi}$	5%
Death in remission or in chronic phase $^{\Phi}$	6%
$^{\Phi}$as defined by European LeukemiaNet 2009	

Summary/Conclusions: A policy of imatinib first-line with switch to 2nd generation TKIs for failure or toxicity, as recommended by European LeukemiaNet 2009, in an unselected (median age 60 years) cohort of patients registered according to population-based criteria showed a high efficacy.

E1106

THE ALTERATION OF IMMUNOPROFILE DURING DASATINIB TREATMENT IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA: A PROSPECTIVE OBSERVATIONAL STUDY

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Background: Some chronic myeloid leukemia (CML) patients treated with dasatinib develop lymphocytosis with an expansion of large granular lymphocytes (LGLs) and natural killer (NK) cells. The lymphocytosis is known to be related to a better response to dasatinib therapy and survival. However, it has been not elucidated whether increased lymphocytes harbor an effective immunological and/or anti-tumor activity.
Aims: The aims of this prospective study were 1) to evaluate changes of NK-cell reactivity and soluble factors and 2) immune cell counts by dasatinib therapy in treatment naive CML patients and those who were treated with dasatinib beyond the first line therapy.
Methods: Thirty patients with CML in chronic phase who started tyrosine kinase inhibitor (TKI) as the first-line treatment or switched to the other TKI were enrolled in this study between September 2010 and December 2013 at our institute. TKIs were; dasatinib (as first line n=7, beyond first line n=12), imatinib (as first line n=3), nilotinib (as first line n=2, beyond first line n=6). We analyzed peripheral blood samples collected from patients at enrollment, 1, 3, 6 and 12 months after the initiation of treatment to determine cell counts, lymphocyte subset (such as NK cells, NK-LGLs, T-LGLs and regulatory T cells) immunophenotyping by flow cytometry, NK cell activity and plasma levels of 27 soluble factors.
Results: The number of lymphocytes, NK cells, T-LGLs and NK-LGLs significantly increased in patients treated with dasatinib (all P<0.01). NK cell activity at effector to target (E: T) ratios of 10: 1 and 20: 1 significantly elevated in patients with dasatinib as the first-line treatment (P<0.01) and beyond first-line treatment (P<0.01). The levels of NK cell activity at diagnosis were below the normal level in all treatment naive CML patients and elevated above the normal level at 12 months after the dasatinib therapy in more than half of the patients. In addition, NK cell activity was significantly negatively correlated with BCR-ABL mRNA transcript levels in the dasatinib group (r= -0.304, P=0.011) but not in patients treated with imatinib or nilotinib. A significant association was found between the numbers of T-LGL before and 3 months (P<0.01), and before and 6 months after dasatinib therapy (P<0.01). There were no significant change in counts of any immune cell in patients receiving imatinib or nilotinib. Furthermore, we could not determine a type of chemokine or cytokine associated with lymphocytosis induced by the dasatinib treatment.
Summary/Conclusions: Dasatinib might increase the numbers of LGL and enhance NK-cell cytotoxicity *in vivo*. Dasatinib might restore the function of exhausted cytotoxic lymphocytes present already at the time of CML diagnosis. Increase in LGL counts was strongly correlated with the number of LGL before dasatinib therapy. We speculated that the number of LGL present before dasatinib therapy is potentially a prognostic factor.

E1107

PLA FLOW; A FLOW CYTOMETRY-BASED ASSAY FOR DETECTION OF BCR-ABL FUSION PROTEIN IN BLOOD CELLS FROM CML PATIENTS

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Background: Chronic myeloid leukemia (CML) is currently diagnosed using RT-PCR and/or FISH to reveal the presence of the fusion mRNA transcripts for BCR-ABL, or of the characteristic Philadelphia chromosome. RT-PCR is also used to monitor the effects of treatment by sensitively measuring transcripts representing minimal residual disease (MRD). It has not been possibly to use flow cytometry to identify the neoplastic cells but such a method would be helpful in the workflow of a hematopathology lab. The PLA flow could provide a strong complement to the powerful RT-PCR method used today, with the advantages of flow cytometry.

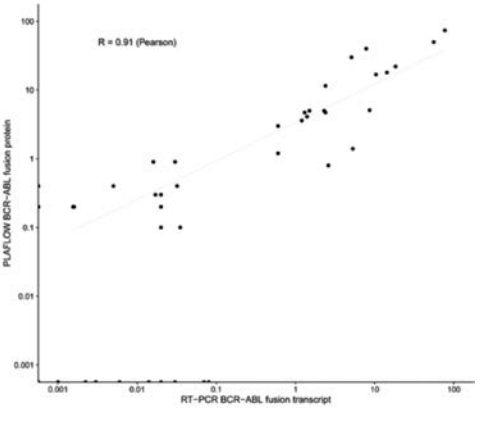

Figure 1. Blood samples from 47 CML patients analyzed for BCR-ABL positive cells, using FlowPLA (y-axis) and routine analysis of the BCR-ABL transcript using RT-PCR (x-axis) and compared. Pearson correlation coefficient was performed.

Aims: We have now developed a method that successfully detects and enumerates cells harboring the fusion protein BCR-ABL by flow cytometry in CML patients.
Methods: The method, PLAflow, uses the *in situ* proximity ligation assay (PLA)

(Söderberg et al 2006, Leuchowius et al 2009), where two antibodies target the BCR and the ABL part, respectively, of the fusion protein. The antibodies are equipped with DNA oligonucleotides that-when brought in proximity-guide the formation of a circular DNA molecule as a template for localized DNA amplification through rolling circle amplification (RCA). Each RCA-product is then labeled with around 1,500 fluorophore-coupled DNA oligonucleotides, allowing cells to be detected by flow cytometry. Using this method we analyzed blood samples from CML patients and compared it to the routine RT-PCR analysis.
Results: The method that was proven to be very sensitive, enable us to detect very low number of cells harboring BCR-ABL in patient samples.
Summary/Conclusions: The PLA flow is a senstive and robust method that could provide a strong complement to the powerful RT-PCR method used today, both at diagnosis and monitoring MRD. Since the PLA flow method is developed for flow cytometry, and now also adapted for CyTOF, all the advantages with multiparametric analysis can be achieved simultaneously. This allows monitoring of patients using their own cytogenetic signatures, both at diagnosis, and during the MRD monitoring. By investigating which cell populations are expressing the BCR-ABL fusion protein, the method may provide tools to identifying patients responding to a specific treatment.

E1108

COMPARISON OF PACE CLINICAL TRIAL *VS* REAL-WORLD PONATINIB PRESCRIBING AND DURATION OF THERAPY IN CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML) PATIENTS

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Background: Ponatinib demonstrated efficacy in patients with highly-resistant CP-CML in the pivotal phase 2 PACE trial (NCT01207440). Introduced to the US market in Dec'12, ponatinib was reintroduced in Jan'14 after an 11-week withdrawal to review data on vascular occlusive events and revise US prescribing information. In the US, ponatinib is available exclusively through a specialty pharmacy that maintains real-world prescribing data for all US patients treated with ponatinib since Jan'14.
Aims: Comparison of real-world pharmacy data to PACE trial data for patients with CP-CML, to identify any differences in duration of therapy due to potential noncompliance or availability of alternative therapies.
Methods: Data from patients with CP-CML enrolled Sep'10-Oct'11 into the PACE trial (all providing informed consent) were compared to real-world data for CP-CML patients starting ponatinib Jan'14-Dec'15. Real-world data source includes referring physicians, pharmacy intake forms and dispensing records. Patient characteristics and dosing were compared overall and by line of therapy using non-parametric tests; average dose was calculated, including therapy gaps as "zero" dose. Duration of therapy was assessed using Kaplan-Meier techniques and log-rank tests.
Results: PACE enrolled 270 CP-CML patients; 333 US real-world CP-CML patients started treatment over the 2-year period. PACE patients were older (median 60 *vs* 55 years; p=0.004). Comparing PACE to 258 real-world patients with known line of therapy, 7% PACE *vs* 16% real-world were in 2nd line, 36% *vs* 28% 3rd line and 57% *vs* 56% ≥4th line (p=0.084). All PACE patients received 45 mg/day as the initial dose of ponatinib; in real-world ponatinib use, 48% of patients initially received 45 mg/day, 30% 30 mg/day, and 22% 15 mg/day; however, average ponatinib dose was similar in PACE *vs* real-world (2nd line: 29.4 *vs* 29.0 mg/day; 3rd line: 26.0 *vs* 25.9 mg/day; ≥4th line: 26.4 *vs* 26.9 mg/day; all p>0.05.) Duration of therapy was similar in 2nd line and ≥4th line patients in PACE *vs* real-world, but longer in PACE *vs* real-world for 3rd line patients (Table).

Table 1.

Duration of Therapy in PACE vs Real-world CP-CML Patients by Line of Therapy

Line of therapy	Population (n)	6month	12month	18month	24month	P
		(%)				
2nd line	PACE (19)	95	89	84	68	0.077
	Real-world (42)	63	63	63	—	
3rd line	PACE (97)	79	69	68	64	0.047
	Real-world (72)	69	59	59	—	
≥4th line	PACE (154)	79	62	58	53	0.311
	Real-world (144)	67	57	56	—	
Total	PACE (270)	80	66	63	58	<0.001
	Real-world (333*)	66	54	52	—	

*Includes 75 patients with missing line of therapy

Summary/Conclusions: Real-world CP-CML ponatinib patients are younger but otherwise similar to PACE patients. Real-world data suggests an increase in earlier (second) line ponatinib use and >50% of initial ponatinb dosing is below 45 mg/day. As expected, the real-world duration of therapy is somewhat shorter than in PACE; however, the majority of real-world ponatinib patients across all lines of therapy were on therapy for >1.5 years.

E1109

PATIENT-REPORTED OUTCOMES FROM AN OPEN-LABEL SAFETY AND EFFICACY STUDY OF BOSUTINIB IN PHILADELPHIA CHROMOSOME POSITIVE CHRONIC MYELOID LEUKEMIA RESISTANT OR INTOLERANT TO PRIOR THERAPY

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Background: Bosutinib (BOS) is a tyrosine kinase inhibitor indicated for the treatment of Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) in adult patients with resistance or intolerance to prior therapy. A 5-year update of the safety and efficacy results from a phase 1/2 study of BOS for Ph+ leukemia resistant/intolerant to prior tyrosine kinase inhibitors (clinicaltrials.gov: NCT00261846) has recently been conducted.
Aims: To assess the long-term health-related quality of life (HRQoL), functioning, and symptoms in patients with chronic phase CML following imatinib resistance or intolerance (chronic phase second line; CP2L) or resistance or intolerance to imatinib plus dasatinib and/or nilotinib (chronic phase third line; CP3L), based on patient-reported outcomes (PROs) from this trial.
Methods: Patients received a starting dose of BOS 500 mg/day and completed the EuroQoL 5 Dimensions (EQ-5D) and the Functional Assessment of Cancer Therapy-Leukemia (FACT-Leu) questionnaires at screening; weeks 4, 8, and 12; every 12 weeks thereafter; and at the end of treatment visit. Mean and 95% CIs were reported. Informed consent was obtained from all patients. The PRO results at 5 years are presented.
Results: A total of 284 patients were enrolled in the CP2L population and 119 patients in the CP3L population. At treatment completion, EQ-5D completion rates were 67.6% and 65.5% in the CP2L and CP3L populations, respectively; FACT-Leu completion rates were 65.7% and 64.7% in the CP2L and CP3L populations, respectively. At baseline, the mean EQ-5D utility score was 0.83 in the CP2L population and 0.80 in the CP3L population. Mean (95% CI) changes from baseline in EQ-5D utility scores ranged from -0.04 (-0.13 to 0.06) to 0.12 (0.03-0.21) in the CP2L population and from -0.05 (-0.16 to 0.07) to 0.01 (-0.15 to 0.16) in the CP3L population. At baseline, the mean EQ-5D visual analog scale (VAS) score was 71.3 in the CP2L population and 72.6 in the CP3L population. Mean (95% CI) changes from baseline in EQ-5D VAS scores ranged from 0 (95% CI not available) to 30.17 (-86.96 to 147.29) in the CP2L population and from -2.64 (-6.14 to 0.85) to 19.69 (-5.30 to 44.68) in the CP3L population. At treatment completion, most patients reported "no problems" on all of the 5 dimensions of the EQ-5D health state profiles (Table 1). At baseline, the mean FACT-General (FACT-G) Total Score and FACT-Leu Total Score were 81.0 and 133.2, respectively, in the CP2L population and 80.8 and 132.5, respectively, in the CP3L population. In the CP2L population, mean (95% CI) changes from baseline in FACT-G Total Score (minimally important difference: 3-7 points) and FACT-Leu Total Score (minimally important difference: 6-12 points) ranged from -1.02 (-2.41 to 0.36) to 9.87 (0.94-18.80) and -4.66 (-14.81 to 5.48) to 17.34 (6.33−28.36), respectively. In the CP3L population, mean (95% CI) changes from baseline in FACT-G Total Score and FACT-Leu Total Score ranged from -2.74 (-5.09 to -0.39) to 9.50 (95% CI not available) and -3.05 (-6.84 to 0.73) to 7.22 (-3.40 to 17.85), respectively.

Table 1.

Table 1. Percentage of Patients on Bosutinib in the CP2L and CP3L Populations Who Reported No, Some, or Extreme Problems on the EQ-5D Health State Profiles

	No Problems			Some Problems			Extreme Problems		
	Baseline	Treatment Completion	Change From Baseline	Baseline	Treatment Completion	Change From Baseline	Baseline	Treatment Completion	Change From Baseline
Second-Line Population, %									
Mobility	75.6	78.0	2.4	24.4	21.5	−2.9	0	0.5	0.5
Self-care	97.6	91.6	−6.0	2.4	8.4	6.0	0	0	0
Usual activities	76.5	72.8	−3.7	23.1	25.1	2.0	0.4	2.1	1.7
Pain/discomfort	61.1	58.9	−2.2	35.2	36.3	1.1	3.6	4.7	1.1
Anxiety/depression	63.2	64.9	1.7	35.6	33.5	−2.1	1.2	1.6	0.4
Third-Line Population, %									
Mobility	77.7	75.3	−2.4	21.4	24.7	3.3	1.0	0	−1.0
Self-care	95.1	92.2	−2.9	4.9	6.5	1.6	0	1.3	1.3
Usual activities	67.0	66.2	−0.8	32.0	32.5	0.5	1.0	1.3	0.3
Pain/discomfort	59.2	55.8	−3.4	35.9	39.0	3.1	4.9	5.2	0.3
Anxiety/depression	56.9	55.3	−1.6	43.1	40.8	−2.3	0	3.9	3.9

Summary/Conclusions: These findings are of considerable importance to patients and physicians and indicate, for the patients who remained in the study, that HRQoL was largely maintained with BOS during the 5-year study duration in both second line and third line patients with Ph+ CML with resistance or intolerance to prior therapy.

E1110

PRELIMINARY FINDINGS FROM A CHART REVIEW OF LOWER DOSING OF PONATINIB IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

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Background: Ponatinib is approved for adult patients with refractory CML or Ph+ ALL and those with the T315I mutation. Current prescribing information recommends a starting dose of 45 mg/day, with consideration of lower doses in patients with selected comorbidities, to manage adverse events, and for patients who achieve response. Post hoc dose-response analyses from the registrational PACE trial suggest lower doses of ponatinib may mitigate safety risk while maintaining response; however, outcomes of patients with lower doses have not been evaluated in clinical practice.
Aims: Characterize response and adverse events of special interest (AESI) in real-world CML patients treated with ponatinib doses ≤45mg/day.
Methods: We conducted a chart review of adult (aged ≥18 years) US CML patients receiving ponatinib. Patients were required to have ≥6 months of follow-up from first dose; however, no minimum ponatinib exposure was required. Data were abstracted from 6 months prior to first dose ("baseline") through date of chart review, death or loss to follow up. Baseline characteristics, treatment response and AESI (including arterial occlusive events [AOEs] and venous thromboembolic events) are reported overall and by average daily dose ≤30 mg ("low dose") and >30 mg ("standard dose"), calculated including therapy gaps as "zero" dose.
Results: Preliminary analysis of 35 patients from 9 US sites found patients did not differ significantly by dosage group in baseline age, CML phase, time since diagnosis or prior therapy lines; however, fewer low dose patients had no response at start of ponatinib therapy (Table). Significantly more low dose patients had hyperlipidemia at baseline (p=0.027), and there was a trend toward more history of myocardial infarction (MI), coronary artery disease and revascularization among low dose patients (all p=0.070). Low dose patients had nominally more baseline risk factors or prior events compared to standard dose patients (Table). Median time on therapy was 11 *vs* 12 months for low dose *vs* standard dose patients, respectively (p=0.336), and dose reduction was common (73% *vs* 60%; p=0.411). Best response of major molecular response (MMR) or better was reported in 40% of low dose patients and 25% of standard dose (p=0.344); major cytogenetic response (MCyR) or better in 53% *vs* 45% (p=0.625), and major hematologic response (MaHR) or better in 80% and 65% of low *vs* standard dose, respectively (p=0.331). AESIs reported in ≥2 patients were: venous recanalization (n=8), mesenteric artery stent insert (n=6), cerebral small vessel ischemic disease (n=6), MI (n=3), increased troponin (n=3), cardiac arrest (n=2), creatine phosphokinase (n=2). There was no statistically significant difference in AESIs between groups; however, a trend toward more AOEs in patients with lower average doses (p=0.076) was noted, perhaps due to patient selection or post-event dose reduction. Power to detect differences was limited by sample size; further analysis of correlation between baseline risk factors and outcomes is ongoing.

Table 1.

Baseline characteristics of patients with average ponatinib dose ≤ 30 mg ("low dose") and >30 mg ("standard dose")

Baseline Characteristics	≤30 mg n=15	>30 mg n=20	All n=35	p-value
Median average daily dose, (mg)	22.4	43.2	31.5	
Starting dose, n (%)				0.202
45 mg	9 (60)	16 (80)	25 (71)	
30 mg	4 (27)	4 (20)	8 (23)	
15 mg	2 (13)	0 (0)	2 (6)	
Median age at ponatinib start, years	43.5	48.3	47.5	0.995
Time since diagnosis, months	48.4	30.8	34.5	0.347
CML Phase at Study Entry, n (%)				0.401
CP	13 (87)	14 (70)	27 (77)	
AP/BP	1 (7)	5 (25)	6 (17)	
Unknown/Missing	1 (7)	1 (5)	2 (6)	
# of prior TKIS, n (%)				0.454
Missing or 0	1 (7)	0 (0)	1 (3)	
1	2 (13)	8 (40)	10 (29)	
2	6 (40)	8 (40)	14 (40)	
3+	6 (40)	4 (20)	10 (29)	
Response at baseline, prior to ponatinib initiation, n (%)				0.014
MCyR or better	0 (0)	2 (10)	2 (6)	
MaHR or better	5 (33)	6 (20)	11 (31)	
No response	5 (33)	10 (50)	15 (43)	
Unknown/Missing	5 (33)	2 (10)	7 (20)	
Any risk factor or history prior of events, n (%)	9 (60)	8 (40)	17 (49)	0.315

Summary/Conclusions: Preliminary data suggest that in clinical practice, response is similar among CML patients treated with low and standard dose ponatinib. Patients with selected risk factors or history of prior event received lower average doses, and the trend toward greater number of AOEs in the low dose group suggests that AOEs may be correlated more with risk than dose. Additional data are needed to fully characterize outcomes of patients treated with ponatinib at doses lower than the currently recommended 45 mg/day.

E1111

IMPACT OF IMATINIB PHARMACOKINETICS ON HEALTH RELATED QUALITY OF LIFE AND ADVERSE EVENTS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Imatinib has improved survival of chronic myeloid leukemia (CML) patients considerably. However, many patients suffer persistent, low-grade adverse events (AEs) and have relatively inferior health-related quality of life (HRQOL). This impairment is in part ascribed to low grade AEs, which is experienced by a large proportion of patients. Studies have demonstrated that imatinib pharmacokinetics may correlate with imatinib-related AEs but none investigated the impact on HRQOL.
Aims: To determine the correlation of imatinib pharmacokinetics with HRQOL, incidence and severity of AEs.
Methods: A prospective cross-sectional study of 70 chronic phase CML patients at Singapore General Hospital was performed. All patients were on imatinib for at least 6 months. Blood samples for imatinib pharmacokinetic analysis were taken at 0 (pre-dose, trough) and 2 hours (peak) after imatinib administration and analyzed via high performance liquid chromatography. Oral clearance was calculated using: $Cl_{oral}=(D/2t)/C_{ss}$, where D=dose (mg), t=dosing interval (hr), and C_{ss}=steady state imatinib concentration (ng/ml). AEs and HRQOL were measured using M.D. Anderson Symptom Inventory Chronic Myeloid Leukemia Module (MDASI-CML), which was filled up within 48 hours of imatinib pharmacokinetic sampling. MDASI-CML is a multi-symptom patient-reported outcome comprising of 20 symptom items (13 core MDASI items, 7 CML-specific symptom items) and 6 HRQOL interference items. Higher scores indicate more severe AEs and worse HRQOL. Clinical responses was determined based on European LeukemiaNet 2013 guidelines. The study was approved by the institutional review board and all participants were required to sign informed consent.
Results: Median daily dose of imatinib was 400mg and the median imatinib trough concentration, peak concentration and clearance were 1365 ng/ml, 2372 ng/ml and 5.74 L/hr respectively. Impairment in at least one of the six HRQOL life activities was experienced by 65.7% of patients while 91.4% of patients experienced at least one AE. Higher HRQOL scores significantly correlated with higher imatinib trough (r=0.274; p=0.021) and slower clearance (r=-0.331; p=0.005) but not with imatinib peak concentrations. Median HRQOL score was significantly higher in the trough Q4 group compared to the Q1 group (1.50 *vs* 0.17; p=0.034) and was significantly lower in the clearance Q4 group compared to the Q1 group (0.17 *vs* 1.50; p=0.010) (see Figure 1). Higher imatinib trough and slower clearance also correlated with increased patient-rated incidence of AEs (r=0.238; p=0.045 and r=-0.278; p=0.020 respectively) and increased severity of AEs (r=0.248; p=0.037 and r=-0.315; p=0.008 respectively), but not with clinical response.

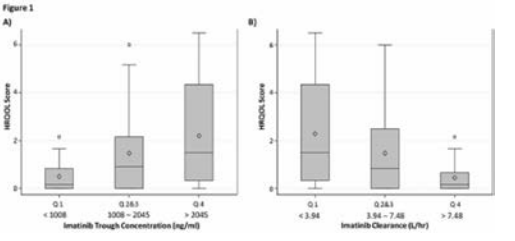

Figure 1.

Summary/Conclusions: Higher imatinib trough levels and slower clearance of imatinib are associated with poorer HRQOL, higher incidence and increased severity of AEs. Dose adjustment using imatinib trough levels may be beneficial in reducing AEs and improving HRQOL.

E1112

CMREGISTRY: AN OBSERVATIONAL, MULTI CENTER, PROSPECTIVE FOLLOW-UP REGISTRY OF PATIENTS WITH CHRONIC PHASE CML WITH A HIGH PROBABILITY OF OBTAINING A STABLE DEEP MOLECULAR RESPONSE >CMR4 (IS)

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Background: Since the advent of Tyrosine Kinase Inhibitors (TKI), many patients diagnosed with Chronic Myeloid Leukemia (CML) in chronic phase could achieve a deep molecular response, defined as at least a 4-log reduction in the bcr-abl transcripts when measured by a standardized PCR on the International Scale (IS). These patients are expected to maintain their molecular response even after discontinuation of their TKI treatment. Several on-going clinical trials are exploring the best way of stopping TKI therapy and evaluating the patient and disease characteristics that could predict long term disease control without treatment.
Aims: The aim of CMRegistry is to collect clinical data and molecular informa-

tion in the international scale for CML patients in Spain that have achieved a series of cytogenetic or molecular responses at different time points to any of the tyrosine kinase inhibitors currently available in Spain in order to monitor their progress and the achievement of a stable deep molecular response >MR^4 $^{(IS)}$. These data may be used to identify patients that would be candidates for inclusion in future discontinuation studies or combination studies with other compounds.

Methods: This is an observational, multi-center, prospective study open to all CML patients that are receiving treatment with any of the tyrosine kinase inhibitors currently available in Spain and are likely to achieve (or have already achieved) a deep molecular response (>$MR^{4\ (IS)}$). This likelihood of achieving >MR^4 is defined for the purposes of the study as a bcr-abl/abl ratio of: 1) ≤1% at 3 months from start of TKI therapy; 2) ≤0.1% at 6 months from start of TKI therapy; or 3) ≤0.01% any time point during treatment. Clinical data have been collected using a specific CRF created exclusively for this study. All data were registered in an anonymous manner. The BCR-ABL ratios in the IS have been provided by standardized labs in Spain.

Results: From June 2014 to January 2016 a total of 732 patients were registered in the study. Median age was 55 years (18-78) and 440 patients were male. The Sokal risk groups were as follows: 254 patients low risk, 230 intermediate risk and 97 high risk. Hasford (Euro) risk stratification showed 223 patients low risk, 225 intermediate and 136 high risk. Eutos classification yielded 341 patients in the low risk and 220 in the high risk categories. The majority of patients received treatment with Imatinib (409 patients), Dasatinib (82 patients) or Nilotinib (122 patients). Of note, 5 patients received Bosutinib, 1 patient Ponatinib and 4 patients were treated with Interferon. So far 722 patients remain alive. 10 patients have died, mostly of non-CML related conditions such as Carcinoma (3 patients), Cerebral Hemorrhage, Ischemic heart disease, respiratory failure and sepsis (1 patient each). Interestingly, 2 patients developed progression of their CML to Accelerated phase and blast crisis (1 patient each). At present, 104 patients (14%) have achieved a MR^4 and 70 (10%) patients a MR^5, while 176 patients (24%) have obtained a complete molecular remission (undetectable bcr-abl transcripts with a sensitivity of at least 10^{-5}).

Summary/Conclusions: In summary, a large number of CML patients have been identified in Spain in a prospective study as having a promising molecular response that would predict for a sustained deep molecular remission. A significant proportion of these patients has already achieved a complete molecular remission and would be potential candidates for discontinuation studies.

E1113

BCR-ABL1 MONITORING ON THE IS USING AN ANALYTICALY AND CLINICALLY VALIDATED MULTIPLEX ASSAY DIRECTLY ALIGNED TO THE WHO PRIMARY STANDARDS

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Background: Detection of BCR-ABL1 e13a2/e14a2 fusion transcripts (major breakpoint, M-BCR) of t(9;22) is important in studying tumor burden in CML. The International Scale (IS) was established to standardize the reporting of these transcripts against a common baseline. As newer TKI therapies create deeper responses, analytical sensitivity has become a critical topic in investigations into TKI discontinuation, where researchers require an assay that confidently calls molecular responses of ≥4.5 logs below baseline (0.0032%IS or MR4.5). This has led to various reporting formats as a patient achieves deep response over time, creating a non-contiguous language of monitoring: baseline, 10%IS, 1%IS, MMR, MR4, and MR4.5.

Aims: We describe the analytical and clinical validation of a multiplex assay system reporting continuous MR values via automated software analysis, clinical accuracy at MR3, analytical sensitivity of MR4.7, and direct traceability to the WHO Primary BCR-ABL1 reference materials without requiring establishment and revalidation of a conversion factor.

Methods: We developed reagents for RT-qPCR, both steps performed on the ABI 7500 Fast Dx. Armored RNA Quant® (ARQ) technology was employed to generate a blend of nuclease-resistant BCR-ABL1 and ABL1 RNA transcripts to calibrate and control the system. A single four-point standard curve using ARQ blends mimics the WHO Primary BCR-ABL1 reference materials and accounts for the relative batch run-specific efficiency of the RT step. cDNA generation and qPCR were optimized, including allowance of high mass of nucleic acid without inhibition. Residual clinical RNAs were tested to estimate LOD at minimum RNA input. Software was developed, including a floating, traceable logic algorithm to ensure that sufficient ABL1 was detected to protect this LOD. A multi-center clinical outcome study was conducted at 3 clinical laboratories to validate clinical monitoring. Performance was assessed by event-free survival (EFS) at 32-40 months against test results at 12-18 months on TKI as estimated by the Kaplan Meier survival function. A total of 139 samples from 98 patients were enrolled at 2 clinical sites.

Results: We surpassed the desired analytical sensitivity: 1680 measurements were generated across 28 levels and yielded an LOD estimate of 95% positivity by probit analysis of MR4.74 (0.0018%IS). LOQ was similar. Despite deep analytical sensitivity, this system maintains analytical specificity (non-leukemic and non-CML leukemic specimens were true negative). Linearity was observed from at least MR0.3 (50%IS) to MR4.7 (0.002%IS). Single-site precision included lot, instrument, operator, and run, and was verified as SD ≤0.13 for MR values ≤3.7. Multi-site precision included site, instrument, operator, and day, and was verified as SD ≤0.10 for MR values ≤3.7. Traceability to the higher order WHO standards was demonstrated. In the clinical study, the difference in EFS between.

Summary/Conclusions: The BCR-ABL1 test improves workflow with its streamlined reagent formulation, multiplex assay format, and automated software analysis. It facilitates assessment on the IS without conversion (through integrated ARQ materials traceable to the WHO Primary), reports results on a continuous scale (as both MR and%IS values), and generates results sufficient for studies in deep molecular responses. Further, it is clinically validated to predict EFS outcomes at the MR3 level.

E1114

RESULTS OF OBSERVATION WITHOUT TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH DEEP MOLECULAR RESPONSE WHO STOPPED THERAPY DUE TO PREGNANCY, ADVERSE EVENTS OR BY OWN CONSIDERATION

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Background: A possibility of observation without treatment in chronic myeloid leukemia (CML) patients is important as the long-term survival on tyrosine kinase inhibitors (TKI) therapy is excellent and a significant proportion of patients (pts) may achieve a deep molecular response (DMR).

Aims: To evaluate the results of observation without TKI treatment in CML patients with DMR who stopped therapy for different reasons.

Methods: Sixty-one CML pts with DMR were observed after TKI cessation. Fifty-nine pts had chronic phase, 2 pts had accelerated phase of CML at initial diagnosis. Sokal risk groups ratio low/intermediate/high was 65%/20%/15%. Male/female ratio was 15/46. Median (Me) age at TKI cessation was 37 years (range 22-76). The reasons for TKI discontinuation were: 1) adverse events (AE) of TKI (n=28), 2) self-made decisions of pts or TKI absence (n=15), 3) pregnancy (n=18). Imatinib and second generation TKI (TKI2) were used in 40(66%) and 21(34%) of pts respectively. Twenty-three (38%) pts were pretreated with interferon prior to TKI. Me treatment duration before TKI discontinuation was 72 months (range 6-159 months). BCR-ABL transcript level was assessed by quantitative Real-time polymerase chain reaction according to international scale (IS). DMR was considered for at least molecular response 4 log (MR4) or BCR-ABL≤0,01%. TKI were resumed after loss of major molecular response (MMR) or BCR-ABL>0,1%. For pregnant pts therapy was resumed at BCR-ABL level>1% if it happened during pregnancy and at *BCR-ABL* level>0,1% if it happened after delivery. MR4 duration >2 years was in 45 (74%) of pts. We evaluated survival probability without MMR loss, terms of MMR loss and BCR-ABL level at MMR loss.

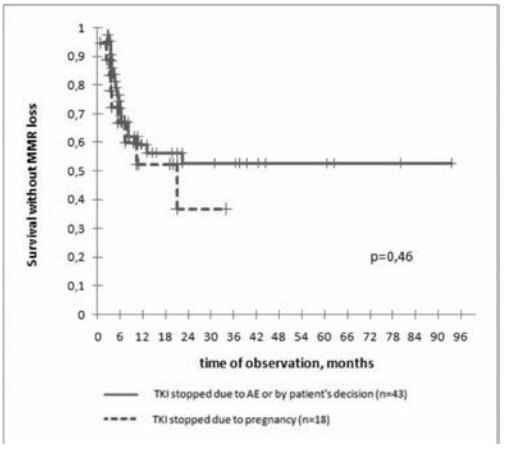

Figure 1. Survival without MMR loss after TKI discontinuation.

Results: Me follow-up after TKI discontinuation was 31 month (range 4-99 months). MMR was lost in 28 (46%) pts, TKI were restarted in all of them. Me time of MMR loss was 5 months (range 1-22 months). MMR loss happened within 6 and 12 months after TKI cessation in 20(33%) and in 5(8%) of pts respectively. In 3(5%) pts MMR loss was at 13,21 and 22 months. No hema-

tologic relapses were observed. At the time of MMR loss BCR-ABL level ranged from 0,11% to 13%. In 6 pts TKI were restarted without MMR loss by physician's decision at BCR-ABL level 0-0,082%. Two pts with DMR died due to concomitant diseases. Twenty-five (41%) pts continued to be treatment free under monitoring for Me 20 months (range 4-93 months), including 4 women without MMR loss after delivery. The probability of survival without MMR loss after TKI cessation at 6, 12 and 24 months was 67%, 57% and 49% respectively. For pregnancy group the probability of survival without MMR loss after TKI cessation at 6, 12 and 24 months was 67%, 32% and 37% respectively. No significant difference with pts in whom TKI were stopped for other reasons was found (p=0,46, figure 1). No MMR loss was observed after 24 months of TKI discontinuation neither in patients who stopped TKI due to pregnancy nor in those who stopped treatment for other reasons.

Summary/Conclusions: Approximately half of CML patients with DMR had a possibility to keep MMR within one year after TKI cessation. MMR loss mostly occurred within first 6 months after TKI discontinuation. No MMR loss was marked after 24 months of treatment free observation. Pregnancy in CML patients with DMR did not influence on the ability to maintain MMR after TKI cessation. A safe treatment free observation in CML patients who stop TKI may be warranted only with accompanying regular BCR-ABL level monitoring.

E1115

CONCURRENT OPTIMAL MOLECULAR AND CYTOGENETIC RESPONSES AT BOTH 3 AND 6 MONTHS PREDICT A HIGHER PROBABILITY OF MR4.5 ACHIEVEMENT IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB

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Background: Tyrosine kinase inhibitors (TKIs) have become the first line treatment of chronic myeloid leukemia (CML). Because early molecular and cytogenetic responses have been demonstrated to strongly predict long-term outcomes, the 2013 European LeukemiaNet (ELN) recommends that the patient early response should be defined by both molecular and cytogenetic tests to optimally direct the selection of subsequent treatments. The rapid achievement of a deep molecular response has become the treatment goal, therefore, the impact of the combination of early responses on it needs to be investigated.
Aims: We tried to evaluate the impact of the combined molecular and cytogenetic responses at 3 and 6 months on the achievement of molecular response of 4.5 (MR4.5) in imatinib treated CML patients.
Methods: A total of 228 newly diagnosed chronic phase CML patients treated with imatinib were included. At 3 and 6 months, they all were detected BCR-ABL transcript levels by real-time quantitative polymerase chain reaction (RQ-PCR) using peripheral blood samples, and performed karyotyping by standard G-banding techniques. Patients were grouped into 3-month and 6-month molecular optimal cytogenetic optimal (MOCO), molecular optimal cytogenetic warning (MOCW), molecular warning cytogenetic optimal (MWCO), molecular warning cytogenetic warning (MWCW) and failure groups according to the 2013 ELN recommendation. BCR-ABL^{IS} (BCR-ABL levels according to the international scale) was serial followed up for molecular response evaluation.

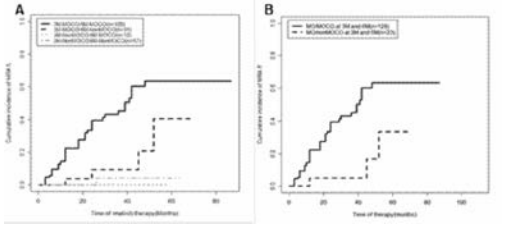

Figure 1.

Results: The median follow-up time was 27 months (range 8-94). For 3 months, both MOCW and MWCO patients had 3-year MR4.5 rates similar to those of MWCW and failure patients (8.3% [95% confidence interval (CI), 0-70.5%], 0, 0 and 0, all P>0.05), and they all were significantly lower than that of MOCO patients (35.5% [95% CI, 23.2-48.0%]; P=0.0047, 0.0057, 0.0089 and 0.0081, respectively). Similarly, for 6 months, both MOCW and MWCO patients had 3-year MR4.5 rates similar to those of MWCW and failure patients (5.6% [95% CI, 0-66.6%], 20.0% [95% CI, 0.2-67.0%], 0 and 0, all P>0.05), and they were all significantly lower than that of MOCO patients (41.6% [95% CI, 29.2-53.5%]; P=0.025, 0.021, 0.0005 and 0.0010, respectively). Next, responses at 3 and 6 months were combined. Three-month MOCO/6-month non-MOCO, 3-month non-MOCO/6-month MOCO and 3-month non-MOCO/6-month non-MOCO patients had similar 3-year MR4.5 rates (9.4% [95% CI, 0-56.5%], 0 and 4.2% [95% CI, 0-64.0%], P>0.05), and they were all significantly lower than that of 3-month MOCO/6-month MOCO patients (45.4% [95%CI, 32.9-57.1%]; P=0.0024, 0.0073 and<0.0001). This patient group also had significantly higher 3-year event-free survival (EFS) and progression-free survival rates (PFS) than those of patients with non-MOCO at 3 and/or 6 months (P<0.0001 and 0.0065) and had a significantly higher 3-year MR4.5 rate than that of patients with a 3-month/6-month molecularly optimal response but not a cytogenetically optimal response at 3 and/or 6 months (including 3-month MOCO/6-month MOCW, 3-month MOCW/6-month MOCO and 3-month MOCW/6-month MOCW, 5.0% [95%CI, 0-65.6%], P=0.0028).
Summary/Conclusions: The combination of early molecular and cytogenetic responses better predicts a deep molecular response in CML patients treated with imatinib. Concurrent optimal molecular and cytogenetic responses at both 3 and 6 months are associated with MR4.5 achievement.
Grant support The Nature Science Foundation of China (81370637 and 81570130) and the Beijing Municipal Science and Technology Program (Z131100004013026 and Z141100000214011).

LB2255

TELOMERE LENGTH SHORTENING IS ASSOCIATED WITH TREATMENT FREE REMISSION IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Telomere biology has been more extensively studied in chronic myeloid leukemia (CML) than in any other blood cancer. Shorter telomeres have been associated to disease progression, poor prognosis, higher Hasford score and acquisition of further cytogenetic aberrations. So far, there are no studies investigating the possible influence of telomeres on treatment-free remission (TFR) after discontinuation of therapy with tyrosine kinase inhibitors (TKIs).
Aims: The aim of the present study was to investigate whether telomere length was associated with durable TFR in a cohort of CML patients after discontinuation of treatment with imatinib.
Methods: Thirty-two chronic-phase CML patients discontinued TKI treatment after achieving complete molecular remission (MR4). All patients were treated with imatinib. Two patients underwent second-line TKI (nilotinib) following molecular relapse. Telomere length analysis was performed as described by Cawthon (2002) using a quantitative PCR (q-PCR). The Relative Telomere Length (RTL) was determined as the Telomere (T) to Single copy gene (36B4) (S) ratio (T/S) normalized to a reference sample (K-562 DNA). Peripheral blood samples were also collected from 32 age- and sex-matched healthy controls. Age corrected RTL (acRTL) represented the difference in telomere length between patients and age- and sex-matched controls.

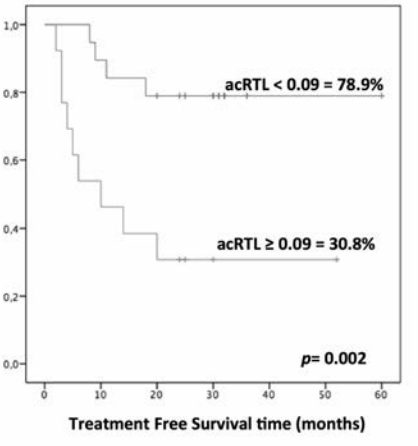

Figure 1.

Results: Complete molecular remission (CMR) was achieved after a mean of 26 months of imatinib (range 3-93). TKI treatment was discontinued after a mean of 84 months from the start of treatment (range 24-143). The median follow-up after discontinuation was 30 months (range 18-60). Thirteen patients (41%) showed loss of CMR. All relapsed patients regained CMR after restarting treatment with TKIs. The 36-month cumulative probability of TFR was 59.4%. RTL was assessed at a mean of 32 months from discontinuation in TFR patients and at a mean of 23 months after relapse in others. The median value of acRTL in the CML cohort was 0.09 (range -0.26, +0.86). The Mann-Whitney U test

showed shorter acRTL in TFR patients compared to patients with molecular relapse (mean±SD = 0.01±0.14 *vs* 0.20±0.21; p=0.01). Patients were stratified according to the median value of acRTL ≤0.09. TFR was significantly higher in CML patients with acRTL ≤0.09 in comparison to those with longer telomeres (78.9% *vs* 30.8%, p=0.002) (Figure 1).

Summary/Conclusions: Achievement of biological recovery through control or eradication of CML stem cells might depend on their proliferative potential in relation to telomere length. Age (a parameter directly proportional to telomere shortening and senescence) has recently been reported as a predictive factor for molecular relapse in CML patients who discontinue imatinib. CML cells would escape senescence through up-regulation of telomerase, restoring telomere length. In our study cohort, TFR patients showed significantly shorter acRTL than patients who presented molecular relapse. It can be postulated that quiescent CML stem cells harboring longer telomeres possibly escape senescence mechanisms and maintain a proliferative potential after discontinuation of imatinib treatment. To avoid such tumor escape mechanisms, anti-telomerase treatment strategies after TKI discontinuation would seem to be a reasonable proposal.

Gene therapy, cellular immunotherapy and vaccination

E1116

THE JAK1/2 INHIBITOR RUXOLITINIB MODULATES ANTIGEN PRESENTING FUNCTIONS OF ACTIVATED PRIMARY B CELLS ON A METABOLIC LEVEL

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Background: B lymphocytes not only balance the physiologic immune response but can also be involved in a variety of immune pathologies. Besides their traditional function as antibody producing plasma cells, it has been shown that B cells, upon activation, can develop into professional antigen-presenting cells (APCs) which mediate further cellular immune responses. Moreover, cytokines modulate B cell activation via the Janus kinase - signal transducer and activator of transcription (JAK-STAT) pathway. Inhibition of the JAK-STAT pathway has been introduced into clinical practice with the specific JAK1/2 inhibitor ruxolitinib. Approved for the treatment of JAK2 mutated myeloproliferative diseases, ruxolitinib has also shown to alleviate immune mediated symptoms such as fatigue and night sweat. Also in steroid-refractive graft-*versus*-host disease, ruxolitinib has shown activity.
Aims: Given the broad employment of JAK-STAT signaling in immune responses and the suggested clinical benefits of ruxolitinib mediated JAK1/2 inhibition in immune pathologies, we analyzed if such "off-target" effects can also mediate B cell activation.
Methods: Primary human B cells from healthy donors as well as from ruxolitinib treated patients were used for in vitro analyses. Here, CD40ligand mediated B-cell activation was applied as a well established model for T-cell dependent B-cell activation. Morphologic, phenotypic and functional changes were analyzed by 10-color flow cytometry. Cell metabolism was analyzed via extracellular flux changes (Seahorse Bio.).
Results: We found that ruxolitinib treatment significantly suppressed CD40L induced B-cell activation. These alterations became manifest in a reduced cell size and less homotypic cluster formation as well as in a reduced expression of co-stimulatory (CD80, CD86) and HLA-class-II molecules. On a functional level, JAK1/2 inhibition significantly reduced the ability of CD40L activated B cells to induce T-cell activation in a mixed allogeneic lymphocyte reaction. As a mechanism of action we found that ruxolitinib reduced glycolysis and the glycolytic capacity of activated B cells. Vice versa, inhibition of glycolysis via 2-deoxyglucose could recapitulate the ruxolitinib induced phenotype. Finally, data from ruxolitinib treated patients suggest that similar alterations of B cells also occur *in vivo*.
Summary/Conclusions: Taken together, our data suggest that ruxolitinib has important effects on B-cell activation which might contribute to its previously reported immunosuppressive features. Furthermore, we show that ruxolitinib can modulate APC-features of activated human B cells via glycolysis.

E1117

INTRA-BONE DONOR LYMPHOCYTE INFUSION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES RELAPSING POST ALLOHSCT

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Background: Donor Lymphocyte Infusion (DLI) is effective in a proportion of patients with leukaemia relapsing post alloHSCT. This effect is over-paid by a high risk of severe aGvHD. Ikehara and Frassoni showed the efficacy of the intra-bone (IB) marrow hematopoietic stem cell transplantation associates with a prompt hematopoietic reconstitution and a low risk of aGvHD in animal experiments and in human setting, respectively. Recurrence and GvHD are major causes of treatment failure post alloHSCT.
Aims: In this study we evaluated a novel technique for relapse treatment in which donor lymphocytes are directly injected into the patient's bone marrow cavity. This study was approved by the local ethic committee.
Methods: Four patients: 3 with AML (2 alloSIB: 50 years old, female, normal karyotype; 22 years, male 7q31 del., CNS lesions post trauma; and one alloMUD: 25 years, male FLT3 ITD), and one with CLL, 64 years old male, *TP53* del, chronic EBV infection. FLT3 ITD AML case relapsed 9 months, other AML cases 2 and 3 yrs and CLL patient 7 years post alloHSCT. The salvage chemotherapy included FLAG for FLT3 ITD case, anti-CD20 MoAb for CLL case, two other AML cases received DLI upfront. All received IB DLI (according to the escalating dose regimen starting from 10E6 for the first and 10E7 for the second and usually 5 times 10E7 CD3+ lymphocytes for the third dose [cells/kg

body weight]). The intervals between IB DLI in 3 patients varied from 1 to 2 months being longer in one AML case in which between IB DLI 5' azacitidine was administrated. The cells for DLI were obtained from the primary PBPC transplant material in alloMUD AML case and from unstimulated PBPC in alloSIB and in two AML cases as well as in one CLL case. The cells were injected directly to the bone marrow cavity under local anaesthesia and a low molecular Heparin prophylaxis.
Results: 1) At 30 check points post IB DLI the marrow and blood lymphocyte profile was investigated in 4 patients. We found the prevalence of CD8+ cells proportions in the marrow over those in the blood (median: 24.3% *vs* 23.8%, p<0.004) but in particular the prevalence of CD279+ lymphocytes (16.3% *vs* 9.5%, p<0.001) and those CD8+CD279+ (6.5% *vs* 3.2%, p<0.001). 2) In CLL cases there was a drop in the count of blood lymphocytes, cellularity of the marrow also decreased and the patient was haematologically stable. The examination 8 months after the last DLI revealed 3300/µl CD5+CD19+ lymphocytes in the blood. The level which was also seen soon after the completion of the IB DLI treatment. 3) AML FLT3 ITD patients responding well to the FLAG salvage therapy followed by IB DLI is leukaemia free 3 months after the relapse, two other cases were stable haematologically (ECOG1) but an increase in the proportions of myeloblasts from 23% to 34% seen during 7 and 6 months of observation time which included IB DLI treatment prompted us to perform the second transplantation in one case (uncomplicated) and to schedule the second transplant in the other case.
Summary/Conclusions: 1) The observation of four patients they completed IB DLI suggests that it is safe procedure and GvHD was not observed. 2) The anti-leukemic effect was seen in CLL case and a stabilization of the haematopoiesis was seen in two patients receiving IB DLI being not in remission. AML FLT3 ITD positive case relapsing soon after transplant responded to the FLAG regimen followed by IB DLI. 3) The higher proportions of CD8+CD279+ lymphocytes in the marrow as compared to the blood suggest the presence in the marrow the cells actively involved in the surveillance of leukaemia.
Supported by INNOMED/I/1/NCBR/2014 grant.

E1118

INTERLEUKIN-2-PRIMED NAÏVE CD8 T CELLS AS A POTENTIAL REMEDY FOR OVERCOMING TUMOR-DERIVED IMMUNOSUPPRESSION IN TUMOR MICROENVIRONMENT

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Background: CD8 T cells have cytotoxic effector functions upon antigen stimulation and receive considerable attention for adoptive immunotherapy against cancer. Despite of their potential implication, however, conventional approaches using in vitro expanded CD8 T cells have not been successful so far, mostly due to lack of functionality with cellular exhaustion.
Aims: In this study, we therefore investigated the phenotypic and functional differences among in vitro activated CD8 T cells of three different sources, namely naïve, memory and tumor-infiltrating lymphocytes (TILs) of human and mice, for better understanding mechanisms behind potent effector functions and overcoming limitations of the current approaches.
Methods: Effector T cell populations were activated from $CD8^+$ naïve, memory T cells, and $CD8^+$ TILs obtained from cancer tissue (T_{eff} N, T_{eff} M, and re-stimulated TILs, respectively). Individual effectors were analyzed for functional difference and cytotoxicity. In murine model, we also checked the same methods for the phenotypes of T cell exhaustion and tumor challenging test using EG7-EL4 cell line (expressing the OVAp) in adoptively transferred OT-1, thy1.1 T_{eff} N, T_{eff} M and activated TILs T to C57BL6 mice.
Results: *In vitro* stimulation of human naïve, memory, and TIL CD8+ T cells using typical anti-CD3/CD28 antibodies were able to generate phenotypically homogenous populations of $CD62L^{lo}CD44^{hi}OX40^{hi}CD27^{hi}$ cells. Notably, proliferation and total yield after the in vitro expansion were far greater in NT_{eff} cells than for MT_{eff} and TIL_{eff} cells, which was well correlated with the enhanced telomere length of NT_{eff} cells compared to the latter two populations. In line with the longer telomere length, NT_{eff} cells exhibited significantly less amounts of T cell exhaustion markers, PD-1, CTLA-4 and KLRG-1, than those of MT_{eff} and TIL_{eff} cells, and moreover, acquired distinct expression patterns of memory-promoting transcription factors, with T-bet and Eomes being highly induced in a rapid, sustainable manner. With regard to tumor-induced immune-suppression, interestingly, NT_{eff} cells appeared to have lower expression of Foxp1, a transcription factor promoting T cell quiescence, and were refractory to apoptosis upon TGF-β conditioning, implying a better survival potential. As with *in vitro* stimulated human T cells, *in vitro* priming of murine naïve CD8 T cells using high doses of IL2 were also found to be effective for generating potent effector cells that can exert tumor-specific cytotoxic activity.
Summary/Conclusions: Of CD8 T cell pools that are different in their activation states, naïve cells other than pre-existing effector/memory and TIL cells were better poised to develop into potent effector cells after in vitro stimulation, which provide an implication for rational design of adoptive immunotherapy against cancer.

E1119

SYNERGISTIC KILLING EFFECT OF A NOVEL TRIPLE-REGULATED ONCOLYTIC ADENOVIRUS CARRYING PROGRAMMED CELL DEATH 5 GENE AND DAUNORUBICIN ON HUMAN LEUKEMIC CELLS

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Background: The expression levels of programmed cell death 5 (PDCD5) are down-regulated in many malignancies. A novel triple-regulated conditionally replicating adenoviruses (CRAd) carrying PDCD5 gene expression cassette, SG611-PDCD5, alone could inhibit tumor growth in vitro and *in vivo* and protect selectively the normal cells.
Aims: The purpose of this study was to investigate the synergistic killing effect of SG611-PDCD5 and low-dose daunorubicin (DNR) on human leukemic cells in vitro and *in vivo*.
Methods: The antitumor efficacy was characterized in several leukemic cell lines in vitro and in xenograft models of human leukemic cell line in nude mice. A panel of leukemic cells was treated with different concentrations of DNR alone or in combination with different multiplicities of infection (MOI) of SG611-PDCD5. The cell viability was determined by using CCK-8 assay. Apoptosis was detected in whole living cells using flow cytometry or in paraffin-embedded tumor tissues using the TUNEL kit.
Results: Combined SG611-PDCD5 and low-dose DNR showed synergetic effects in inhibiting the proliferation of human leukemic cell lines K562, MEG-01, KG-1a and TF-1. Synergistic effects in inducing apoptosis were observed both in K562 cells in vitro and in tumor tissues from xenograft models of K562 cells in nude mice. In K562 cells, the apoptotic percentages of SG611-PDCD5 at an MOI of 240 pfu/cell in combination with 0.12 ug/ml DNR were 72.5%, significant higher than that of SG611-PDCD5 alone (49.4%) or DNR alone (16.7%). TUNEL assay showed significantly more apoptotic cells in the SG611-PDCD5 plus DNR group than in SG611-PDCD5 group or in DNR group (25.0, 12.5 and 7.8 apoptotic cells/field, respectively, $P<0.05$). As shown *in vivo* experiment (Figure1), the tumor sizes were significantly decreased in combined treatment group on days 8 and 10 after treatment than those in DNR alone and SG611-PDCD5 alone (all $P<0.05$). Tumor sizes in the control, DNR, SG611-PDCD5 and DNR plus SG611-PDCD5 groups were 175.9±82.5, 80.9±42.8, 51.6±17.4, and 27.6±11.9 mm^3 on day 10, respectively.

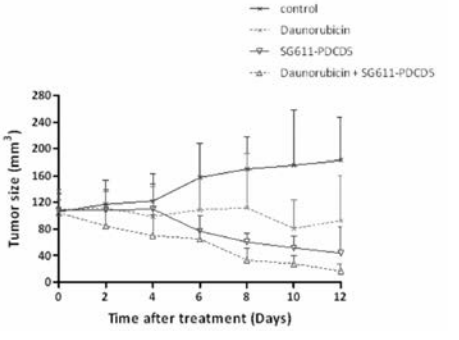

Figure 1.

Summary/Conclusions: Combined treatment of SG611-PDCD5 and DNR achieves synergistic effects in inhibiting human leukemic cell growth in vitro and *in vivo*. This study may lead to development of new strategies for effective leukemia treatment with a potential reduction in systemic toxicity.

E1120

TRIPLE-REGULATED ONCOLYTIC ADENOVIRUS-MEDIATED VSTM1 OVEREXPRESSION EXHIBITS POTENT ANTITUMOR ACTIVITY ON COMMON HUMAN LEUKEMIC CELL LINES

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Background: VSTM1 (V-set and transmembrane domain-containing 1), as a potential myeloid differentiation antigen gene, is downregulated in bone marrow cells from myeloid leukemia patients. Restoration of VSTM1 expression inhibited myeloid leukemia cells' growth. VSTM1 might provide a potential target for the diagnosis and treatment of leukemia.
Aims: The purpose of this study was to construct a triple-regulated oncolytic adenovirus carrying VSTM1 gene expression cassette, SG611-VSTM1, and to explore its antitumor efficacy on human leukemic cell lines.

Methods: In SG611-VSTM1, the E1a gene with a deletion of 24 nucleotides within CR2 region was controlled under the human telomerase reverse transcriptase promoter, the E1b gene expression was directed by the hypoxia response element, whereas the VSTM1 gene was controlled by the cytomegalovirus promoter. The insertion and orientation of all recombined plasmids were confirmed by restriction enzyme digestion and polymerase chain reaction (PCR). The relative VSTM1 expression level in human leukemic cell line K562 after infection with SG611-VSTM1 was detected by real-time quantitative PCR (RQ-PCR) and western blot. The tumor-selective replication of this virus and its antitumor efficacy were characterized in several leukemic cell lines with different multiplicities of infection (MOI) in vitro. Cell viability was detected by using Cell Counting Kit-8 (CCK-8). Cell apoptosis was analyzed by flow cytometry (FCM).
Results: A triple-regulated oncolytic adenovirus carrying VSTM1 gene expression assette, SG611-VSTM1, was completed and confirmed. VSTM1, hTERTp, HRE, skeleton and fiber 11 of recombinant adenovirus SG611-VSTM1 were successfully amplified. SG611-VSTM1 expressed VSTM1 with high efficiency in leukemic cells as compared with SG611 (P<0. 001). The expression level of VSTM1 increased gradually along with the increase of MOI. In CCK-8 assay, the cell viabilities were decreased gradually along with the increase of MOI in leukemic cells with SG611-VSTM1 in comparison with SG611, while the cell viabilities were maintained a relatively higher level in the normal cell lines BJ (about 50%) and L-02 (about 60%) with SG611-VSTM1. Similarly, the proapoptotic effect of SG611-VSTM1 on leukemic cells was superior to SG611. In K562 cells at 48 h after infection (Fig.1), the apoptotic percentage of SG611-VSTM1 at a MOI of 40 and 160 pfu/cell were 19.3% and 70.9%, significantly higher than that of SG611 (11.0% and 46.6%), respectively.

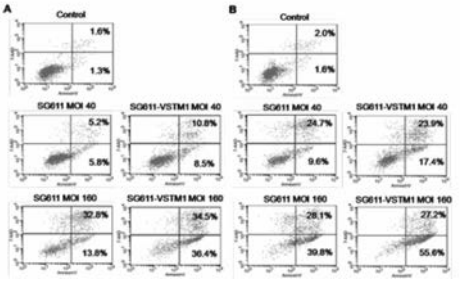

Figure 1. Apoptosis of K562 cells infected with SG611-VSTM1 or SG611. A: at 48 h after infection; B: at 72 h after infection. The apoptotic percentage was increased gradually along with the increase of MOI and infection time prolonging, especially in the group treated with SG611-VSTM1.

Summary/Conclusions: It is concluded that the triple-regulated adenovirus of SG611-VSTM1 containing the VSTM1 has been successfully established with high VSTM1 expression level in leukemic cells. SG611-VSTM1 holds an increase of anticancer efficacy and might be an improvement of the safety as a new anticancer agent.

E1121

COMBINED ISOLATION OF MULTI ANTIGEN SPECIFIC T CELL PRODUCTS CONTAINING NAÏVE AND/OR MEMORY VIRUS-SPECIFIC T CELLS AND T CELLS SPECIFIC FOR TUMOR ASSOCIATED AND MINOR HISTOCOMPATIBILITY ANTIGENS

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Background: T cell depleted allogeneic stem cell transplantation (aIloSCT) followed by a donor lymphocyte infusion (DLI) is a potential curative treatment for patients with hematological malignancies. However, between the aIloSCT and the DLI patients are vulnerable to viral infections and disease relapses. Within a clinical phase I/II study in the EU FP7 program T-control we isolate and adoptively transfer multi-antigen specific T-cell products to prevent these complications. Therefore, we isolate donor T-cells directed against HLA-A1, A2, A24, B7, and/or B8-restricted peptides of CMV, EBV and Adenovirus and HLA-A2-restricted peptides from the tumor-associated antigens (TAA) NY-eso, WT-1, RHAMM, PRAME and proteinase 3, and the minor histocompatibility antigen (MiHA) HA-1h. Due to the relatively high precursor frequency of virus-specific T cells in seropositive donors, memory virus-specific T cells can be easily isolated.
Aims: To investigate the presence and functional activity of the specificities with a very low precursor frequency, e.g. TAA and MiHA specific T cells, and virus specific T cells from seronegative donors.
Methods: HLA-A2, B7, and/or B8-positive CMV seronegative donors (n=5) and EBV seronegative donors (n=2) were selected for isolation of CMV, EBV and AdV-specific T cells. From 4 HLA-A2 positive donors TAA and MiHA specific T cells were isolated. The reversible HLA/peptide streptamer and magnetic nanobead technique (Juno) was used for the isolation out of 1-2*10^9 donor PBMC, using all relevant streptamers according to the HLA-type of the donor. The positive fractions were non-specifically expanded using PHA, IL-2 and allogeneic feeder cells. The presence of the different specificities was assessed 10-14 days after the isolation by tetramer staining. To confirm the presence of the invisible very low frequency T cell specificities, subsequent enrichment and expansion rounds were performed until populations were visible. Specificities that were present in a frequency >1% were clonally expanded and tested for their functional potential.
Results: After 2 or 3 enrichment and expansion rounds, we were able to demonstrate specific T cell populations for 6 CMV specificities from 4 seronegative donors (pp65 A2 NLV (3x), pp65 B7 TPR, 1E-1 A2 VLE, 1E-1 B8 QIK) and 4 EBV specificities from 2 seronegative donors (BMLF-1 A2 GLC, EBNA-3a B7 RPP (2x), BZLF-1 B8 RAK). The CD8/tetramer positive clones from two CMV specificities (pp65 A2 NLV and pp65 B7 TPR) and one EBV specificity (EBNA-3a B7 RPP) were tested for antigen specific reactivity measured by cytokine release (IFN-gamma and GM-CSF) after 24 hours of stimulation with TAP deficient T2 cells and/or allogeneic EBV-LCL exogenously loaded with the relevant peptide. This analysis revealed the presence of clones of high avidity recognizing up to 10^-9M peptide on T2 cells and 10^-8M peptide on allogeneic EBV-LCL. Besides this, all mentioned TAA and MiHA specificities could be isolated from all four HLA-A2 positive donors after 2 to 4 enrichment and expansion rounds, and comprised clones with a range of different functional avidities.
Summary/Conclusions: In conclusion, the HLA/peptide streptamer technology allows the simultaneous isolation of multi antigen specific T cell products containing not only highly frequent virus-specific memory T cells, but also EBV and/or CMV specific T cells from the naïve T cell repertoire of seronegative donors, as well as TAA and MiHA specific T cells with differential functional avidities.

E1122

THE IGE REPERTOIRE IN HEALTHY, NON-ALLERGIC INDIVIDUALS: IMPLICATIONS FOR EFFICIENT ALLERGEN DESENSITATION IMMUNOTHERAPY

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Background: Type I allergic disease is a hypersensitivity reaction mediated by allergen-specific IgE. Allergen desensitation immunotherapy consists of serial injections of increasing doses of the allergen. A possible mechanism for the restriction of the severity of type I allergy by desensitation is the induction of allergen-specific neutralizing antibodies of non-IgE isotype. It is unclear whether allergen-specific IgG clones in desensitised individuals have been recruited form the naive repertoire or from pre-existing clones from which the IgE clones originated. The largest reported IgE repertoire from a single person comprises 32 immunoglobulin sequences.
Aims: To characterise the IgE repertoire in healthy donors in-depth by an unbiased approach.
Methods: Peripheral blood CD19+ cells from six healthy donors were isolated by magnetic bead separation and divided into aliquots of $2x10^6$ cells. For each donor, mRNA was amplified from 5 aliquots by ARTISAN PCR, an anchored RT-PCR primed on constant immunoglobulin regions and employing a template-switching reverse transcriptase. ARTISAN PCR was performed in parallel to obtain IgE, IgG, and IgM libraries. PacBio sequencing of the resulting 15 amplicon libraries per donor yielded a median of 284 (range: 100-743) full-length sequences/aliquot and 27500 sequences in total.
Results: A median of 261 (range: 175-358) unique IgE sequences were obtained per donor. 9.1-61.8% of individual IgE sequences were represented in ≥2 aliquots of the same donor. In contrast, 1.4-7.8% IgM and 5.7-16.3% IgG sequences, respectively, were represented in multiple aliquots. 29 unique VDJ sequences were identified in both IgM and IgG compartments. All IgE sequences were strictly restricted to their isotype. Somatic mutation loads per sequence differed significantly between IgE (median: 5.6%), IgM (0.3%), and IgG (8.3%). Intraclonal sequence diversity in IgE clones was predominantly due to silent framework region mutations. IGHV3-11, IGHV3-9, and the IGHV3 family were significantly overrepresented in IgE compared to IgM/IgG. IGHV3-30, IGHV3-33, IGHV4-59, and IGHV2, 4, 5, and 6 families were underrepresented.
Summary/Conclusions: These data provide a reference for investigations of the IgE repertoire in allergy and parasitology. In healthy donors, the peripheral IgE repertoire is less diverse than IgM/IgG, displays a strong IGHV bias, and carries an intermediate mutation load without evidence for ongoing affinity maturation. IgE and IgG/IgM repertoires apparently do not overlap. The lack of overlap between IgE sequences and expanded IgG clones, as well as the higher mutation load of the latter, suggest that most IgE sequences have undergone class switch recombination directly from IgM, rather than from class-switched expanded IgG clones as intermediates. These findings suggest that desensitisation strategies should focus on recruiting novel allergen-specific cells from the naïve B-cell compartment, rather than attempting to activate and expand existing IgG-expressing B cells that belong to the same B-cell clone and have the identical antigen specificity as the allergy-causing B-cells that have undergone alternative or subsequent class switching to IgE.

E1123

THE EFFECTS OF VARIOUS BLOOD PROTEINS ON THE ZETA POTENTIAL OF POLYMER NANOPARTICLES AND PROTEIN CORONA FORMATION: AN ANALYSIS BY TUNABLE RESISTIVE PULSE SENSING

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Background: Nanoparticles are increasingly used as delivery vehicles for targeted drug delivery, gene delivery, imaging and theranostics. Upon introduction of nanoparticles into biological fluids such as blood, the particles interact with the constituents of the environment, in particular with proteins. The interaction of nanoparticles with the proteins leads to formation of a protein-corona and change in the surface charge of the particles. These changes significantly affect the pharmacokinetic, pharmacodynamic and toxicological properties of the nanoparticles. Tunable Resistive Pulse Sensing (TRPS) technology, based on Coulter principle, enables particle-by-particle measurement of the size and zeta potential of nanoparticles during their passage through a tunable pore (Langmuir (2016) 32,1082-1090.
Aims: To ascertain the effects of three predominant proteins in blood, namely albumin, immunoglobulin and fibrinogen, in biologically relevant concentrations on the zeta potential of carboxylated polymer nanoparticles (CPNPs) in saline, serum and plasma at room temperature and 37ºC using TRPS
Methods: The particle size and zeta potential of carboxylated polymer nanoparticles were studied in phosphate buffered saline, albumin (40g/l), immunoglobulin (20g/l), fibrinogen (4g/l), normal human serum and normal human plasma at room temperature and 37ºC using TRPS (qNano, Izon Sciences, Oxford, UK). The kinetics of the protein corona reorientation for particles initially placed into serum and then adding 5%(V/V) plasma was then studied by monitoring changes in size and zeta potential of the particles using qNano.
Results: We observed a significant difference in distributions and zeta values between room temperature and 37ºC assay. The effect was protein dependent, and the largest difference between the two temperatures was recorded for the γ-globulin protein where the mean zeta potential changed from -16.7 mV to -9.0 mV for 25 and 37ºC, respectively. This method was further applied to monitor particles placed into serum and/or plasma. A temperature dependent change was again observed with serum showing a 4.9 mV difference in zeta potential between samples incubated at 25ºC and 37ºC, this shift was larger than that observed for samples in plasma (0.4 mV). Finally we monitored the kinetics of the corona reorientation for particles initially placed into serum and then adding 5%(V/V) plasma.
Summary/Conclusions: The technology presented offers an interesting insight into protein corona structure and kinetics of formation measured in biologically relevant solutions *i.e.* high protein, high salt levels and its particle-by-particle analysis gives a measure of the distribution of particle zeta potential that may offer a better understanding of the behaviour of nanoparticles used for drug/gene delivery in blood.

Hematopoiesis, stem cells and microenvironment

E1124

EXPANDING THE KNOWLEDGE OF BLOOD TRANSCRIPTOME: CIRCULAR RNAS IN HEMATOPOIESIS

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Background: Circular RNAs (circRNAs) are a class of covalently closed RNA molecules generated by back-splicing joining exons in a non-collinear way. circRNAs have been found throughout the tree of life and are conserved in mammals. As reported circRNAs may decoy miRNAs thus de-repressing miRNA targets and regulating cellular processes and may interact with RNA-binding proteins and other RNAs. High stability and complex cell type- and differentiation-specific expression patterns make circRNAs promising disease biomarkers. In cancer circRNAs are highly relevant since they associate with cancer miRNAs and regulate cancer-related pathways. Moreover, specific circRNAs exhibit anti-cancer effects, whereas others discriminate malignant cells from healthy ones. Recent studies demonstrated that the circRNA number and expression level in normal blood is reproducible and higher than in other tissues and incipient observations detected circRNAs in B-ALL.
Aims: We aim to detect, discover and characterize circRNAs expressed during hematopoiesis in order to expand the knowledge of transcriptome complexity and variations in normal differentiation to set the basis for circRNA studies in hematologic malignancies.
Methods: CircRNA detection grounds on identification of RNA-seq reads that contain a back-splice junction. Different programs rely on slightly different approaches, such as using a pre-compiled dataset of all possible exons of each gene coupled in reverse order, or by *de novo* identifying the back-splice junction from signals in the alignment data coupled with signals of canonical splicing. Combining the output of at least two programs increases the accuracy of true circRNA detection, as suggested by recent literature. Poly-A enriched RNA-seq data of eight cell populations of the hematopoietic tree (Figure 1A) were collected (HSC, MPP, CMP, CLP, GMP, MEP, MK, EB cells; three biological replicates per cell type; ID: EGAS00001000284, by Chen L et al., 2014, Science).
Results: A bioinformatics pipeline for detection and analysis of cirRNAs from RNA-seq data was set up that brings together published circRNA detection tools (CIRI, find_circ and testrealign.x) and custom made scripts, with a modular design expandable to novel programs. Preliminary analyses performed with CIRI allowed us to detect 11,079 putative circRNAs expressed during hematopoiesis, of which 41% are exonic, 42% are intronic and 17% fall in regions annotated as intergenic (Figure 1B). Expressed circRNAs are associated to 4,317 known genes, 58% of which produce only one circRNA, whereas 837 (19%) genes produce two different circular isoforms each, and 959 (22%) even more than three isoforms. The large majority (87.5%) of circRNAs was detected only in one cell type, and in each cell population on average 15% of the total detected circRNAs is present. We identified 20 circRNAs expressed in all cell stages (Figure 1C) and defined several patterns of circRNAs that are up or down regulated in less differentiated cells compared to more mature stages (e.g. HSC *vs* CMP), specifically in the lymphoid compared with the myeloid branch of the tree (CMP *vs* CLP) and, through the myeloid lineage, from CMP to more and terminally differentiated cells.

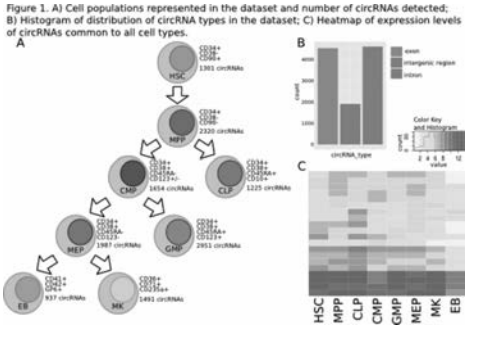

Figure 1.

Summary/Conclusions: Recent studies have unveiled a great potential of circRNAs in hematology. Our results, although preliminary, confirm that a high number of circRNAs are expressed during hematopoietic cell differentiation. We identified circRNAs expressed by all cell stages in the dataset, and several expression patterns that seem to be specific of stem cells, of differentiated cells, or of cellular lineages. We plan to conduct additional RNA-seq data analyses with different approaches to enrich the set of detected circRNAs in true positive hits, in order to validate them, to confirm some of the more striking expression variations observed and to predict functions and interactions of expressed circRNAs.

E1125

THE APC/C COACTIVATOR CDH1 CONTROLS MAINTENANCE OF THE HEMATOPOIETIC STEM CELL POOL *IN VIVO*

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Background: Cdh1 is a co-activator of the anaphase-promoting complex (APC/C) and highly active during G1 to target cell cycle proteins for proteasomal degradation thereby regulating cell proliferation. Recently, evidence accumulates, that Cdh1 may play a role in proliferation, self-renewal and multilineage differentiation of hematopoietic stem cells (HSCs).
Aims: Our objective was to examine the functional role of Cdh1 in the hematopoietic compartment in *vivo*.
Methods: We used a murine transplantation model in combination with targeted genetic RNAi approach for Cdh1 deletion in a competitive transplantation (TX) assay. Therefore, 5-FU enriched murine bone marrow cells (BMCs) were lineage depleted and retrovirally infected either with pLMPmiR^{Ctrl} or pLMPmiR^{CDH1}. Primary BMCs and equal proportions of stem and progenitor cells with significant Cdh1 knockdown were transplanted in competition with non-infected wildtype BMCs into lethally irradiated wildtype recipients.
Results: Peripheral blood (PB) chimerism of recipient mice revealed a significant higher ratio of EGFP+ leukocytes in recipients of pLMPmiR^{CDH1} BMCs compared to controls (p=0.009). Moreover, spleen and BM chimerism showed significantly enhanced repopulation of Cdh1 knockdown cells at week 3 compared to pLMPmiR^{Ctrl} BMC recipients (p=0.004). Furthermore, Cdh1 knockdown leads to a significant decrease of apoptotic cells compared to control BMCs. Additionally, a highly significant decrease in the Sub-G1 cell population in Cdh1 knockdown BMC recipients confirms enhanced viability of Cdh1 knockdown cells. In contrast to apoptosis alterations, no obvious difference in cell cycling could be monitored between sorted EGFP+ pLMPmiR^{Ctrl} or pLMPmiR^{CDH1} infected BMCs of recipient mice 3 and 8 weeks after TX by dynamic 2-dimensional EdU/FxCycle violet assays. Interestingly, the difference in chimerism vanished during the course of the TX assay. To evaluate differences in the repopulation capacity of Cdh1 knockdown and control BMCs in transplanted animals, FACS analysis were performed and showed a significant reduction of LK- (lineage-, cKit+, Sca1-) and LSK- (lineage-, cKit+, Sca1+) cell frequency within pLMPmiR^{CDH1} BMC recipient mice compared to controls at the end of TX indicating that Cdh1 knockdown leads to an initial increase in repopulation capacity followed by exhaustion of hematopoietic stem and progenitor cells in a competing situation. To examine whether Cdh1 modulates lineage differentiation of HSCs we performed FACS analyses and detected a significantly higher proportion of B-cells in recipient mice transplanted with pLMPmiR^{CDH1} BMCs compared to control animals. Analyses of the myeloid compartment displayed differences with reduced levels of granulocytes as well as monocytes in the PB of Cdh1 knockdown transplanted BMCs compared to the pLMPmiR^{Ctrl} group. T-cells were not affected significantly by Cdh1 knockdown, at least in this short transplantation setting.
Summary/Conclusions: Taken together, Cdh1 knockdown leads to enhanced viability as a consequence of less susceptibility to apoptosis *in vitro* and *in vivo*, suggesting that Cdh1 plays an important role in maintaining the stem cell numbers within the HSC pool. Furthermore, Cdh1 knockdown regulates lineage differentiation in favor to the B-cell differentiation, whereas the myeloid lineage is repressed in Cdh1 knockdown cells in competitive transplantation experiments.

E1126

THE TRANSCRIPTION FACTOR C/EBPG IS RESPONSIBLE FOR RAPID PRODUCTION OF NEUTROPHILS DURING EMERGENCY GRANULOPOIESIS

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Background: CCAAT/enhancer binding protein γ (C/EBPγ) is a member of the C/EBP family of myeloid transcription factors, which are known as master regulators of hematopoiesis. Recently, it has been shown that increased C/EBPγ levels contribute to the block of neutrophilic differentiation in acute myeloid leukemias characterized by C/EBPα hypermethylation. Hovewer, the function of C/EBPγ in normal and stress-induced hematopoiesis remains largely unknown.
Aims: In this study we investigate the role of C/EBPγ in hematopoiesis both in steady-state and under stress conditions.
Methods: We generated a C/EBPγ conditional knockout murine model, which allows tissue and time specific excision of C/EBPγ. These mice were crossed to Vav-1Cre deleter strain to excise C/EBPγ specifically in the hematopoietic system from early embryonic stages. We employed C/EBPγ f/f Vav-1Cre- and C/EBPγ f/f Vav-1Cre+ mice, refered here as wt and C/EBPγ ko respectively. First, frequency of distinct hematopoietic lineages in bone marrow, spleen, and blood of C/EBPγ ko mice was determined by flow cytometry. Next, to elucidate whether C/EBPγ is necessary for stress induced granulopoiesis, *in vivo* administration of different stimuli, including lipopolysacharide (LPS), granulocyte colony-stimulating factor (G-CSF) and *Candida albicans*, was used to model bacteria- or candidemia-induced emergency granulopoiesis. Mice were sacrified and examined 24 hours after infection since we focused on early phases of granulopoiesis. Differential cell counting and flow cytometric analysis were performed to assess whether C/EBPγ is involved in demand-accelerated neutrophilic production.
Results: Mice lacking C/EBPγ within the hematopoietic system presented partially reduced levels of blood neutrophils (wt 1.438x10^6/ml -/+ 0.114 *vs* CEBPγ ko 0.988x10^6/ml -/+ 0.078, p=0.0021), although this reduction did not result in any signs of sickness. Analysis of other cell populations, including progenitors and hematopoietic stem cells, did not reveal further differences between wt and C/EBPγ ko mice. These data suggest that hematopoietic-specific genetic ablation of C/EBPγ is not critical for steady-state hematopoiesis. Since C/EBPγ forms heterodimers with another C/EBP member, C/EBPβ, we determined whether C/EBPγ can modulate the function of this transcription factor. C/EBPβ is critical for emergency granulopoiesis, and therefore we investigated whether the absence of C/EBPγ would affect this process. Deletion of C/EBPγ did not compromise the ability of the hematopoietic system to respond to G-CSF or LPS, since flow cytometric analysis revealed similar hematopoietic response in C/EBPγ ko mice in comparison to wt control animals. Interestingly, C/EBPγ ko mice showed reduced numbers of blood neutrophils during early phases of candidemia (wt 2.44x10^6/ml +/- 0.78, *vs* CEBPγ ko 1.55x10^6/ml +/- 0.59, p=0.031). In addition, the distribution of different developmental stages within the neutrophilic compartment in bone marrow was perturbed, leading to the accumulation of more immature granulocytic populations and reduced percentage of mature neutrophils. Altogether, these results indicate that C/EBPγ promotes accelarated neutrophilic production during early phases of emergency granulopoiesis upon *Candida* stimulation.
Summary/Conclusions: Taken together, our data unravel C/EBPγ as a player in the expansion of granulocytes during early phases of candidemia-induced emergency granulopoiesis. On the other hand, C/EBPγ appears to be dispensable for the regulation of steady-state hematopoiesis and bacteria-induced granulopoiesis.

E1127

Abstract withdrawn.

E1128

TELOMERASE MRNA IN T CELL LEUKEMIA CELL LINE (JURKAT) DERIVED EXOSOMES TRANSFORMS NONMALIGNANT FIBROBLASTS INTO TELOMERASE POSITIVE CELLS

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Background: Exosomes are small (30-100nm) vesicles secreted from all cell types serving as inter- cell communicators by affecting biological processes in "recipient" cells upon their uptake. Telomerase is transcriptionally repressed in non malignant somatic cells thus enabling cellular senescence caused by telomere shortening. Telomerase activation is a hallmark of cancer cells providing them with endless replicative potential. Telomerase activation is associated also with other major cellular changes such as resistance to DNA damage, protection from apoptosis and others. We demonstrate that hTERT mRNA is transferred via exosomes from Jurkat cells into telomerase negative primary fibroblasts. The mRNA is translated into active telomerase causing several major changes in those cells. These findings have potential implications for tumor cells-microenvironment interactions.
Aims: To assess the presence of hTERT mRNA, the transcript of the enzyme telomerase, in exosomes secreted from Jurkat cells; to study the possible effects of this transcript on the phenotype of fibroblasts cells upon exposure to exosomal hTERT mRNA.
Methods: Exosomes were isolated by exosome isolation kit (exoquick). Real time PCR was performed to assess the hTERT mRNA levels in exosomes and in the cells. Verification of exosome isolation and telomerase levels were estimated by Western blotting. Telomerase activity was assessed by Real time PCR based TRAP assay. Cellular proliferation and senescence status were assessed by Trypan blue exclusion and b-Galactosidase activity assays, respectively. Nuclear morphology was visualized after DAPI staining. DNA damage response was evaluated by counting gH2AX foci accumulation following phleomycin insult.
Results: The current study demonstrates for the first time that hTERT mRNA, the transcript of the enzyme telomerase, is shuttled from "donor" cancer cells via exosomes into "recipient" telomerase negative fibroblasts, where it is translated into a fully active enzyme. All tested cells secreted exosomal hTERT

mRNA in accordance with the endogenous levels of their hTERT mRNA and telomerase activity. A non-cancer fibroblasts cell line in which telomerase was ectopically expressed secreted relatively high levels of exosomal hTERT mRNA as well. A similar *ex-vivo* shuttle of exosomal hTERT mRNA isolated from the serum of a patient with pancreatic cancer into primary fibroblasts was demonstrated as well. Telomerase activity induced phenotypic changes in the recipient fibroblasts including extension of life span and postponing senescence. In addition, telomerase activity protected the fibroblasts from DNA damage induced by Phleomycin and also protected from apoptosis, indicating that telomerase extracurricular activities are also manifested in the recipient cells.
Summary/Conclusions: The shuttle of telomerase from cancer cells into fibroblasts and the induction of these changes may contribute to the formation of cancer associated fibroblasts representing a unique aspect of the cross talk between tumors and their microenvironment. These processes bear a therapeutic potential targets and as such serve as a promising research avenue.

E1129

PERIPHERAL BLOOD LEUKOCYTE TELOMERE LENGTH AND RISK OF INFECTIOUS DISEASE: A PROSPECTIVE STUDY OF 75,919 INDIVIDUALS FROM THE GENERAL POPULATION

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Background: In the general population, older age is associated with short leukocyte telomere length and with high risk of infections. In a recent study of allogeneic hematopoietic cell transplantation for severe aplastic anemia, long donor leukocyte telomere length was associated with improved long-term survival in the recipients. These findings suggest that leukocyte telomere length could possibly be a marker of overall immune competence. However, previous studies examining this hypothesis among individuals from the general population have produced conflicting results.
Aims: We tested the hypothesis that short telomere length in leukocytes is associated with high risk of infectious disease hospitalization.
Methods: Peripheral blood leukocyte telomere length was measured using quantitative polymerase chain reaction in 75,919 individuals from the general population. The participants were followed for up to 23 years.
Results: During follow-up, 9671 individuals were hospitalized due to infections. Short telomere length was associated with high risk of any infection (hazard ratio 1.05 per 1000 base pair shorter telomeres; 95% confidence interval 1.03-1.08), pneumonia (1.08;1.04-1.12) and endocarditis (1.32;1.06-1.66) after adjustment for other infectious disease risk factors. Telomere length was not associated with risk of skin infection, urinary tract infection, sepsis, diarrhoeal disease, meningitis or other infections.
Summary/Conclusions: Short leukocyte telomere length was associated with high risk of any infection, pneumonia and endocarditis. These findings indicate that leucocyte telomere length may be a marker of immune competence among individuals from the general population. Further studies are needed to determine whether risk of infections in allogeneic hematopoietic cell transplant recipients can be reduced by considering donor leukocyte telomere length when selecting donors.

E1130

ABNORMAL EXPRESSION OF PYRUVATE KINASE M2 IN MYELOID DENDRITIC CELLS OF THE PATIENTS WITH SEVERE APLASTIC ANEMIA

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Background: Severe aplastic anemia (SAA) is a hematologic disease characterized by pancytopenia with severe bone marrow failure. dendritic cells was found to be an antigen-presenting cell (APC) with a most powerful function.Our previous studies have demonstrated that activated myeloid dendritic cells (mDCs) increased in the bone marrow of SAA patients, which could accelerate the polarization of Th1 from Th0 cells, and then initiate the cellular immunity which played an important role in the primary stage of the immune response.
Aims: We further analyzed the protein components of mDC in SAA and explored the possible pathogen that activated mDC.
Methods: Basing on our previous research, we further analyzed the protein components of mDC in SAA as well as normal control, furtherly we applied both FACS western and qRT - PCR technology from a perspective of mRNA and protein to validate our discovery.to explored the possible reason that activated mDC.
Results: After 7 days of culture, the purity of mDC cells was more than 90% determined by flow cytometry. Results revealed that expression of pyruvate kinase M2(PKM2) were enhanced in mDCs from SAA patients at an early stage of the onset. Concurrently, the level of PKM2 in mDC of SAA patients (59.1±15.8)% was conspicuously higher than that in normal control (32.7±20.2)% at the protein level. We measured the levels of mRNA of *PKM2* genes in our patient samples and identified significantly higher expression of *PKM2* in the SAA untreated group (1.50±0.84) relative to the recovering group (0.81±0.24) and the control group (0.32±0.11, $p<0.05$). In addition, a similar phenomenon was seen by Western blot. As expected, the percentage of PKM2 in mDCs of SAA patients appeared to be strongly correlated with proliferous degree of bone marrow. The percentage of PKM2 in mDCs in patients with SAA was positively correlated with the proportion of reticulocytes absolute neutrophil counts and quantity of mDCs in peripheral blood. Additionally, the ratio of CD4+ T-helper lymphocytes to CD8+ T-suppressor lymphocytes ($T_{H/S}$) was correlated with the percentage of PKM2 in mDCs. However, no significant difference in platelet counts was detected in SAA patients.

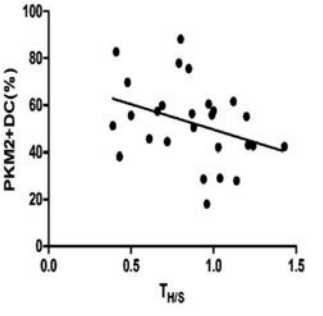

Figure 1. The purity of mDC cells was more than 90% determined by flow cytometry.

Summary/Conclusions: These findings demonstrated that dysregulation of PKM2 expression and activation in mDCs might exert an impact on the immune status of SAA patients by enhancing the cellular function of mDC.

E1131

ANALYSIS OF T CELL SUBSETS IN G-CSF PRIMED BONE MARROW FOR ALLOGENEIC STEM CELL TRANSPLANTATION AND ITS ASSOCIATION WITH CHRONIC GVHD INCIDENCE

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Background: There is a subset of T cells which modulate the immune system, maintain tolerance to self-antigens, and abrogate autoimmune disease, called regulatory T cells (Tregs). These cells generally suppress or downregulate induction and proliferation of effector T cells. Regulatory T cells come in many forms with the most well-understood being those that express CD4, CD25, and Foxp3 (CD4+CD25+ regulatory T cells). Mouse models of bone marrow transplantation as well as some clinical trials, have shown that the administration of Tregs in combination with the harvested bone marrow prevent and decrease graft-*versus*-host-disease (GVHD) and facilitate engraftment. At our institution the population transplanted with G-CSF primed bone marrow, has a lower incidence of chronic GVHD compared to those transplanted with peripheral blood and not primed bone marrow. Some microenvironment characteristics of this hematopoietic stem cells (HSC) source remain unknown, as well as the quantitative profile of different T cell subsets in patients who develop chronic GVHD and those who do not.

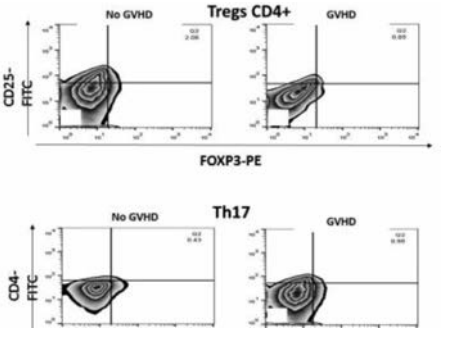

Figure 1.

Aims: To analyze the characteristics of G-CSF primed bone marrow, identifying different T cell subsets, such as Th1, Th2, Th17, and Tregs. in 29 donor samples, between patients who developed chronic GVHD and those who did not.
Methods: A prospective analysis was performed in 29 G-CSF primed bone marrow samples from donors from 1999 to 2012. Mononuclear cells were

defrosted, counted, and viability was assessed by trypan blue exclusion After a 24 hour resting time (RPMI), mononuclear cells were stimulated with PMA (50 ng/ml) for 48 hours and cells were harvested and stained for FACS analysis. Supernatants were collected and measured by Cytometric Bead Array. From each sample, one million mononuclear cells were permeabilized, fixed, and stained with CD4-FITC, IL17A-PE, *IFN*-γ APC, and IL-4 PECy7, and were analyzed by FACS. All samples were obtained in a BD LSR Fortessa cytometer and analyzed with the Flow-Jo software. Patients (recipients) demographic and clinical data were analyzed with support of the software SPSS v.21.
Results: GVHD incidence was reported as following: Ten patients (34.5%) developed chronic GVHD (10% extensive, and 90% limited), and 16 patients did not present either. There was no difference in TH1 and TH2 numbers between both groups, but mononuclear cells from donors of patients who developed chronic GVHD had a higher percentage of Th17 cells (0.99% *vs* 0.44%, p<0.001), as well as decreased Tregs (0.91% *vs* 2.00%, p<0.001), compared to those who did not developed GVHD. It was not possible to relate T cell subsets with chronic GVHD severity since only one patient developed extensive disease.
Summary/Conclusions: With the usage of G-CSF primed bone marrow as a source of HSC, the incidence of chronic GVHD at our institution was 34.5%. Patients who develop chronic GVHD are characterized by a pro inflammatory response with a higher percentage of Th17 cells, as well as a decreased suppressive response characterized by reduced Tregs levels. The low incidence of chronic GVHD show that G-CSF primed bone marrow is an excellent source for allogeneic HSC transplantations, and would be useful to compare its lymphocyte subset profile and cytokines levels with other HSC sources.

E1132

DYSFUNCTION OF BONE MARROW ENDOTHELIAL PROGENITOR CELLS FROM SUBJECTS WITH POOR GRAFT FUNCTION FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION CAN BE IMPROVED BY ATORVASTATIN

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Background: Poor graft function (PGF) is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and the mechanisms are poorly understood. Murine studies suggest that endothelial progenitor cells (EPCs) are preferential supporting cells for hematopoietic stem cells (HSCs) in the bone marrow (BM) microenvironment. Our previous prospective nested case-control study suggested that a lower frequency of EPCs, which represent a key cellular component of the BM microenvironment, was an independent risk factor for the occurrence of poor graft function after allo-HSCT. However, the functional role of bone marrow EPCs in the subjects with poor graft function has never been reported. Moreover, approaches for improving the dysfunction of bone marrow EPCs in subjects with PGF are lacking. Atorvastatin has been reported to improve the mobilization and function of circulating EPCs in a number of diseases, such as heart disease, diabetes, ischemic stroke and others. Nevertheless, no previous studies have focused on the roles of atorvastatin on bone marrow-derived EPCs in subjects with poor graft function following allo-HSCT.
Aims: In this study, we evaluated the function of bone marrow EPCs in subjects with poor graft function post-allotransplant. Moreover, we investigated whether atorvastatin could enhance the number and function of bone marrow EPCs derived from subjects with poor graft function in vitro.
Methods: Three cohorts were included: subjects with poor graft function (N=15), subjects with good graft function (N=15), defined as persistent successful engraftment after allotransplant, and transplant donors as normal controls (N=15). Atorvastatin (0.5nM,5nM,500nM) was administrated to the bone marrow EPCs in subjects with poor graft function cultured for 7 days. The number and functions ofCD34(+)/CD133(+)/KDR(+)EPCs were evaluated by flow cytometry, cell counting, DiI-Ac-LDL and FITC-lectin-UEA-1 double staining, migration, tube formation test and apoptosis. Reactive Oxygen Species (ROS) level was evaluated by flow cytometry and DCFH-DA staining. Cell proliferation was determined by cell counting kit-8 assay. Protein expression for p-p38,p38, p-akt, akt was measured by flow cytometry and western blots.
Results: Dysfunctional bone marrow EPCs, which were characterized by decreased migration and angiogenesis and higher levels of reactive oxygen species and apoptosis, were found in subjects with poor graft function following allo-HSCT. The phospho-p38 expression was significantly elevated in subject with poor graft function but all groups showed similar intracellular levels of phospho-Akt. Furthermore, bone morrow EPCs derived from subjects with poor graft function were enhanced both quantitatively and functionally by 500nM atorvastatin treatment in culture on day 7 through down-regulation of the p38 MAPK pathway.
Summary/Conclusions: In summary, the current study is, to our knowledge, the first to demonstrate the dysfunctions of bone marrow EPCs in the bone marrow microenvironment of subjects with poor graft function following allo-HSCT. Moreover, atorvastatin treatment in vitro quantitatively and functionally improved the bone morrow EPCs derived from subjects with poor graft function through down-regulation of the p38 MAPK pathway. Our results indicate that atorvastatin represents a promising therapeutic approach for repairing the impaired EPCs in subjects with poor graft function post-allotransplant.

E1133

MULTIPOTENT PROGENITOR CELLS AND ACQUIREMENT OF HEMATOPOIETIC CAPACITY IN THE BONE MARROW OF XENOPUS LAEVIS

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Background: Hematopoietic stem cells (HSCs) are characterized by their capacity for long-term repopulation and multilineage differentiation upon cytokine stimulation. Thrombopoietin (TPO) is one of the key regulators of HSC maintenance and platelet production. Previously, we cloned *Xenopus laevis* (*X. laevis*) TPO (*xl*TPO) and demonstrated that differentiation and proliferation in thrombocyte progenitors and hematopoietic progenitors are regulated by *xl*TPO. Unlike that of mammals, bone marrow (BM) of *X. laevis* is filled with adipose cells, and blood cells are mainly produced in the liver. However, functional contribution of fatty marrow for hematopoiesis is unclear, and multipotent hematopoietic progenitor is not yet identified in *X. laevis*.
Aims: In this study, we attempted to identify multipotent hematopoietic cells and evaluate the ability of hematopoiesis in fatty marrow in *X. laevis*.
Methods: The suspension cells in *X. laevis* were cultured in semi-solid culture system in the presence of *xl*TPO. Laparotomy was performed in *X. laevis* under anesthesia with MS222. The left liver was resected, and cultured with *xl*TPO for 24 days. The cultured cells were labeled by PKH26 and autologously transplanted by intracardiac injection. After 30 days, the *X. laevis* were killed and their right liver was analyzed by FACS. PKH26 labelled cultured cells were also injected into the blastocoels of *X. laevis* embryos at stage 8, which have no immune system. After 3 days, the labeled cells were analyzed by fluorescence microscope. For low-temperature exposure, cage containing *X. laevis* were transferred to an incubator set at 5°C for 12 days. For micro-computerized tomography (mCT) analysis, *X. laevis* were restrained in polystyrene foam restrainers, and the femurs were imaged by three-dimensional X-ray CT scan.
Results: Hepatic colonies stimulated by *xl*TPO could be cultured for more than 3 months, during which the cell number reached 1×10^6, indicating that the cells divided at least 20 times. These colonies expressed erythrocyte-, thrombocyte-, leukocyte-, and HSC-specific markers. Moreover, these cells differentiated to thrombocytes and leukocytes in the presence of splenic-conditioned media. Liver cells obtained by partial hepatic resection were cultured in the semi-solid culture system in presence of *xl*TPO, and cells labeled with PKH26 were autologously transplanted. After 30 days, PKH26-positive cells were detected in the sinusoids of liver and spleen. Flow cytometric analysis showed that the PKH26-positive cells displayed low forward (FSC) and side (SSC) scatter and had thin-layered cytoplasm and round nuclei, which are typical features of mammalian HSCs. These results indicated that *xl*TPO regulates proliferation of hematopoietic progenitors, which can be engrafted and differentiated to multiple lineages. To enrich multipotent hematopoietic cells, we generated anti-*xl*Mpl monoclonal antibodies and showed that anti-thrombocyte antibody $(T12)^-$/*xl*Mpl^+/FSC^{low} population was enriched in high nuclear/cytoplasm ratio-hematopoietic progenitors, and the ratio of these cells to all hepatic cells was 0.28%. Surprisingly, $T12^-$/*xl*Mpl^+/FSC^{low} cells were identified in the liver, spleen, and BM in normal state. Although BM in *X. laevis* comprises mostly of adipocytes, large colonies induced by *xl*TPO were identified in the BM. The protein expression patterns in these cells overlapped with those in colonies derived from the liver, demonstrating that multipotent hematopoietic progenitors were localized in the BM. To explore the hematopoietic capacity in the BM, *X. laevis* were exposed to 5°C, which led to pancytopenia. After exposure to low temperature for 12 days, the numbers of erythrocyte and multipotent progenitors were increased. mCT revealed that femoral bone density was higher than that before exposure to 5°C, indicating change in the BM microenvironment.
Summary/Conclusions: In conclusion, multipotent hematopoietic progenitors in *X. laevis* are enriched in $T12^-$/Mpl^+/FSC^{low} cells and these cells are mainly localized in the liver and BM. These findings suggested that $T12^-$/Mpl^+/FSC^{low} cells in BM is dormant, and changes in the BM microenvironment by low-temperature stimulation induced proliferation of hematopoietic progenitors.

E1134

BONE MARROW STROMAL CELLS FUNCTIONAL CHARACTERISTICS STUDY IN RECIPIENTS BEFORE ALLO-BMT

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Background: The role of the stromal microenvironment in hematopoietic stem cell transplantation (HSCT) is based on nonlineage-specific effects on proliferation and differentiation of HSCs. Deficient graft functioning observed in some cases necessitates development of functional tests for the stromal cells, in order to provide clinical indications for co-transplanting of hematopoietic and bone marrow stromal cells (BMSC), and evaluable introduction of alternative therapeutic approaches.

Aims: The aim of this study was to investigate the role of bone marrow stromal cells in the course of donor marrow cells engraftment and their significance in post-transplant complications.
Methods: The study included clinical observation of the post-transplant course in ten patients with acute myeloid leukemia (AML) and 7 healthy donors. Bone marrow nucleated cells were selectively harvested two weeks prior to BMT, followed by monolayer culture in alpha-MEM culture medium with 20% fetal bovine serum. Upon growth of fibroblast-like cell colonies (CFU-F), their hematopoiesis-supporting activity was determined in the agar-drop/liquid culture system, as well as their differentiating ability along adipogenic and osteogenic pathways. We have also analyzed the relative expression of *selectin* and *CXCR4* genes in these cells.
Results: When comparing functional characteristics of BMSC from healthy donors and AML patients, an increased hemostimulatory activity of the latter was noted, as reflected by an increase in large and small CFU-GM numbers ($p<0.02$). In addition, an increase in differentiation along adipogenic and osteogenic pathways was observed in AML patients ($p=0.03$). Moreover, the number of CFU-Fs, capable for adipogenic differentiation was inversely correlated with platelet recovery time ($p=0.05$). In contrast, higher numbers of osteogenic colonies in culture were associated with an increased time to leukocyte lineage recovery ($p=0.05$). When analyzing gene expression in BMSC population, a decreased expression of the *CXCR4* gene responsible for the homing effect, with age of the patient's ($p=0.05$) was noted. The *selectin* gene expression in BMSC was higher by AML patients as compared to healthy donors.
Summary/Conclusions: Stromal cells derived from the patients' bone marrow exhibit higher proliferative activity and marked expression of molecules mediating HSC homing, as compared with a group of healthy donors. That finding could be explained by affection of stromal cells by previous chemotherapy and myelosuppression. BMSC from AML patients taken before bone marrow transplantation are characterized by a more pronounced capacity to osteogenic and adipogenic differentiation than those from healthy donors. Increased adipogenic differentiation ability of the BMSCs is associated with a more rapid recovery of hematopoiesis.

E1135

G-CSF TREATMENT IN VIVO INDUCES BONE MARROW RELEASE OF PD-L1 POSITIVE MYELOID DERIVED SUPPRESSIVE CELLS

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Background: For its two main adverse effects which engage the vital prognosis, graft *versus* host disease (GVHD) and graft *versus* leukemia (GVL), tuning the immune system is the major challenge for successful cure of the patient who benefits from Hematopoietic Stem Cell (HSC) transplantation. Myeloid derived suppressor cells (MDSCs) are supposed to be an immature leukocyte subset composed of granulocytic cells (GrMDSC) and monocytic cells (MoMDSC). They are well described in different pathologies such as cancer, burns and sepsis. Few publications suggest that G-CSF induces bone marrow release of myeloid derived suppressive cells (MDSCs). But, phenotypically, it is hardly possible to distinguish them from other myeloid precursors with the classical lineage markers. Recently, immunosuppressive action of MDSCs was reported to be due to the PD-L1 molecule that renders these cells able to kill T-cells through the PD-1/ PD-L1 axis.
Aims: Using PD-L1 as an additional marker, the aim of this study was to quantify MDSCs subsets (GrMDSC and MoMDSC) before and after G-CSF treatment as well as in bone marrow of donors prior Hematopoietic Stem Cell (HSC) transplantation.
Methods: Recruitment of donors was done in two hospitals university centers (Bordeaux and Limoges, France).We analyzed 10 samples of peripheral blood (PB) before and 17 after G-CSF treatment, 27 samples of cells collected after apheresis (PSC) and 5 samples of bone marrow (BM) as control. We measured the rate of GrMDSC ($CD45^+$ Lin^- $CD11b^+$ $CD14^-$ $CD33^+$ $CD16^-$ HLA-DR^- $PD\text{-}L1^+$) and MoMDSC ($CD45^+$ $CD11b^+$ $CD14^+$ $CD33^+$ $CD16^-$ HLA-DR^- $PD\text{-}L1^+$) in BM, PB and PSC sample by flow cytometry. Percentage and absolute counts CD34 and CD3 positive cells in G-CSF treated donors have been also collected. Differences between groups were tested for significance with the Student t-test.
Results: White blood cell count was 5.09 +/- 2.55 G/L and 45.77 +/- 25.5 G/L before and after G-CSF treatment. Levels of $PD\text{-}L1^+$ HLA DR^- MoMDSCs and $CD33^+$ Lin^- $PDL1^+$ GrMDSCs were 0.0013 +/- 0.0027 G/L (0.023% +/- 0.042%) and 0.058 +/-0.048 G/L (1.15% +/- 0.91%) in PB before and were 0.078 +/- 0.085 G/L (0.21% +/- 0.25%) and 12.31 +/- 6.52 G/L (28% +/- 13.3%) after G-CSF treatment ($p=0.002$ and $<10^{-4}$ respectively). Percentages of MoMDSCs and GrMDSCs with PDL1 expression in bone marrow were 0.0033% +/- 0.0071% and 0.84% +/- 1.21%. Apheresis treatment was associated with enrichment in $PDL1^+$ MoMDCs but with a loss of $PDL1^+$GrMDSCs. Nevertheless, when compared to bone marrow, percentages and total numbers of both PDL1+ MoMDSC and GrMDSC in apheresis products were still much higher than in bone marrow ($p<0.0001$ and $p=0.0006$). There was no correlation between levels of $CD34^+$ stem cells, $CD3^+$ or $CD19^+$ lymphocytes and MDSCs after G-CSF treatment.
Summary/Conclusions: In this study we have shown that $PDL1^+$ MDSCs were virtually absent in normal bone marrow and that G-CSF treatment induced a strong MDSC production. We have also demonstrated that, compared to PB, the product of apheresis was even more enriched in MoMDSC but was partially depleted in GrMDSC. These results point a major difference between bone marrow HSCs and PSCs in terms of immunomodulation capacities of the engraftment product.

E1136

ALTERATIONS OF BONE MARROW MICROENVIRONMENT IN PATIENTS WITH HEMATOLOGICAL DISORDERS AT THE DIAGNOSIS AND DURING THE TREATMENT

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Background: Bone marrow (BM) microenvironment is involved in the initiation and propagation of normal hematopoiesis and hematological diseases. Leukemia and chemotherapy affect hematopoietic and stromal precursor cells. Multipotent mesenchymal stromal cells (MMSCs) are the essential element of hematopoietic microenvironment.
Aims: The study aimed to characterize MMSCs and their more differentiated progeny CFU-F derived from the BM of the patients with hematological disorders at diagnosis and during the treatment.
Methods: 74 newly diagnosed cases (33 AML, 21 ALL, 20 CML) were involved in the study after informed consent. BM was aspirated prior to treatment (time-point 0) and at days 37, 100 and 180 since the beginning of treatment of acute leukemia and +3, +6 and +12 months for CML (time-points 1-3). MMSCs were cultured in aMEM with 10% fetal calf serum. Time to P0 and cumulative MMSC production after 3 passages were evaluated and CFU-F concentration was analyzed. The relative expression level (REL) of genes was measured by TaqMan RQ-PCR. As a control MMSCs and CFU-Fs from 88 healthy donors were used.

Table 1.

	Time-point	AML	ALL	CML
Time to P0, days	0	17.6 ± 0.6 *	19.8 ± 1.4 *	17.7 ± 0.8 *
	1	15.5 ± 0.9	14.1 ± 0.9	16.7 ± 0.8 *
	2	16.6 ± 0.6 *	16.0 ± 0.9	18.2 ± 1.0 *
	3	16.8 ± 0.5 *	16.2 ± 0.7 *	17.6 ± 0.7 *
Cumulative cell production for 3 passages, $x10^6$	0	4.8 ± 0.7 *	5.2 ± 1.4	7.5 ± 1.6
	1	7.9 ± 1.4	7.7 ± 1.5	7.1 ± 1.1
	2	10.1 ± 1.7	8.2 ± 1.4	6.7 ± 1.2
	3	9.8 ± 1.4	9.4 ± 2.3	6.5 ± 0.7
CFU concentration per 10^6 BM cells	0	11.5 ± 4.6 *	8.3 ± 3.6*	15.8 ± 4.2*
	1	27.7 ± 4.4	30.3 ± 8.2	21.2 ± 4.4
	2	16.3 ± 3.5	18.5 ± 6.5	12.6 ± 2.8 *
	3	21.2 ± 3.7	28.9 ± 7.8	4.9 ± 1.1 *

*statistically significant difference from control group($p<0.05$)

Results: Time needed to reach P0 reflects the quantity of MMSC in the BM sample. The time to P0 in control group was 13.7±0.3 days. Its elongation in acute leukemia cultures at the time of the diagnosis (table) suggested the reduction of MMSC number probably due to leukemic expansion, while the longer time to P0 at time-points 2 and 3 could be explained by the therapy influence. In CML cultures time to P0 was significantly longer during the whole observation period due to the continuous therapy and maintaining disease. Cumulative MMSC production in control group was 7.1±1 x 10^6 cells. In patients with AML it was 1/3 of the donor's at the disease manifestation with no difference at time-points 1-3, indicating the impaired proliferative abilities of MMSC at the AML manifestation. Cumulative MMSC production in patients with ALL and CML didn't differ from donor's. BM blast count did not correlate with MMSC production. CFU-F concentration in the BM of acute leukemia patients was significantly lower than donor's (25.4±3.1 per 10^6 cells) at the time-point 0 with no difference at time-points 1-3. CFU-F concentration in the BM of CML patients was also nearly 40% lower than in control group at the time-point 0 with its following restoration at time-point 1 and subsequent drop (up to 5 fold lower) at time-point 3, reflecting the long-lasting lesion of CFU-Fs during the course of the disease. Gene expression analysis of MMSC from all patients revealed significant decrease in REL of VEGF and SOX9 genes at the disease manifestation that did not restore after the treatment. The REL of LIF was significantly increased at the disease manifestation, reflecting the efforts of MMSCs to inhibit leukemic proliferation. REL of PDGFRB and IL6 increased during the treatment. The treatment lead to the downregulation of FGF2, TGFB1 and 2. As FGF2 and TGFB inhibit the differentiation of MMSCs, their downregulation may refer to the effectiveness of therapy. In MMSCs from acute leukemia patients the ICAM and SPP1 genes were downregulated at all points, reflecting the mechanism of the blocking of MMSCs migration and differentiation during the stress conditions. The influence of chemotherapy lead to decrease in REL of SDF1, IL8, VCAM, IL1b1R1, JAG1, IGF1, FGFR1 and 2.

Summary/Conclusions: The study supports the major influence of leukemic cells and chemotherapy on the BM microenvironment. The two types of studied precursors are affected differently. Future studies are needed to evaluate the role of MMSCs in leukemia pathogenesis.

E1137

COMPARISON OF MOLECULAR AND FUNCTIONAL PROFILES OF WHARTON'S JELLY AND BONE MARROW MESENCHYMAL STEM CELLS

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Background: We have previously shown that *ex vivo* expanded human mesenchymal stem/stromal cells (MSCs) derived from the Wharton's jelly (WJ) of the umbilical cord exhibit increased proliferative capacity and reduced differentiation potential to adipocytes and osteocytes, compared to bone marrow (BM) derived-MSCs. This altered differentiation capacity has been attributed, at least in part due to reduced expression of Wnt antagonist sFRP4 (a promoter of adipogenesis) and WNT-induced secreted protein-1 (a regulator of osteogenesis in WJ-MSCs), thereby supporting the contribution of WNT-pathways in human MSC biology (Batsali A et al.,Blood 2014:124,21).
Aims: In order to extent the comparative study between BM and WJ-MSCs we seek to estimate the telomere length of cultured MSCs and analyze the cell-cycle signaling pathway. Moreover, we aim to investigate the capacity of WJ-MSCs to support the growth of hematopoietic stem cells (HSCs).
Methods: MSCs were isolated and expanded from healthy donors' BM aspirates (n=15) and from the WJ of full-term neonates (n=15) after written informed consent. MSCs were *in vitro* expanded and re-seeded for a total of 10 passages and were verified phenotypically by flow cytometry. The expression of 84 genes related to cell-cycle signaling pathway was assessed using a PCR array (Fold Change (FC) $=2^{-\Delta\Delta Ct}$). DNA was isolated from culture expanded MSCs (at P2, P6 and P10) and telomere length was measured by means of real-time-PCR using β-globin as control. Telomere length is proportional to the relative telomere/single-copy-gene ratio, $T/S= 2^{-\Delta Ct}$ ($\Delta Ct= Ct^{telomere} - Ct^{\beta\text{-globin}}$). Levels of human hematopoietic associated cytokines (Flt-3 ligand/G-CSF/GM-CSF/SDF-1a) were measured by means of enzyme-linked-immunosorbent assay, from supernatants of MSC cultures (from P2, P6 and P10). MSC hematopoietic supportive capacity was evaluated in two-week co-cultures with allogeneic BM or UC-blood CD34+ cells.
Results: Culture-expanded cells from both WJ and BM displayed typical morphological and immunophenotypic MSC characteristics and were able to differentiate into osteoblasts and adipocytes. Regarding the cell-cycle signaling pathway many genes essential for mitosis and genome stability were up-regulated in WJ-MSCs, such as ATM (FC=4.04), CCND2 (FC=4.55), AURKB (FC=3.15) and MKI67 (FC=2.86), while the anti-apoptotic gene BCL2 was down-regulated (FC=4.07). Moreover the relative telomere length of WJ-MSCs was higher comparing with BM-MSCs through all passages, with a significant difference in P2 ($p=0.032$). The expression of hematopoietic associated cytokine Flt-3 ligand, an important cytokine for development of several hematopoietic populations, was expressed in both MSC-sources. G-CSF and GM-CSF, growth factors of HSCs involved in the production of granulocytes, macrophages and dendritic cells, were significantly up-regulated in WJ-supernatants but were almost absent in BM-cultures ($p<0.001$). The regulator of migration, adherence and engraftment of HSCs, SDF-1a, was significantly overexpressed in BM-supernatants through cultivation ($p<0.05$). Nevertheless, WJ-MSCs seemed not to support CD34+ cell growth as evidenced by the numbers of total colony-forming units (CFUs), at least in the current experimental setting.
Summary/Conclusions: Collectively, our results suggest that WJ-MSC population exhibit greater proliferation potential and decreased apoptosis, longer telomeric length and higher genome stability. The observed differences of CFUs in the co-culture systems could be attributed to the differences in cytokines secreted by WJ and BM-MSCs. Our results point out that WJ- and BM-MSCs exhibit unique characteristics and therefore the appropriate source should be selected for a specific clinical application.

E1138

IMPACT OF LEUKEMIA STEM CELLS PHENOTYPE EXPRESSION ON RESPONSE TO INDUCTION THERAPY IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Laboratory data suggest that acute myeloid leukemia AML originates from a rare population of cells, termed leukemic stem cells (LSCs) or leukemia-initiating cells, which are capable of self-renewal, proliferation and differentiation into malignant blasts. There's a universal agreement that LSCs lie within the CD34+ compartment of hemopoietic cells and most of leukemic stem cells express the interleukin-3 alpha chain receptor, CD123 and lack CD38. The standard model of leukemogenesis posits the stepwise acquisition of genetic mutations in a susceptible cell and its progeny, leading to the development of an autonomous clone that expands enough to cause a clinical syndrome. The genetic events occur in susceptible cells that are either hemopoietic stem cells or its progeny. The resultant leukemic cell has the properties of a stem cell in terms of self-renewal, but with persistent uncontrolled division and resistance to chemotherapy. This leukemia stem cell theory has been proved by proliferation of proposed leukemic stem cell after its injection into lethally irradiated mice. This finding inspired scientists and researchers to explore new fields for leukemia treatments because with the current regimes, the relapse rate is high and death frequently happens due to treatment failure or consequences of bone marrow blast invasion. In the same perspective, researchers began to assess the prognostic value of leukemic stem cells at diagnosis and to weigh its significance with other known prognostic factors of AML.
Aims: This study aimed to estimate the expression of LSC phenotype in AML patients and to correlate it with response to induction therapy.
Methods: A cohort of 41 patients older than 15 years with newly diagnosed de novo AML were enrolled in this study. They were obtained from the National center of hematology in Baghdad and Baghdad teaching hospital between February and July 2013. The expression of CD34, CD38 and CD123 was assessed by multi-color flow cytometry. LSC positive (LSC+) samples must express CD34 and CD123 and lack the expression of CD38 in >1% of cells. French American British (FAB) classification system was used in this study. After four weeks of induction therapy; three groups were found: those who reached the complete morphological remission (CR), those who failed to reach CR and those who died before assessment of morphological remission. The last two groups were merged for statistical purposes. All statistical operations were done by Microsoft excel 2010 and SPSS programs. The p-value for significance was calculated from Chi square test and Fisher Exact test for 2X2 contingency tables when the Chi square test was not applicable. The percentage of LSCs was calculated as the percentage of cells in CD34+CD123+ quadrant X the percentage of CD38- cells X the blast% of the individual patient
Results: The mean expression of CD34+CD38-CD123+ cells at diagnosis was 3.85 ± 7.14 (mean$\pm$SD), range=0% to 42.05%, median=2.15%. A cut off value of 1.0% was designed to separate two groups of patients; those with LSCs of less than or equal to 1.0% and those with more than 1.0%. In the first group (LSCs<1.0%), the CR rate was 53.33%, while it was 34.61% in the second group. Percentage of cases who sustained NR was 46.67% in the first group and 65.39% in the second age group. Regarding gender, LSC+ male patients were more (70.83%) than LSC+ females (52.94%). Patients with a low (<1%) percentage of CD123+CD34+ cells had a CR rate of 50% and an NR rate of 50%. On the other side, in patients who had a higher expression (>1%) of CD123+CD34+ cells, the CR rate was less (7 of 21, 33.33%) and NR was higher than the other group (14 of 21, 66.67%).
Summary/Conclusions: 1. LSCs were expressed in 63.41% of AML cases and were distributed among FAB subtypes without preference to any FAB subtype. 2. The expression of LSC phenotype was associated with poor response to induction therapy in AML patients.

E1139

VASCULAR ENDOTHELIAL CELL-RELATING MOLECULES EXPRESSED IN AN ADULT HUMAN DERMAL FIBROBLAST WHEN CULTURED WITH INTERLEUKIN-1-BETA

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Background: We previously reported that when an adult human dermal fibroblast (HDF) was cultured with interleukin (IL)-1-beta (b), vascular endothelial growth factor (VEGF)-A, erythropoietin (EPO), anti-human VEGF-C antibody (Ab) and anti-human tumor necrosis factor (TNF)-alpha (a) Ab, hematopoiesis-related genes expressed; however, culturing cells were morphologically fibroblasts (56th ASH). We also reported that hemogenic endothelium-relating molecules expressed in HDF with poly (I:C) culture and an electrical stimulation (44th ISEH).
Aims: In this report we observed the biological effects of IL-1-b to HDF for up to one month-culture.
Methods: HDF was cultured with CELRENA medium, used for induced pluripotent stem (iPS) cell-culture, on 0.1% gelatin-coated plate (porcine skin). Cultures were added with recombinant human IL-1-b for up to four weeks with splitting each one week. Morphological changes and the expression of vascular endothelial cell-related genes, and hematopoiesis-related ones were observed.
Results: When HDF was cultured with IL-1-b, CD31, CD34, and CD41 molecules expressed for two weeks' culture, which are vascular endothelial cell-markers. Also, VEGF-receptor type-1, and -2, and VE-cadherin expressed significantly. Morphologically, HDF was changed to an endothelial cell. When HDF was further cultured, blast cells were attached to an endothelial cell, and SCL, and GATA-2 molecule expressed significantly.
Summary/Conclusions: Recent reports reveal that a kind of vascular endothelial cells, hemogenic endothelium, can be converted into a hematopoietic cell. We identified that IL-1-b worked an important role in vascular endothelial cell-formation.

LB2256

EXOSOMES SECRETED BY STROMAL CELLS CONTRIBUTE TO THE HEMATOPOIETIC STEM CELL NICHE

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Background: Hematopoietic stem cells (HSCs) are identified by their ability to self-renew and to differentiate into all blood cell lineages. *In vivo*, hematopoietic stem/progenitor cells (HSPCs) are in close association with stromal cells that constitute a supportive microenvironment also called niche. Recently, exosomes that are small microvesicles enclosed by a lipid bilayer and enriched in cytoplasmic proteins, mRNAs, microRNAs, have emerged as major communication mediators between cells. However, their implication in the cross-talk between HSCs and stromal cells is still largely unknown.
Aims: This study aims to assess the existence and the functionality of stromal cell-derived exosomes in the HSPC support.
Methods: To address this issue, we used two murine stromal cell lines derived from the fetal liver and with differing capacity to maintain HSPCs *ex vivo* as revealed by repopulation assay and long-term cultures. AFT024 (AFT) harbors a potent HSPC supporting capacity *in vitro* whereas BFC012 (BFC) is non supportive. For each cell line, the exosome fractions were isolated from culture supernatant by ultra-centrifugation. Electron microscopy, western blot, and flow cytometry were performed to characterize the exosomal fraction isolated. Using PKH67 staining we analyze the uptake of exosomes after co-culture with Lin-Sca1+ cKit+ (LSK) cells, total bone marrow or after *in vivo* injection. Clonogenic assay and FACS analysis were performed after co-culture of LSK cells with exosomes to assay their biological effect. High-throughput sequencing was realized to explore the molecular signature followed by several bioinformatic analyses. Finally, RNA transfer from exosomes to recipient cells was analyzed by qPCR.
Results: Electron microscopy performed on stromal cells showed multivesicular bodies containing exosomes. Additional electron microscopy, FACS and western blot analyses performed on the fraction isolated by ultracentrifugation revealed that both AFT and BFC stromal cells secrete exosomes. We then investigate if these exosomes could be taken up by HSPCs. Interestingly, using PKH67 stained exosomes, we demonstrated that bone marrow $Lin^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$ (LSK) cells preferentially uptake AFT-derived exosomes. This observation might be related to the different tetraspanin compositions of AFT and BFC derived exosomes as observed by flow cytometry. Furthermore, *in vitro* and *in vivo* assays showed that AFT exosomes specifically target hematopoietic CD45+ cells. We then showed an increase in cell viability and clonogenic potential when LSK cells were exposed to AFT-derived exosomes for 96 hours in cytokine-free medium as compared to controls. Moreover, cultures with AFT-derived exosomes exhibited a 3.5 fold increase in the number of LSK cells as compared to untreated conditions. We then used high-throughput sequencing to explore the molecular signatures of AFT and BFC derived exosomes, as well as their cells of origin. We identified a list of 324 mRNAs and 23 microRNAs specifically expressed in exosomes and correlated to the HSPC support. Gene ontology analysis revealed that the apoptotic regulation, cell survival and proliferation pathways were significantly enriched in the AFT-derived exosomal signature. In addition, we showed the transfer of mRNAs involved in these pathways from the AFT-exosomes to the LSK recipient cells. Together with our observation of a decrease in the LSK apoptotic cells after co-culture with AFT-derived exosomes, these data suggest that exosomes released by AFT cells may protect HSPCs from apoptosis.
Summary/Conclusions: Collectively, our results revealed an important role for exosomes in the HSPC supporting capacity of stromal cells. This work provides new insights in our understanding of the molecular and cellular mechanisms involved in the cross-talk between HSPCs and their niches. It may also have interesting applications in regenerative medicine, regarding the *ex vivo* manipulation of HSCs in stromal-free conditions for cell therapy.

LB2257

HUMAN UMBILICAL CORD BLOOD STROMAL CELLS PROMOTE HEMATOPOIETIC RECONSTITUTION AND REDUCE GVHD BY AFFECTING THE TH CELL SUBSETS IN HAPLOIDENTICAL-HSCT

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Background: Microenviroment palys very important role in the regulation of graft-versus-host desease (GVHD).
Aims: To investigate the effect of human cord blood–derived stromal cells (hUCBDSCs) combined with haploidentical-hematopoietic stem cell transplantation (haplo-HSCT) on GVHD and hematopoietic reconstitution of severe bone marrow radiation disease.
Methods: The hybrids F1 generation mice were taken 8.0Gy of 60Coγ ray total body irradiation (TBI). Haploidentical-HSCT were taken after TBI. Grouping: mBMSC (mouse bone marrow-derived stromal cells) + HSC(Hematopoietic stem cells) group, hUCBDSCs + HSC group, HSC group and the control group (saline group). Peripheral blood were taken at 0,3,7,10,14,21,28d days respectively after transplantation to count WBC, RBC, Hb, PLT. Bone marrow, liver, spleen, intestine and skin were collected and paraffin-embedded after transplantation at each point to observe pathologic changes. The changes of body weight, postural, activity, hair, toilet and survival were observed after transplantation at each point. Cell colony culture were taken at 7,14,21d after transplantation. IL-2, IL-4, IL-10, IL-12, IL-17, IL-21, IL-22, IL- 23, IL-33, TGF-β, IFN-α and IFN-γ in serum were detected by ELISA at 0,7,14,21d after transplantation.
Results: All mice in control group died at 9d to 11d after TBI. The hemogram began to recover at 7d in hUCBDSCs + HSC group and mBMSC + HSC group after transplantation which was earlier than that in HSC group (10d). Cell colony of CFU-E, CFU-GM, CFU-GEMM and CFU-G in hUCBDSCs + HSC group was more than mBMSC + HSC group ($p < 0.05$). And mBMSC + HSC group was more than HSC group ($p < 0.05$). The death rate of hUCBDSCs + HSC group and mBMSC + HSC group were (20.00±5.00)% and (21.67±12.58)% which were lower than (38.33±7.64) % in HSC group ($p < 0.05$). The GVHD of hUCBDSCs + HSC group was not obviously which was better than HSC group. There were no significant differences of IL-12, IL-17 and IL-23 in each group. IL-2, IFN-γ expression were increased after transplantation, which is higher in HSC group than hUCBDSCs group($p < 0.05$); IL-10 expression in the peripheral blood were decreased in each group after transplantation. The recovery of IL-10 in hUCBDSCs + HSC group was faster than HSC group ($p < 0.05$). TGF-β expression were increased faster in hUCBDSCs + HSC group than the other two groups($p < 0.05$) which was the same as TNF -α.
Summary/Conclusions: HUCBDSCs promote hematopoietic reconstitution and reduce the incidence of GVHD in severe bone marrow radiation disease in vivo. The main mechanism of this may be associated with the decrease of Th1 cells and increase of Th2 cells expression.

Hodgkin lymphoma - Clinical

E1140

THYROID DISEASES IN LYMPHOMA SURVIVORS

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Background: Improvements in the treatment of both Hodgkin's (HL) and non Hodgkin's Lymphomas (nHL) have resulted in an increasing number of long term survivors. However this patient's population is at high risk of developing late therapy related complications that can negatively affect their survival and quality of life. In this report we focus on thyroid diseases.
Aims: In our institution the HL and aggressive nHL long term survivors are followed up in a dedicated clinic since September 2014. Here we report preliminary data on thyropathies.
Methods: We have reviewed our lymphoma survivors database
Results: We have analyzed data regarding 469 consecutive patients coming in our clinic from 15 September 2014 to 18 February 2016, 247 were affected by HL and 222 by nHL. Two hundred thirty three were females, 236 males; median age at diagnosis was 29 years for HL (range 13-84), 48 years for nHL (range 12-83); median age at last observation in the follow up clinic was 50 years for HL (range 21-89) and 62 years for nHL (range 24-88). The median duration of follow up was 18 years for HL (range 5-40) and 13 years for nHL (range 5-37). One hundred eight patients (23%) experienced thyroid disorders; they were 85 females (79%) and 23 males (11%), 77 have been treated for HL (71%) and 31(29%) for nHL. Thyroid diseases observed in our patients were: hypothyroidisms in 54, autoimmune thyroiditis in 24, nodules in 10, goiters in 10,carcinomas in 8, hyperthyroidism in 2 (table1). Thirteen of these patients had thyroid exeresis after thyroid disease diagnosis (table2). Regarding the previous therapies in thyroid disease patients: mediastinal and/or neck radiotherapy has been administered to 83 (77%) of patients and chemotherapy in 99 (92%). The median time between diagnosis of lymphoma and diagnosis of thyroid problems was 10 years (range 0-33). Thirty four of these disorders (31%), including one carcinoma, have been detected during planned controls (hormonal dosage and echotomography) in absence of symptoms.

Table 1. Type of thyroid disorders in our lymphoma survivor patients.

Type of thyropathies	Patients	%
Hypothyroidis	54	50
Autoimmune thyroiditis	24	22,2
Nodules	10	9,2
Goiters	10	9,2
Carcinomas	8	7,4
Hyperthyroidism	2	2

Table 2. Causes of thyroidectomy.

Causes	number	%
Carcinoma	8	62
At diagnosis of lymphoma	2	15
Goiter	2	15
Basedow disease	1	8

Summary/Conclusions: In our Department we described a high number of cases of thyroid disorders in the lymphoma survivors population. These were more common in females, as in general population, and in previous HL, probably because of higher rate of radiotherapy received in the treatment of HL compared to nHL. Almost a third of these disorders were found by tests done for early diagnosis of late complications in asymptomatic patients. These results outline the importance of screening of thyropaties in this setting of thisspecial population.

E1141

Abstract withdrawn.

E1142

PROGNOSTIC RELEVANCE OF SERUM FERRITIN LEVELS IN HODGKIN LYMPHOMA (HL) UNDER TREATMENT WITH ABVD OR EQUIVALENT REGIMENS WITH OR WITHOUT RADIOTHERAPY (RT)

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Background: Cytokine-driven inflammatory processes dominate the clinical and laboratory expression of HL, especially in advanced stages. Serum ferritin (SF) levels increase in the context of "acute phase reaction" along with fibrinogen, ESR, cerulloplasmin, haptoglobins etc. Although SF elevations are a well known feature of HL, their prognostic significance has not been adequately evaluated. Recently, Fernandes-Alvarez et al reported that elevated SF levels (>350 μg/L) were an independent prognostic factor in 173 optimally treated patients with classical HL (Leuk Lymphoma 2015).
Aims: To evaluate the prognostic significance of SF levels in a large series of patients with HL treated with ABVD or similar chemotherapy with or without radiotherapy (ABVD±RT).
Methods: We evaluated 309 pts with HL, treated with ABVD or equivalents (±RT), who had available data for SF levels. Median age was 33 yrs (15-87), 54% of patients were male, 34% had B-symptoms, 17% had stage IV disease, 50% had advanced stage (IB,IIB,III,IV), 33% had IPS ≥3, 67% nodular sclerosis histology and 19% mixed cellularity. Failure-free survival (FFS) was defined as time between treatment initiation and toxic death, failure to achieve remission requiring switch to salvage therapy, relapse after remission or last follow-up; deaths in remission of unrelated causes were not counted as events.
Results: Median follow-up of currently alive patients was 41 months. The median SF levels were 149 μg/L (range 6.6-6709.0, interquartile range; IQR 63.9-316.5). As expected, SF levels were lower in females than males (medians 70.6 *vs* 240.2, p<0.001). Increased SF levels were highly correlated (p<0.001) with older age, advanced stage, increased IPS and markers of systemic disturbance (B-symptoms, anemia, ESR, CRP, low serum albumin), as well as with beta2-microglobulin, elevated serum LDH (p=0.01) and mixed cellularity (p=0.01 compared to nodular sclerosis). However, there was no correlation with leukocytosis or lymphocytopenia. At a cutoff of 150 μg/L (almost the median), the 5-year FFS was 85% *vs* 67% for patients with lower and higher SF levels (p=0.0005). If a gender-based median cutoff was adopted (high levels above the median of each gender), the results were similar (84% *vs* 68%, p=0.0006). At the published cutoff of 350 μg/L only 21% of patients had high SF levels and the difference in the outcome was less pronounced (79% *vs* 64%; p=0.01). In both early (IA/IIA) and advanced (IB, IIB-IV) stages, SF ≥150 μg/L provided an 8-9% absolute difference in 5-year FFS, which was not however statistically significant (p=0.18 and p=0.16). In multivariate analysis, SF ≥150 μg/L was an independent prognostic factor (p=0.04; HR=1.79) along with advanced stage (p<0.001; HR=2.66), when adjusted for B-symptoms, anemia, LDH and gender. Further adjustment for CRP or ESR did not result to any meaningful alteration of the magnitude of the HR. However, the significance of SF became marginal if stage IV was used instead of "advanced stage". In models containing SF levels, B-symptoms and IPS, SF displaced IPS.
Summary/Conclusions: Elevated SF levels may be an independent prognostic factor in HL treated with ABVD or equivalent regimens±RT after adjustment for other important variables, including B-symptoms, anemia, ESR and CRP. However, larger patient series are needed in order to extract safe conclusions and determine the optimal cutoff.

E1143

INFRADIAPHRAGMATIC HODGKIN LYMPHOMA: A LARGE SERIE OF PATIENTS AT THE ERA OF TEP-CT. A PARTICULAR SUBSET OF HL?

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Background: Infradiaphragmatic Hodgkin Lymphoma (IDHL) accounts for 3-11% of adult cases of stage I-II Hodgkin Lymphoma. The strategy of treatment has been improved and standardized along the last decades in most clinical subsets of HL, while it remains heterogeneous in IDHL these patients being often excluded from clinical trial. Moreover, in previous studies focused on IDHL, the patients were not staged by PET-CT and the risk was considered localized stages as advanced.
Aims: Thus, this study aimed to include PET staged patients by comparing with PET non staged patients for demographic, clinical and biological data the outcome of these patients.
Methods: The clinical, biological data at baseline, the details of treatment and

outcome of patients with a first diagnosis of stage I-II of IDHL were retrospectively collected in 8 french departments of hematology. During the same period, patients with a positive HIV serology and those treated with radiotherapy alone were excluded. Also, we reported only the patients treated with ABVD regimen of chemotherapy.
Results: From 1986 to 2014, 100 patients were included whose 65 of them staged with PET. The characteristics between patients staged with or without PET-CT were significantly different for age (higher median for PET staged, 53 years *vs* 47 years, p=0.041), ESR (higher median for PET non staged; 27 *vs* 58mm, p= 0.044), haemoglobin (lower for PET non staged, 13.6 *vs* 12.8g/dL, p=0.0267) and frequence of central adenopathy involvement (60% *vs* 80%, p=0.042). Indeed, the following analyses were made in each group separately in univariate and after adjustment with these four parameters. In the PET-staged patients group, which were considered as real pure infra-diaphragmatic, the median of follow-up was 3.8 years. At five years, the PFS was 78% (IC95% 0.64-0.87) and OS was 88% (IC95% 0.73-0.95). Thirteen relapses occurred (20%) whose eleven (16%) in the five-years after diagnosis, and five died (8%), 3 from HL progression and 2 from toxicity of chemotherapy. In univariate ananalysis, bulky mass at baseline (p=0.03) and the chemo-radiotherapy combination (p=0.024) influenced the PFS and only bulky mass and the combination of treatment remained significant (p=0.008) and at the limit of statistical significativity (p=0.069) in the modelisation analysis, respectively.
Summary/Conclusions: This multicenter retrospective study including PET-staged patients, considered as pure IDHL, shows clinical and biological specific characteristics, which confirm that PET was very useful for management of these patients. Thus, IDHL could be identify as a subset of HL, in particular of elderly HL. The chemo-radiotherapy combination seemed be related to a better PFS in these patients.

E1144

RESULTS AND OUTCOME WITH ABVD-PLATINE PROTOCOL IN HODGKIN LYMPHOMA WITH BULKY MEDIASTINAL INVOLVEMENT
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Background: Mediastinal lymph node involvement can be large in 50-60% of Hodgkin lymphoma (HD) wich may be discovered on imaging in asymptomatic patients (pts) or pts with respiratory signs or chest pain. Bulky form is defined as a mediastinal mass exceeding one third of the maximum transverse thoracic diameter on standard chest X-Ray at the level of 5th and 6th thoracic vertebra=mediastinal tumor ratio (MDR) ≥35.
Aims: We report the results of 179 HD with bulky mediastinal treated by ABVD-Platine protocol.
Methods: Over a period of 9 years (2004-2012), 341 pts / 674 (50,6%) with HD had mediastinal involvement with bulky's one in 200 (58,6%), median MTR=0,43 (0 -35- 0,80) among them 179 were treated with ABVD-P: median age 25 years (11-64), sex ratio 1,03. The most frequent revealing symptom was lymphadenopathy (51%), fonctionnal respiratory signs (23%) and superior cava vein syndrome in 5 pts. The histopathologic patterns are nodular sclerosis (65%), mixed cellularity (28%) lymphocyte depleted 06 pts or predominant 1 pt and unclassified 5 pts. The clinical Ann Arbor staging was stage II: 53%, stage III 32% and stade IV 15%. According to prognostic score GHSG (German Hodgkin Lymphoma Study Group): 18 pts (10%) were in intermediate stage and 161 (90%) in advanced stage. The protocol treatment is ABVD with platine 30 mg/m^2 D2, D3 and D16, D17) monthly: 3 cycles for stage II, 4 for stage III and IV. After chemotherapy (CT), 122 pts received radiotherapy (RT), 24 pts high dose CT (HDC) with autologous stem cell transplantation(ASCT) and 25 pts were followed after CT alone. At december 2015, the median follow-up is 98 months (39-143).
Results: After CT, 177pts / 179 are evaluable, the overall response (OR) is 96,6% with 32,2% in complete remission (CR) and 64,4% partial remission (PR),6 pts are in failure treatment. At term follow-up 158 pts are alive, among them 155 CR, 3 relapse and 19 died. The overall (OS) and event free (EFS) actuarial survival are 90% and 83% respectively at 8 years. Among the 171 pts in response after CT: 122 pts with CT-RT, 24 pts with CT-ASCT and 25 pts followed after CT alone, the OS were 94,8%, 71,6%, 77,4% respectively and EFS 93,8%,46,8% and 49,7%.
Summary/Conclusions: The protocol ABVD-Platine in HD with bulky mediastinal involvement allowed good results in association with RT better than in association with CT-ASCT (p<0,001), but no with CT alone (p=0,08) on OS. Concerning EFS the association CT-RT is better than CT-ASCT and CT alone p<10^{-8}).

E1145

ADDITION OF ABSOLUTE LYMPHOCYTES/MONOCYTES RATIO IMPROVES THE STANDARD PROGNOSTIC SCORES FOR ADVANCED AND LOCALIZED HODGKIN LYMPHOMA PATIENTS TREATED WITH ABVD +/- RADIOTHERAPY
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Background: Hodgkin lymphoma (HL) is a lymphoproliferative malignancy constituted by a few malignant cells (Reed-Sternberg cells) surrounded by an inflammatory microenvironment. The amount of monocytes infiltrating HL is a known prognostic factor but its study implies immunohistochemistry. Lymphopenia is a recognized adverse prognostic factor included in the International Prognostic Score (IPS). In the last years the clinical value of the absolute lymphocytes/monocytes ratio (LMR) has been reported as an alternative way to consider previous prognostic factors.
Aims: We aim to evaluate the combination of the absolute lymphocytes/monocytes ratio with the standard prognostic scores for advanced (IPS) and localized (GHSG) HL in order to improve definition of prognosis.
Methods: We retrospectively selected from the Pathology and Pharmacy registries of Son Espases University Hospital those patients with HL homogenously treated with ABVD +/- radiotherapy (RT). All standard prognostic factors were obtained from the records included in IPS and GHSG prognostic scores, as well as the absolute value of lymphocytes and monocytes. COR curves were used to determine the cutoff value for the LMR. IPS and GHSG scores were combined with LMR as follows: IPS 0-2=0; IPS>2=1; GHSG 0=0; GHSG>0=1; LMR≤cutoff=1; LMR>cutoff=0. LMR-IPS and LMR-GHSG were the sum of IPS or GHSG with LMR previous values (ranging from 0 to 2). Survival analysis was performed using Kaplan-Meier curves with the Log-rank test.
Results: From January 1990 to June 2015, 124 patients fulfilled inclusion criteria. Median age was 37 years (15-75), 62% males, 48% Ann Arbor (AA) stage III-IV, 13% with ECOG performance status >1, 53% with IPS >2. Median LMR was 2.19 (0.2-7.7). Response was as follows: 105 (87%) achieved complete response (CR), 2 (2%) partial response (PR) and 17 (12%) stable disease or progression (SD/P). Using COR curves we set the cutoff for LMR in 1.56. Response rate, CR and SD/P were significantly better in patients with LMR >1.56: respectively 95% *vs* 72%, 94% *vs* 69% and 5% *vs* 29% (p=0.001); as well as patients younger than 45 (p=0.024) and 60 (p=0.003) years, with ECOG 0-1 (p=0.001), LMR-GHSG 0 (p=0.037) and LMR-IPS 0-1 (p=0.007). Univariate survival analysis showed several factors significantly influencing OS: age, ECOG PS, GHSG and IPS, ALC, AMC, LMR, LMR-IPS and LMR-GHSG (Figure 1). PFS was significantly influenced by age, AA stage, ECOG PS, IPS, LMR and LMR-IPS (Figure 1). In multivariate analysis for early HL age >45 (HR 13.1; p<0.001), ECOG PS>1 (HR 9.7; p=0.034) and LMR-GHSG>0 (HR 19.8; p=0.045) (Figure 1) where independent predictors of worse OS while in advanced HL only remained independent age>45 (HR 4.8; p=0.028) and LMR-IPS 2 (HR 14; p=0.041). In the PFS multivariate analysis only LMR-IPS showed to be an independent adverse prognostic factor both for the whole group (HR 4.5; p<0.001) and the advance HL patients (HR 3.5; p=0.013).

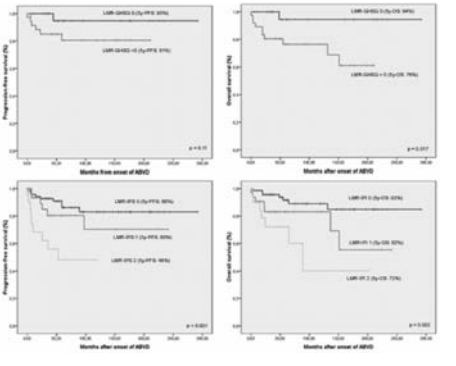

Figure 1.

Summary/Conclusions: Addition of absolute LMR improved the ability of IPS and GHSG scores in identifying HL patients with worse responses and survival after standard ABVD +/- RT. Particularly, LMR-IPS was able to identify a high risk subgroup of patients with a 5y-PFS of 48% inside the patients with IPS>2.

E1146

BRENTUXIMAB VEDOTIN-BENDAMUSTINE COMBINATION FOR HODGKIN LYMPHOMA: EXPERIMENT WITH 8 PATIENTS
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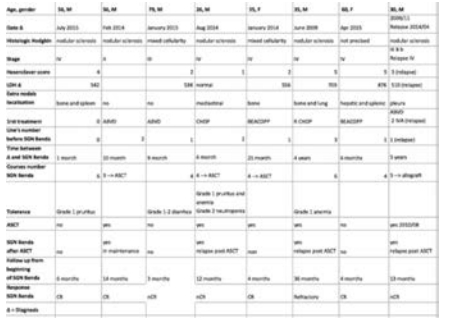
Background: Optimal treatment for patients (pts) with heavily pretreated Hodgkin lymphoma (HL) is controversial. Long term outcomes from autologous stem cell transplant (ASCT) in relapsed/refractory (R/R) HL are significantly better in pts achieving complete remission (CR) from salvage chemotherapy prior to ASCT. Brentuximab vedotin and bendamustine are highly active, as single agents, for pts with R/R HL and have manageable safety profile. Bren-

tuximab is an antiCD30 monoclonal antibody conjugated to monomethyl auristatin E. Patients in CR after Brentuximab, particularly young pts and low risk early stage disease, did not necessarily benefit additional consolidative therapy (ASCT or allograft). Elsewhere, Brentuximab consolidation following ASCT should be proposed for pts with primary refractory disease, early relapse, or extra nodal disease.
Aims: To point brentuximab-bendamustine combination efficiency and safety for R/R HL pts.
Methods: We retrospectively analyzed 8 HL pts in 2 centers, treated with 90mg/m^2 bendamustine on days 1 and 2, and 1.8mg/kg brentuximab on day 1, every 3 weeks. Six pts were R/R (including 1 post ASCT), 1 in partial response post chemotherapy and 1 in first line (contraindication to chemotherapy, heart and respiratory failures).

Table 1.



Results: Median follow-up was 12 months [3-36] from beginning of treatment. Median chemotherapy treatment was 4 courses [2-6]. Four pts were in CR, 3 in nearly CR, 1 refractory. Three pts underwent subsequently ASCT (2 where in CR and 1 in nCR): 1 of them had brentuximab maintenance and 1 relapsed post ASCT. One other pt in nCR beneficiated from reduced intensity-conditioning allograft. All pts remained alive at follow-up. This combination was well tolerated, without grade 3-4 toxicities: 2 pts had grade 1 anemia, 1 of them grade 2 neutropenia.
Summary/Conclusions: This regimen represents a promising approach to optimize response rate prior to ASCT or allograft in pts with R/R HL. It could also be proposed to frail pts as frontline treatment.

E1147

HODGKIN LYMPHOMA: A RETROSPECTIVE ANALYSIS OF SURVIVAL AND TREATMENT RELATED TOXICITIES OF A COHORT OF PATIENTS DIAGNOSED BETWEEN 2005-2015

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Background: Hodgkin Lymphoma is highly sensitive to chemotherapy and radiotherapy. Treatment related toxicity is a major concern and have a negative impact on quality of life and overall survival of these patients.
Aims: To evaluate chemotherapy and radiotherapy attributable toxicity in a cohort of Hodgkin Lymphoma survivors.
Methods: We performed a retrospective analysis of a Hodgkin Lymphoma cohort diagnosed and treated in a Portuguese tertiary hospital centre, from 2005 to 2015. The toxicities assessed were: secondary neoplasia, infertility, thyroid, cardiac, pulmonary, cerebrovascular and neuropsychiatric disorders.
Results: From 2005 to 2015, 151 patients were diagnosed and received first treatment at our centre. 87 (56,7%) patients were male. At diagnosis, the median age was 34 (18-88) years. Prevalence of HIV infection was 9,9% (n=15). Among 151 patients, 138 (91,4%) were treated with a curative intention: 12 (8,6%) had early-stage favourable disease; 39 (28,2%) early-stage unfavourable; 87 (63%) advanced stage. As first line approach, 81 (58,7%) were treated with chemotherapy alone and 57 (37,7%) with combined chemotherapy and radiotherapy. The ABVD protocol was the chemotherapy regimen in 122 (88,4%) patients, followed by BEACOPP in 7 (5,1%). Radiotherapy was performed in 60 (41,7%) patients, with Involved Field protocol in 59. Overall Response Rate after first line treatment was 92% for early-stage favourable, 86% for early-stage unfavourable, 71% for advanced-stage. Autologous Transplantation was performed in 17 patients (12,3%), all in context of relapse or refractory disease. The 5-year Overall Survival was 86,3%. Among patients diagnosed throughout reproductive age, 10,4% (n=10) underwent gamete cryopreservation, while 24,4% (n=10) of females in fertile age underwent on GnRH agonists for ovarian protection. The overall incidence of reported treatment attributable toxicities was 57% (n=86). Pulmonary and neuropsychiatric disorders were the most commonly reported, 25,8% (n=39) and 29,8% (n=45), respectively. Pulmonary complications developed independently of previous thoracic radiotherapy exposure (p>0,05). Other reported toxicities were: infertility in 1% (n=1) of individuals in reproductive age; secondary neoplasia in 4,6% (n=7) with median presentation after diagnosis of 36 months; thyroid disorders in 7,9% (n=12), of which 75% (n=9) were exposed to cervical radiotherapy; cardiac complications in 4,6% (n=7), the majority (n=5) was not exposed to mediastinal radiotherapy; cerebrovascular disease developed in 3,3% (n=5).
Summary/Conclusions: In our cohort of Hodgkin Lymphoma survivors, chemotherapy and radiotherapy attributable toxicities had a significant prevalence. The short follow-up period and the lack of a fully applied surveillance protocol might be the reason for the lower prevalence of toxicities observed in this cohort, in comparison with the most relevant published studies. The development of surveillance protocols for detection of short and long-term toxicities are of major importance to understand their true impact in overall survival and quality of life of Hodgkin Lymphoma survivors.

LB2258

BENDAMUSTINE-BRENTUXIMAB COMBINATION IS EFFECTIVE AND HAS A FAVOURABLE TOXICITY PROFILE IN THE TREATMENT OF REFRACTORY AND RELAPSED HODGKIN LYMPHOMA

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Background: The management of patients with refractory or relapsed Hodkin lymphoma (HL), expecially if after autologous stem cell transplantation (ASCT) remains controversial. Bendamustine has demonstrated efficacy in several lymphoproliferative disorders but limited data are available regarding the schedule in patients with HL, in particular its dosage and the possibile combinations for a synergistic effect. Brentuximab Vedotin is a CD30-directed antibody-drug conjugate, currently approved for the treatment of relapsed or refractory Hodgkin lymphoma (HL), as single-agent at the dose of 1.8 mg/kg.
Aims: The objective of this phase II study was to evaluate efficacy and safety of salvage cytotoxic regimens in patients with refractory and/or relapsed HL. Three different schedules were evaluated.
Methods: From May 2011 to February 2016, 24 patients (13 M/11 F) (Table 1) with a median age of 32.5 years (range 16-73) received a salvage regimen after failure of ASCT. Patients were by chance assigned to one of these three arms : Standard dose Bendamustine (90 mg/sqm) days 1, 2 + DHAP x 3 cycles (Arm A), brentuximab single agent 1.8 mg/kg x 4-8 cycles (Arm B), High dose Bendamustine (120 mg/sqm, days 1, 2) + brentuximab 1.8 mg/kg (day 3) x 4-6 cycles (Arm C). Each cycle was repeated every 28 days and growth factor support was sistemically administered, in association with antimicrobial prophylaxis. The treatment efficacy in each group was evaluated according to Revised Response Criteria for Malignant Lymphoma. Any adverse event occurred was recorded and classified for type and grade using NCI-CTCAE criteria (v 4.0).
Results: In arm A, which included 10 patients, the overall response ratio was 40%, with 4 complete remission (CR) and 6 progressive disease (PD). Extra-hematological toxicities were grade 3 thrombocytopenia in 4 patients and bone marrow aplasia in 1 patient. In arm B, which included 6 patients, the overall response ratio was 66%, with 4 CR and 2 PR. Extra-hematological toxicities were grade 3 neuropathy in 1 patient, hematological toxicity was grade 2 neutropenia in 2 patients. In arm C, which included 8 patients, the overall response ratio was 100%, with 8 complete remission (CR) and then SCT (4 autologous and 4 haploidentical-SCT) with persistence of complete remission in all patients. Median overall survival was 32.2 months (r. 27-44) and median progression free survival 15.3 months (r. 9-18.4). Extra-hematological toxicities were increase of transaminase in 2 patients and CMV reactivation in 2 patients, treated successfully with valgangiclovir. Hematological toxicity was grade 3 thrombocytopenia in 2 patients.

Table 1.

	Arm A (High dose Benda + other agents)	Arm B (Brentuximab single agent)	Arm C (High dose Bendamustine)
Median age	31.5	25.3	35.3
Median number of previous treatments	3 (r. 2-6)	4 (r. 2-7)	6 (r. 2-8)
Median number of cycles of treatment	3 (r. 1-6)	8 (r. 1-16)	6 (r. 1-8)
ORR	40%	66%	100%
Response	4 CR 6 PD	4 CR 2 PD	8 CR
SCT + type	2 Auto-BMT 1 Haploidentical SCT	2 Haploidentical SCT	4 Auto-BMT 4 Haplo-SCT
Median overall survival	22.3 (r. 18-28)	28.1 (r. 23.9-34.5)	32.2 (r. 27-44)
Median progression free survival	10.2 (r. 8-19.1)	12.8 (r. 8.5-20.5)	15.3 (r. 9-18.4)
Extrahematological toxicities + grade	/	1 grade 3 Neuropatia	2 Increase AST/ALT 2 CMV reactivation
Hematological toxicities + grade	4 grade 3 Thrombocytopenia 1 bone marrow aplasia	2 grade 2 Neutropenia	2 grade 3 thrombocytopenia

Summary/Conclusions: High dose Bendamustine plus Brentuximab has shown significant efficacy in a particular severe setting of heavily pretreated patients, and it could be considered as a bridge to allogenic BMT.

LB2259

UPDATES ON EFFICACY AND SAFETY OF DOSE-DENSE AND DOSE-INTENSE (DD-DI) ABVD AS FRONTLINE THERAPY OF ADVANCED CLASSICAL HODGKIN LYMPHOMA (HL)

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Background: The optimal therapeutic upfront strategy in advanced classic HL hasn't yet been standardized. In recent years, the scientific community has given greater attention to the concept of dose-intensity and several studies demonstrated that intensifying therapy through dose-escalation of drugs and shortening cycle intervals yields best results than standard ABVD. However, the best frontline approach is still debated and the dispute remains between 2 strategies: 1)The "first hit principle" followed by dose reduction in early responders, 2) The "standard dose" followed by the "second hit" in poor responders.
Aims: Based on the promising results of our recent experience (D'Arco et al, 2015) and of a phase II trial of DD-DI ABVD in advanced HL (Russo et al, 2014) we expanded our previous series and updated follow-up to confirm our findings.
Methods: We treated in frontline, 22 consecutive cases of advanced HL with the ABVD $_{DD-DI}$ protocol (Russo et al, 2014), between June 2011 and December 2015. The drugs were given on days 1 and 11, at the doses of standard ABVD except for doxorubicin (escalated to 35 mg/m2, in the first 4 of 6 cycles). Cycles were administered every 21 days plus granulocyte-colony stimulating growth factors. Patients received prophylaxis with cotrimoxazole and lamivudine (as required).
Results: 22 patients were treated in frontline. The median age was 43 years (range: 23-67) and unfavourable risk factors such as erytrocyte sedimentation rate>50 mm/h, ≥3 nodal areas, International Prognostic Score≥3 were present in 68%, 77%, 59% of patients, respectively. All patients completed the planned six courses of therapy without dose reductions or delay. Toxicities were: grade III-IV neutropenia and anemia (9%). Other relevant toxicities were: grade 2 mucocutaneous changes (23%) consisting of skin rash, skin hyperpigmentation and nail alterations, grade 2 transaminases elevation (14%), grade 2 and 3 vomiting (27%) and grade 2 fatigue (18%). We didn't observe severe or prolonged infections or acute/delayed cardiac or pulmonary toxicities. The overall response rate (ORR) was 100%, complete response (CR) were 91%, partial response (PR) were 9%. In patients with bulky disease, 83% achieved CR at the end of treatment. Positron emission tomography (PET) after 2 and 4 cycles was negative in 68% and 91% patients, respectively. Only 2 patients were in PR at the end of cycle 6 and underwent to consolidation treatment: 1) Radiotherapy (RT); 2) High-dose therapy followed by autologous stem cell transplantation (ASCT), improving PR, then salvage with brentuximab vedotin, reaching CR. At a median follow-up of 31.5 months (range: 4-53), all patients were alive and in continuous CR and the median progression free survival (mPFS) hasn't been reached.
Summary/Conclusions: On a larger series, we confirm the safety and feasibility of ABVD$_{DD-DI}$, in the context of "real life" clinical practice. This intensified schedule seems to be promising in increasing the efficacy of standard ABVD, in the subset of advanced, high risk HL, in frontline therapy. Our results reinforce the critical role of dose-intensity to achieve CR and probably to reduce the need for salvage therapy followed by ASCT. Probably, the role of RT may be limited to patients with positive PET disease sites, at the end of treatment, as already demonstrated for BEACOPPescalated in the same context (Engert et al, 2012). Finally, even with a longer median follow-up (31.5 months), mPFS hasn't yet been reached and relevant toxicities haven't been highlighted.

Indolent Non-Hodgkin lymphoma – Clinical

E1148

HIGH THROUGHPUT TCR SEQUENCING PROVIDES ADDED VALUE IN THE DIAGNOSIS OF CUTANEOUS T-CELL LYMPHOMA

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Background: Diagnosis of early-stage CTCL can be challenging because skin lesions contain a mixture of both diverse benign and clonal malignant T cells. High throughput sequencing (HTS) of the TCR gamma (TCRG) and TCR beta (TCRB) CDR3 regions is a technique that provides comprehensive analysis of the total repertoire of T cell clones in a specimen, the breadth of repertoire diversity, and an exact quantitation of individual T cell clone
Aims: We investigated whether the Adaptive Biotechnologies multiplex PCR and high throughput sequencing assays of TCRB and TCRG could discriminate between benign and malignant T-cell skin dyscrasias
Methods: We analyzed skin biopsies from 46 patients with histopathologically and clinically (based on presentation, history, and course) labelled CTCL (over 80% Stage I or Stage II), 23 patients with psoriasis, 11 eczematous dermatitis, 10 allergic dermatitis, and 6 with normal skin findings.
Results: The TCRB and TCRG assays were able to define the dominant clonal sequences in every CTCL case and to distinguish likely gamma/delta from alpha/beta lymphoma. Using the fraction of the top two TCRG sequences as a fraction of the total nucleated cell population defined a cut off of approximately 1/1000 above which the biopsy was highly specific for malignant disease and below which the assay approached 100% specificity for non-malignant disease. The discrimination afforded by reference to the per cent comprised by the top two TCRG sequences of all TCRG sequences generated from the sample (most comparable to data generated by TCR PCR) was not nearly as robust. TCR PCR failed to provide relevant categorization in approximately 30% of the same CTCL cases. This approach also was able to identify low level presence of CTCL-correlated clones in systemic circulation and to distinguish subsequent inflammatory reactions from disease recurrence. Analysis of CTCL TCRG genes was consistent with CTCL being a malignancy derived from mature T cells.
Summary/Conclusions: Multiplex PCR and high throughput sequencing of TCRG and TCRB loci in suspect skin lesions was able to impact and add value to the diagnosis and monitoring of patients with early stage cutaneous T-cell lymphoma.

E1149

SENSITIVITY OF ULTRASOUND-GUIDED 16 G CORE-NEEDLE CUTTING BIOPSY AND EXCISIONAL BIOPSY FOR THE CHARACTERIZATION OF LYMPHADENOPATHIES IN PATIENTS WITH SUSPECTED LYMPHOMA: A RANDOMIZED SUPERIORITY TRIAL

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Background: The performance of lymph node excisional biopsy requires validation. Although fine-needle aspiration under image guidance offers an alternative to open biopsies, its sensitivity in lymphoma diagnosis is still a matter of debate. New approaches to imaging-guided methods are needed. The introduction of new generation ultrasonographic devices (particularly effective in predicting the malignant status of lymphadenopathies), and progress in tissue sampling with needles with cutting edge of adequate diameter (particularly effective in providing architectural information of the core of nodal lesion) have enabled to obtain enough tissue for a definitive diagnosis (according to the WHO Classification) by mini-invasive procedures, without the need of excisional biopsy.
Aims: The primary endpoint was to demonstrate the superiority in terms of sensitivity in detecting malignancy of ultrasound (US)-guided core-needle cutting biopsy (CNCB) compared with standard excisional biopsy of lymphoadenopathies suspected for lymphoma. Secondary endpoints were: specificity, positive predictive value (PPV), negative predictive value (NPV), complication rate related to each diagnostic procedures and relative cost analysis.
Methods: From January 2009 to December 2015, in this single centre trial, patients having lymphoadenopathies with clinical suspicion of lymphoma were randomly assigned (1:1) to either US-guided CNCB or standard excisional biopsy. In the US-guided group, patients underwent baseline US exploration of all superficial lymph node areas and any abnormal lymph node underwent power-Doppler study to select the site of CNCB. The CNCB were all performed using a 16 gauge modified Menghini-type needle 150 mm in length with automatic aspiration (Biomol HS-Hospital). The selection of lymph node in the exci-

sional biopsy (standard) group was suggested by the physical examination (at surgeon's discretion).
Results: Overall, 372 patients were randomized into two well-matched arms. Histology showed a malignancies in 93% (172/185) of patients in the US-guided group (lymphoma, 151 patients; carcinoma, 21 patients) and in 80% (149/187) of patients in the standard group (lymphoma, 122 patients; carcinoma, 27 patients). During the follow-up of the patients with lymph nodes reported as being reactive, 19 of 38 patients in the standard group were rebiopsied and were found to have lymphoma or carcinoma at the subsequent lymph node histology, whereas three of the six patients in the US-guided group requiring a second biopsy were found to be positive for lymphoma diagnosis. Thus, sensitivity in detecting malignancy was higher in the US-guided group compared with the standard group (172/175 [98%] *vs* 149/168 [89%]; p=.0003), demonstrating the superiority of US-guided CNCB in reaching a definitive histopathological diagnosis for malignancies. Biopsy provided false-negative results for malignancy in 10.2% of patients affected by lymphoma in the standard group and 1.6% in the US-guided group (p=.0005). Furthermore, US-guided CNCB showed higher NPV than traditional biopsy, 80% (12/15) *vs* 50% (19/38; p=.04), respectively. No differences were found in terms of specificity and PPV between the two diagnostic techniques, by the time that no false positive were detected in both groups. Estimated cost per diagnosed with traditional biopsy was 11-fold higher compared with US-guided CNB group (p<.0001). Overall complication rate was significantly higher in patients who received excisional biopsy (78%) than in patients who underwent US-guided CNCB (15%), p<.0001.
Summary/Conclusions: US-guided CNCB has proven to be a quick, safe, and efficient technique and has radically altered the diagnostic strategy of enlarged lymph nodes at our institution, avoiding unnecessary lymph node excisions.

E1150

MCL-002: UPDATED EFFICACY AND SAFETY RESULTS FOR LENALIDOMIDE *VS* INVESTIGATOR'S CHOICE MONOTHERAPY IN RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA

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Background: Although most patients with mantle cell lymphoma (MCL) respond to initial therapy, relapses are common and lead to increasingly shorter remissions. Lenalidomide, an IMiD® immunomodulatory agent with direct and immune-mediated mechanisms of action, has demonstrated efficacy and safety in relapsed/refractory (R/R) MCL in multiple studies, including heavily pretreated patients.
Aims: Evaluate results after an additional 1 year of follow-up after primary analysis for the randomized MCL-002 (SPRINT) study comparing lenalidomide *vs* investigator's choice (IC) monotherapy in R/R MCL patients.
Methods: MCL-002 is a multicenter, open-label study in MCL patients who had 1-3 relapses or failed prior therapy (including an alkylating agent and an anthracycline, and/or cytarabine, and/or fludarabine [± rituximab]) and were ineligible for intensified chemotherapy or stem cell transplantation (NCT00875667). Upon informed consent, oral lenalidomide was initiated at 25 mg/day on days 1-21 of 28-day cycles until disease progression or as tolerated. IC treatment included single-agent rituximab, gemcitabine, fludarabine, chlorambucil, or cytarabine. The primary endpoint was progression-free survival (PFS, central review per modified 1999 IWG criteria); secondary endpoints included ORR, time to response, OS, and safety. Post-primary analysis (07March2014) all subsequent analyses are based on investigator's assessment.
Results: 254 patients were randomized 2:1 to lenalidomide (n=170) or IC (n=84). As of the current data cut-off of March 7, 2015, 21 (12%) patients remain on lenalidomide and 2 (2%) patients on IC. With an additional 1 year of follow-up from the primary analysis (median 21.0 month follow-up [range, 0.02-69.6]), the median PFS by investigator assessment continues to show improvement for lenalidomide over IC (8.6 *vs* 5.4 months, respectively), with an HR=0.67 (95% CI, 0.48-0.89; sequential log-rank *P*=0.012). Response rates showed a similar trend with an ORR of 46% *vs* 23% (*P*<0.001) and CR/CRu of 12% *vs* 8% (*P*=0.336) for lenalidomide *vs* IC, respectively. Median time to best response was 5.9 *vs* 13.1 months and median OS was 27.8 *vs* 21.1 months. Among the safety population (250 patients receiving ≥1 dose of study drug), 68% of lenalidomide and 40% of IC patients with ≥1 adverse event (AE) had dose reductions/interruptions, in part due to a longer duration of lenalidomide treatment and strict dose modification requirements. At a median time of 2.9 months (range, 0.7-37.8) from randomization, 39 patients (46%) crossed over from IC to lenalidomide. The most common grade 3/4 AEs were neutropenia (45% for lenalidomide *vs* 34%% for IC, without increased risk of infection), thrombocytopenia (20% *vs* 28%), anemia (9% *vs* 7%), and leukopenia (8% *vs* 11%). Non-hematologic AEs with lenalidomide were primarily grade 1/2. Any-grade tumor flare reaction was observed in 10% of lenalidomide patients (2% grade ≥3), and 1 patient in each arm experienced tumor lysis syndrome. Invasive second primary malignancies were identified in 4% of lenalidomide and 5% of IC patients.
Summary/Conclusions: Lenalidomide continues to show significantly improved PFS and ORR by investigator assessment compared with IC in patients with R/R MCL, which was similar to previous central review. The consistent safety profile of lenalidomide partly reflected longer treatment exposure; AEs were manageable with dose modifications or supportive therapy. Overall, long-term follow-up of the MCL-002 trial confirms the improved efficacy of lenalidomide relative to IC monotherapy in patients with R/R MCL.

E1151

POSITIVE BENEFITS OF CHANGING FROM INTRAVENOUS RITUXIMAB ADMINISTRATION TO SUBCUTANEOUS ADMINISTRATION: A SINGLE UK CENTRE EXPERIENCE

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Background: Addition of the anti CD20 monoclonal antibody, rituximab, to chemotherapy regimens for the treatment of B cell non Hodgkin lymphomas has been standard practice in the UK since 2003. The initial licence for rituximab was for intravenous (IV) administration. Subcutaneous (SC) rituximab was granted marketing authorisation in March 2014. Administration of IV rituximab takes between 90 and 270 minutes depending on a number of factors including the chemotherapy regimen used and the number of doses a patient has already received. SC rituximab takes 5-10 minutes to give. Prior to July 2014 80% of rituximab doses given at University Hospital of Wales (UHW) were prepared by our in house pharmacy staff and 20% of doses were bought in ready prepared bags, to allow flexibility in chemotherapy scheduling. SC rituximab is given as a standard dose for all patients and can be made up by nursing staff in the chemotherapy day unit.
Aims: To investigate cost savings and reduction in chair times for patients treated with chemotherapy regimens containing SC rituximab therapy compared to IV rituximab therapy in a single centre in the UK. Infusion related adverse events were also recorded for the study period.
Methods: Following local governance approval, the UHW department of haematology switched from IV to SC rituximab in July 2014. The policy change only applied to patients with a diagnosis of diffuse large B cell lymphoma (DLBCL) or follicular lymphoma (FL) receiving treatment with R-CHOP, R-CVP or R-bendamustine chemotherapy regimens plus any maintenance doses. All dispensed SC doses of rituximab were retrospectively identified from our pharmacy system in 2015. The actual cost of the SC doses was compared to projected drug costs and staff time for doses given IV with 20% pre prepared drug being bought in as prior to 2014. The chemotherapy day unit audited chair time for the same patient group receiving rituximab containing regimens during a 12 month period in 2013 prior to the change in practice and another 12 month period in 2015 post the changes. Patient notes were retrospectively audited for the same 2 time periods in 2013 and 2015 to record documented complications of rituximab administration.
Results: 92 patients with DLBCL or FL were treated in 2015, receiving a total of 372 doses of SC rituximab. The annual drug cost savings were estimated at £20K. The reduced time spent by pharmacy staff in the aseptic unit was estimated at 160 hours annually. The chemotherapy administration time audit showed that prior to the policy change, average administration times were as follows: R-CHOP 260 minutes (mins), R-CVP 135 mins, R-bendamustine 180 mins and maintenance rituximab 150 mins. Post July 2014 the average administration times were as follows: R-CHOP 130 mins, R-CVP 50 mins, R-Bendamustine 45 mins and maintenance rituximab 11 mins. This reduction in treatment administration translated into a saving of chair times of 842 hours in 12 months (approximately 72 hrs per month) (Fig 1). Retrospective review of patient notes did not show any increase in adverse events relating to SC rituximab administration.
Summary/Conclusions: The introduction of SC rituximab at the UHW resulted in reduced drug costs, reduced staff time in drug preparation and significant reductions in chemotherapy chair time for patients with no evidence of an increase in adverse events relating to drug administration. These savings have freed resources to allow for other therapies to be accommodated into the existing service.

E1152

IMPACT OF STABLE DISEASE OR BETTER RESPONSES TO LENALIDOMIDE ON SURVIVAL OUTCOMES IN PATIENTS WITH RELAPSED/REFRACTORY MANTLE CELL LYMPHOMA: MCL-001 (EMERGE) AND MCL-002 (SPRINT) STUDIES

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Background: Patients with relapsed/refractory mantle cell lymphoma (R/R MCL) show poor overall survival (OS) after experiencing failure to immunochemotherapy and other treatment regimens, including bortezomib. Lenalidomide is an IMiD® immunomodulatory agent with direct and immune-mediated mechanisms of action that has demonstrated safety and efficacy (including meaningful disease stabilization rates) in multiple studies of R/R MCL.
Aims: Post hoc analysis of lenalidomide-treated patients from the MCL-001 and MCL-002 studies to determine the survival benefit based on response status (focusing on patients with stable disease [SD]) at landmark points in time.
Methods: Patients receiving oral lenalidomide 25 mg/day on days 1-21 of each 28-day cycle until disease progression or as tolerated were evaluated from 2 phase II studies: MCL-001 (post-bortezomib failure) and MCL-002 (randomized *vs* investigator's choice monotherapy). Patients provided informed consent for each study. Kaplan-Meier methods and unadjusted Cox regression models were used to support the landmark analyses at the start of cycles 3, 5, and 7. Survival data for patients with SD were compared with those with progressive disease (PD) or those responding with at least a partial response (PR) for each time point. Data cutoff for MCL-001 was March 20, 2013 and for MCL-002 was March 7, 2015.
Results: 304 patients who received lenalidomide in MCL-001 (n=134) and MCL-002 (n=170) were included in the analysis. At baseline, patients had a median age of 68.0 years (66% were ≥65 years of age), 76% were male, 91% had stage III/IV MCL at diagnosis, and 52% had high tumor burden. Patients had received a median of 2 prior lines of antilymphoma therapy (range, 1-10); 48% were refractory (≤SD) to their last therapy. Baseline demographics and characteristics were similar for patients with ≥PR, SD, and PD. Response status at each cycle is shown in Table 1, with no response data for 24% of patients at cycle 3 due to early discontinuation, censoring or response being outside the time point window. Median OS in months by response group at cycle 3 was 28.5 for ≥PR, 34.0 for SD, and 11.3 for PD; at cycle 5: 45.3 for ≥PR, 34.7 for SD, and 13.4 for PD; and at cycle 7: not reached for ≥PR, 37.5 for SD, and 17.4 for PD. At all three time points, there was no significant difference in OS for patients with ≥PR *vs* SD, whereas the median OS for patients with SD was significantly prolonged *vs* PD. It is important to note that the SD and PD groups had a small number of patients at the later cycles.

Table 1. Response status and OS at cycles 3, 5 and 7.

Response status, n (%)	≥PR	SD	PD
Cycle 3	78 (26)	124 (41)	30 (10)
Cycle 5	78 (26)	69 (23)	18 (6)
Cycle 7	80 (26)	32 (11)	10 (3)
Median OS, months (95% CI)	**≥PR**	**SD**	**PD**
Cycle 3	**28.5 (21.3-40.4)**	**34.0 (27.8-42.0)**	**11.3 (6.7-18.7)**
≥PR vs. SD	HR=1.23 (95% CI, 0.83-1.81); *P*=0.2997		
PD vs. SD		HR=2.75 (95% CI, 1.73-4.38); *P*<0.0001	
Cycle 5	**45.3 (29.6-NR)**	**34.7 (28.4-44.1)**	**13.4 (8.8-29.8)**
≥PR vs. SD	HR=0.74 (95% CI, 0.45-1.20); *P*=0.2247		
PD vs. SD		HR=2.57 (95% CI, 1.38-4.78); *P*=0.0028	
Cycle 7	**NR (33.8-NR)**	**37.5 (30.0-NR)**	**17.4 (9.6-23.4)**
≥PR vs. SD	HR=0.66 (95% CI, 0.35-1.25); *P*=0.2036		
PD vs. SD		HR=4.24 (95% CI, 1.77-10.15); *P*=0.0012	

Summary/Conclusions: Landmark analyses for lenalidomide-treated R/R MCL patients with SD at cycles 3, 5, and 7 had OS comparable to patients who achieved ≥PR. Overall, these data show that R/R MCL patients with either SD or ≥PR at these time points have significantly improved long-term outcomes compared to those with PD.

E1153

TREATMENT AND PROGNOSIS OF STAGE I FOLLICULAR LYMPHOMA IN THE MODERN ERA - DOES PET-CT MATTER?

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Background: Follicular lymphoma (FL) is the most common indolent non-Hodgkin Lymphoma. Treatment of stage I disease is usually radiation monotherapy, but debate over the role of additional chemo-immunotherapy exists. Most of the data on treatment and prognosis are based on retrospective historical cohorts before PET-CT was introduced into routine staging procedures. In these previous cohorts, 5 year freedom from treatment failure (FFTF) was 60-80% and the 5 year overall survival (OS) was 80-93%.
Aims: The current study assessed the outcome of patients diagnosed with stage I FL who were treated with radiotherapy with or without rituximab, in the PET-CT era.
Methods: Patients diagnosed with stage I FL based on PET-CT and bone marrow biopsy (BMB), who were treated with involved field radiotherapy between 2002-2015, were retrospectively reviewed in this multi-center study. Patients were treated with either radiation monotherapy or radiotherapy and rituximab.
Results: Ninety one patients from 9 centers were identified. The median age at diagnosis was 60 years (range: 31-93) years. FLIPI score was compatible with a low risk disease (0 or 1) in 93% and with an intermediate risk (FLIPI =2) in 7% of the patients. Forty eight percent of the patients had FL Grade 1, 39%>Grade 2 and 13% -Grade 3a FL, with 30% presenting with an extra-nodal disease. Median radiation dose was 30.2 (18-50) Gy. Most patients were treated with radiation monotherapy (71 patients, 78%), additional rituximab was administered to 20 patients (22%). Within a median follow-up of 57 months, the 5 year FFTF and OS survival rates were 76% and 97% respectively (Figure 1). Twenty one percent of the patients (n=19) experienced relapse; most relapses (n=14) were outside the radiation field. An intermediate risk FLIPI score was the only independent significant factor for a shorter FFTF [HR=2.3, 95% CI= 1.096 - 4.89, p=0.028].Radiation dose or adjuvant rituximab therapy did not significantly affect outcome.

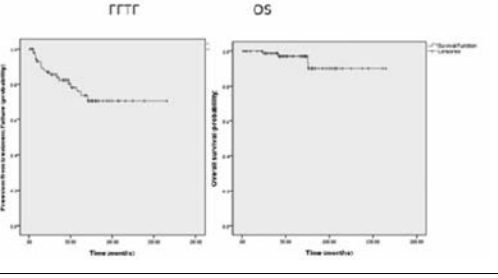

Figure 1. Outcome of patients with FL stage I, determined by PET.

Summary/Conclusions: Our findings show that staging with PET-CT and BMB of stage I FL may result in improved FFTF and OS in patients treated with radiotherapy compared to historical cohorts, possibly due to better identification of a true stage I disease. The distant nature of recurrences suggests that occult distant disease foci, not diagnosed even by PET-CT, are still possible.

E1154

PATTERNS OF HEMOGRAM CHANGES IN PATIENTS WITH RELAPSED/REFRACTORY INHL AND CLL TREATED WITH IDELALISIB

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Background: Chronic lymphocytic leukemia (CLL) and indolent non-Hodgkin lymphoma (iNHL) are B-cell malignancies associated with neutropenia, anemia and thrombocytopenia. Although the etiology of cytopenia is not well understood, bone marrow leukemia/lymphoma cell infiltrates are thought to contribute. Idelalisib (IDELA) is a selective, first-in-class oral PI3Kδ inhibitor. In preclinical studies, IDELA selectively targeted malignant B cells with minimal toxicity to non-malignant B cells and other hematopoietic cell types. In clinical studies evaluating IDELA in B-cell malignancies, hematologic responses across all 3 lineages were observed in a majority of patients (pts) with baseline (BL) cytopenias.
Aims: The objective of this post hoc analysis was to evaluate hemogram changes in pts with relapsed or refractory (R/R) CLL or iNHL treated with IDELA in 2 pivotal studies.
Methods: In phase 3 Study 116, frail pts with CLL were randomized to receive rituximab (R) in combination with IDELA 150 mg BID or placebo (PBO). In phase 2 Study 101-09 (NCT01282424), pts with iNHL received IDELA monotherapy 150 mg BID. Trial inclusion criteria allowed enrollment of pts with BL cytopenias of any grade (CLL) or grade <3 (iNHL). Hematologic profiles for pts were categorized as normal or abnormal (any grade of cytopenia) at BL and assessed while on treatment. Supportive care utilization was assessed by use of blood product transfusions, hematopoietic growth factors, and immunosuppressant therapies. Treatment-emergent de novo autoimmune cytopenias (AIC) and/or worsening of pre-existing AIC were summarized. All analyses excluded pts from the iNHL study with PD at the first assessment to avoid confounding by underlying uncontrolled disease.
Results: A total of 345 pts participated in these trials. The overall response rates for IDELA-treated patients in the CLL and iNHL studies were 84% and 58%, respectively. For pts with CLL on IDL+R (n=110), BL cytopenias (grade ≥1) included anemia (76%), thrombocytopenia (62%), and neutropenia (34%). For pts with iNHL on IDELA-monotherapy (n=115), BL cytopenias included anemia (50%), thrombocytopenia (36%), and neutropenia (24%). In patients with a normal hemogram at BL, median hematologic values remained unchanged over time with IDELA treatment. In patients with BL cytopenia, IDELA treatment was associated with improved Hgb and platelet counts and a reduction in supportive care utilization for anemia, thrombocytopenia, and neutropenia. For pts with CLL, median peak values for Hgb (24% above BL) and platelets (120% above BL) were observed within 6 months of IDELA initiation. For pts with iNHL, median peak values for Hgb (20% above BL) and platelets (64% above BL) were observed within 3 months of IDELA initiation. ANC remained stable over time in IDELA-treated pts with CLL and increased in pts with iNHL; a reduction in G-CSF utilization was observed for pts with CLL or iNHL and BL cytopenias while on treatment with IDELA. A history of AIC was reported in 6.4% and 16.4% of pts with CLL on IDELA+R and PBO+R, respectively. As of the data cutoff, no pt had experienced treatment-emergent AIC and/or worsening of pre-existing AIC.

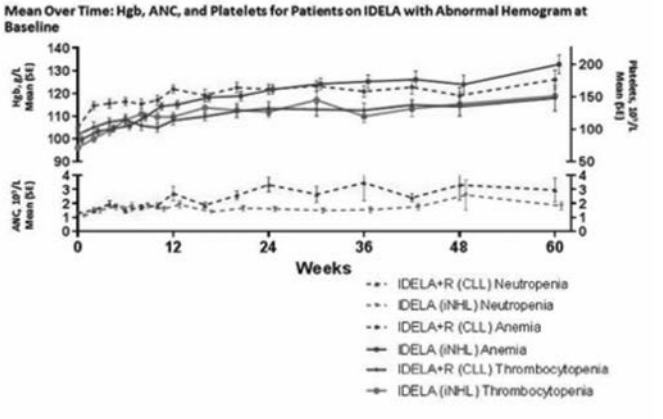

Figure 1.

Summary/Conclusions: This analysis indicates improved cytopenia in 2 lineages for pts with R/R CLL and iNHL treated with IDELA. Thus, pre-existing cytopenias, including those due to advanced disease, myelosuppression from prior chemotherapy, and AIC, do not by themselves preclude treatment with IDELA.
Studies included in this analysis: 101-09: NCT01282424; GS-US-312-0116: NCT01539512

E1155

A PHASE II STUDY OF THE COMBINATION OF OFATUMUMAB WITH FLUDARABINE AND CYCLOPHOSPHAMIDE IN PATIENTS WITH RELAPSED OR REFRACTORY WALDENSTRÖM'S MACROGLOBULINEMIA
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Background: Ofatumumab is a novel anti-CD20 monoclonal antibody which has been approved for the treatment of chronic lymphocytic leukemia, but limited data exist for patients with Waldenström's macroglobulinemia (WM). Ofatumumab targets CD20 in a different epitope than rituximab and may also have a more favorable profile regarding infusion related reactions since, in contrast to rituximab, it is a human and not chimeric antibody. Fludarabine with cyclophosphamide (FC) is a very effective treatment when combined with rituximab (FCR) in patients with relapsed or refractory WM.
Aims: to evaluate the combination of ofatumumab with FC (OFC) in symptomatic patients with relapsed or refractory WM requiring therapy
Methods: This is a phase 2 single center trial. Treatment consisted of four cycles of the OFC combination. A single dose of 100 mg of ofatumumab was given I.V. on day 1 of the first cycle followed by 1000 mg on day 8. Fludarabine 25 mg/m^2 and cyclophosphamide 250 mg/m^2 were given intravenously for 3 consecutive days every 28 days for 4 cycles, followed by ofatumumab (1000 mg) in cycles 2 to 4. Primary end point was overall response rate (complete (CR)+very good (VGPR)+partial (PR)+minor (MR) response) and secondary endpoints included safety and toxicity, occurrence of "IgM flare", duration of response, progression free and overall survival
Results: From 2013 until 2014, twelve patients were treated with OFC; 75% and 17% were high and intermediate risk per the International Prognostic Scoring System for WM. All patients had previously been exposed to rituximab and 4 (33%) were refractory to the last course of rituximab, while 4 (33%) had previously received bortezomib. On intent to treat, 11/12 (92%) patients responded (17% VGPR, 67% PR and 8% MR). Median time to first response (≥MR) was 2.2 months and median time to ≥PR was 8.5 months. In 11 (92%) patients a continuing decrease of IgM levels has been observed and at 6 months post OFC completion 6 (50%) patients have improved their response from MR to PR or VGPR. "IgM flare" was not observed. All patients completed the planned 4 cycles of treatment. Infusion related reactions occurred in only one patient (grade 2) who had a history of severe rituximab intolerance, during the day 8 dosing (1000 mg); the patient completed the full schedule without further reactions. After a median follow-up of 23 months, patients have progressed or died and the median progression-free survival is 21 months. The 2-year duration of response for patients with ≥PR is 70%, and 2-year survival is 83%. Neutropenia grade 3 occurred in 67% and grade 4 in 25% of patients. One patient experienced neutropenic fever and the dose of FC was reduced according to the protocol.
Summary/Conclusions: OFC is a rapidly acting combination, inducing high response rates, including deep and durable responses in heavily pretreated WM patients, even in those refractory to rituximab and/or bortezomib. Ofatumumab is well tolerated with low incidence of infusion related reactions, and although the combination has myelotoxicity, it is manageable.

E1156

MOLECULAR ACTIVITY IN FOLLICULAR LYMPHOMA MONITORED BY BCL2/IGH IN THE PERIPHERAL BLOOD. THE OLD STORY IN A NEW LIGHT?
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Background: Follicular lymphoma (FL) is highly associated with the molecular rearrangement BCL2/IGH. Although BCL2/IGH has been studied many times, its real clinical value remains still matter of debates.
Aims: In our work, we focused on the mutual relationship between molecular levels of BCL2/IGH measured in peripheral blood (PB) and clinical course of FL.
Methods: We performed qualitative and quantitative testing of BCL2/IGH by nested and real-time PCR in FL patients (grade 1-3B). PB samples were tested before the start of therapy (or watch and wait period, 12.8% cases), during treatment and during follow up at the time of routine clinical controls. According to mutual correlation of clinical course FL and molecular activity in PB, we divided patients into five groups (A, B, C, D, E): Group A included patients in a long-term molecular and clinical remission. Group B was defined by long-term remission with sporadic molecular activity. Group C included patients with concordant clinical and molecular remission and progression. Group D included patients with discordant clinical and molecular activity; there are two subgroups: 1) DM defined as evident molecular positivity in lasting clinical remission, and 2) DR defined by a clinically evident relapse in lasting molecular remission. Group E includes non-evaluable patients with the initial molecular negativity in PB, patients without treatment (watch and wait) and patients with lack of samples. Groups A and B with lasting molecular and clinical remissions brought no correlation information, the only informative population is represented by subgroups C and D.
Results: Totally, 187 patients with initial BCL2/IGH positivity were included, median of molecular follow up was 4.7 ys (range 1.1-14.8 ys) and median clinical follow up 5.3 ys (range 0.4-18.4ys); systemic treatment (chemotherapy or

immunochemotherapy) was administered in 173/187 (92.5%) cases. There were 83 men (44%) with median age of 57 ys (range 28-79 ys), clinical stage III-IV was determined in 144/187 (77%) patients. Median number of PCR samples per patient was 15 (range 3-67). There were 60 (32.2%) patients in group A, 16 (8.7%) pts in group B, 39 (20.8%) pts in group C, 26 pts (13.9%) in group D and 46 (24.6%) pts in group E. Groups C and D (65 pts) gave a relevant correlation information only; the proportion of discordant cases was 26/65 (40%) pts. As mentioned, group D was divided into two subgroups: DR group (clinical relapse in molecular remission) with 19 (10.2%) patients, 17/19 (89.5%) patients remain alive with median overall survival (OS) of 8.5 ys. DM subgroup (molecular activity in lasting clinical remission) consisting of 7 pts, all of them remain alive with median OS of 12.1 ys. Interestingly, 10/39 (25.6%) patients subscribed into group C died (median OS 4.5 ys; 7 cases with confirmed death due to FL progression), compared to 2/26 died patients from group D (7.7%, 1 died probably due to progression only) with median OS of 8.8 ys (p 0.067).
Summary/Conclusions: Based on our results, molecular activity of BCL2/IGH in blood does not correlate sufficiently with clinical activity of FL in about 40% cases. However, patients with correlating molecular activity in their clinical relapses seem to have a tendency to a worse prognosis with more deaths related to FL and shorter overall survival. We can speculate, that release bcl2/IGH+ cells into PB mirrors the real aggressiveness of FL.

E1157

THE IMPACT OF HISTOLOGIC GRADING ON THE OUTCOME OF FOLLICULAR LYMPHOMA: IS GRADE 3 FOR BETTER OR FOR WORSE?

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Background: Follicular lymphoma (FL) is an indolent lymphoma with an excellent prognosis. With the incorporation of chemoimmunotherapy, more than 70% of the patients survive at 10 years. The clinical significance of FL histologic grading according to the WHO Classification into grades 1/2 and grades 3A and 3B is still a matter of debate.
Aims: To study the clinical and laboratory characteristics of grade 3A and 3B FL compared to grades 1 and 2 and to explore whether higher grades are associated with a worse outcome.
Methods: We retrospectively studied all patients with FL diagnosed and treated in a single University Hematology Unit between 2001 and 2015.

Table 1. Patients' Characteristics (N=53)9 (103-556).

Characteristic	N (%)
Median age in years (range)	59 (25-83)
Male gender	27 (51)
Stage	
- I-II	25 (47)
- III-IV	28 (53)
FLIPI	
- Low	26 (49)
- Intermediate	13 (24)
- High	14 (26)
Median hemoglobin in g/dL (range)	13.5 (7.8-15.8)
Median lymphocyte counts –x10^9/L (range)	1.76 (0.64-45.8)
Median platelet counts - x10^9/L (range)	229 (103-556)
Elevated LDH	11 (21)
Bone marrow involvement	17 (32)
Bulky disease	9 (17)
Treatment	
- Watch and wait	1 (2)
- Radiotherapy/Surgery	5 (10)
- Rituximab plus radiotherapy	1 (2)
- Alkylators (chlorambucil/CVP)	2 (4)
- CHOP	1 (2)
- Rituximab monotherapy	1 (2)
- Rituximab-fludarabine based	1 (2)
- Rituximab-alkylators	9 (17)
- RCHOP	31 (60)

Results: Among 200 consecutive patients with FL with a median follow-up of 76 months (0.1-186), 53 patients with grade 3 FL were identified (47 with grade 3A and 6 with grade 3B). Patients' characteristics are shown in the table. The comparison between grade 3 and grades 1 and 2 patients revealed the following significant differences: Grade 3 cases had more frequently Ki-67>30% (p<0.0001), CD10 negativity (p=0.009), high LDH levels (p=0.009) and lymphocytopenia <0.8/μL (p=0.05). FLIPI score and bulky disease did not differ significantly. There was a trend that did not reach statistical significance for male gender, localized stage and absence of bone marrow involvement to be more frequent in grade 3 compared to grades 1/2. Treatment also differed significantly between the two groups (p<0.0001): 60% of grade 3 patients were treated with Rituximab (R)-CHOP, while 52% of grade 1/2 patients were treated with R-alkylators (chlorambucil or CVP). The outcome of grade 3 patients was not inferior: 10-year PFS was 64% *vs* 55.6% and 10-year OS was 83.7% *vs* 84.4% for grade 3 and grade 1/2 patients, respectively. Moreover, a plateau in the survival curves of grade 3 patients was observed in contrast to a constantly relapsing course of grades 1 and 2 cases. Among the 53 patients with grade 3, prognostic factor analysis revealed that Ki-67, age, gender, stage, LDH, bulk and FLIPI score did not have any significance for outcome, while there was a trend for superior PFS for R-CHOP treated patients (p=0.08) with a 10-year PFS of 67.3% *vs* 31.3% for patients treated with other modalities. However, overall survival did not differ between aggressive and low intensity treatment strategies. Of note, stage and FLIPI were well balanced between the two groups.
Summary/Conclusions: Grade 3 FL patients show more frequently high Ki-67 expression, CD10 negativity, elevated LDH and lymphocytopenia compared with lower grade FL patients. However, the long-term outcome of grade 3 FL is equally favorable with plateaus in the survival curves.

E1158

THE IMPROVING OUTCOME OF NON-HODGKIN LYMPHOMA (NHL) WITHIN 15 YEARS PERIOD-REAL WORLD DATA OF NATIONAL-WIDE LYMPHOMA PROJECT

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Background: Non-Hodgkin's lymphomas (NHL) represent the most common hematologic cancer with increasing incidence over the years. Number of clinical trials demonstrated improved outcome of different NHL subtypes, the nationalwide studies describing real world data are however limited. Lymphoma Project established by Czech Lymphoma Study Group (LP-CLSG) in 1999 is focused on prospective collection of NHL patients data since that time.
Aims: The aim of this study is to analyze key data of patients diagnosed and entered into this project during the period 1999-2014.
Methods: The data National Cancer Registry (NCR) were used for incidence and mortality population based figures. Web based utility was developed for LP-CLSG, each center (17 with >50 pts.) is responsible for entering the data of consecutive newly diagnosed lymphoma patients, the central data office is responsible for validation of data. Pathology review were performed in dedicated reference centers. The project is approved by ECs.
Results: The NHL incidence and mortality resp. has been observed to increase from 6.1 and 3.6 resp. in 1981 to 10.8 and 6.0 resp. in 2001 and to 13.5 and 5.3 (decrease) per 100,000 resp. in 2011 (data from population based NCR). Altogether 11 828 patients were recorded in the specific Lymphoma Project in the period 1999-2014, 10 333 with B or T-cell NHL had the reference center pathology review and were included into the analysis. More than 2/3 of all newly diagnosed cases in Czech republic is covered by the project. There were 4187 (40.5%) DLBCL pts., 1969 (19.5%) FL, 885 (8.6%) MZL, 857 (8.3%) MCL, 393 (3.8%) SLL and 1252 (12.5%) other or unclassified B-NHL pts. T-NHL cohort consisted of 283 (2.7%) PTC NOS pts., 198 (1.9%) ALCL and 309 (3,0%) other T-NHL. There was no trend for increase or decrease of any NHL subtype during the observation period. The median age at the diagnosis was 62 (17-97) and there were similar proportion of men (51.2%) and women (48.8%). Altogether 9,718 had sufficient data for therapy and survival. The median OS for B-NHL and T-NHL was 13.0 and 3.4 y resp. The median OS for B-NHL patients treated with chemotherapy (CHT) and rituximab-chemotherapy (R-CHT) were 8.7 and not reached resp. with probability death risk reduction (DRR) of 36% (HR 0.64 p<0.0001). It was mainly due to DLBCL outcome improvement with median OS 5.9 years for CHT group *vs* unreached for R-CHT group, with DRR of 44% (HR 0.56, 95%CI; 0.44-0.57, p<0.0001). The OS was significantly improved by rituximab addition to chemotherapy in FL with HR 0.76 (95%CI; 0.59-0.92, p-0.012), MCL with HR 0.67 (95%CI; 0.49-0.82, p<0.001), SLL with HR 0.60 (95%CI;0.40-0.85, p<0.01), MZL did not show rituximab benefit in this analysis. The significant OS improvement in all NHL diagnosed in current period (2009-2014) was observed compared to period 15 years ago (1999-2002) with DRR by 14% (HR 0.86, 95%CI; 0.74-0.91, p<0.001) This is mainly due to the B-NHL outcome improvement with HR 0.87 (95%CI;0.75-0.92, p<0.005), there was

trend for T-NHL outcome improvement (HR 0.82) but it was not significant (p 0.20).
Summary/Conclusions: The analysis of the large national-wide population has revealed: 1. The lymphoma incidence is more than doubled during last 30 years whereas the mortality started decrease since 2001 after rituximab introduction. 2. The significant NHL outcome improvement in B-NHL mainly due to rituximab addition with HR 0.56 for DLBCL, 0.76 for FL, 0.67 for MCL and 0.60 for SLL. Other factors could however play the role since 3. There is insignificant trend for outcome improvement of T-NHL as well. 4. The lymphoma project allows the detailed subanalysis of different lymphoma subtypes and different therapeutic approaches, which will be presented.
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E1159

THE IMPACT OF FDG PET-CT IN THE STAGING OF FOLLICULAR LYMPHOMA, A SINGLE CENTRE EXPERIENCE
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Background: 18-F-fluorodeoxyglucose positron emission tomography-CT (FDG PET-CT) is now the recommended imaging technique for staging and response assessment in follicular lymphoma (FL) (Cheson et al. 2014). However, the relevance of FDG PET-CT in the staging of FL is not well established, apart from selected cases for specific purposes (accurate assessment of stage I and for guidance of biopsy when transformation is suspected). PET scanning is readily available at our centre and it is often included as part of standard staging procedures in FL patients (pts).
Aims: We aimed to investigate the value of FDG PET-CT scans in the staging of FL pts in routine clinical practice.
Methods: Retrospective study of pts with grade 1-3a FL who had undergone conventional staging workup (contrast-enhanced CT and bone marrow biopsy [BMB]) along with FDG PET-CT at our centre. Pts with concurrent diagnosis of diffuse large B cell lymphoma were excluded. We compared the disease extent based on conventional investigations with the incorporation of PET to staging.
Results: A total of 55 pts (female - 29, male - 26) were included, with a median age of 64 years (range, 33-82). Forty-eight pts (87%) were staged at the time of initial diagnosis, whereas 7 pts (13%) had received prior treatment and were staged at relapse. By conventional staging, 42 pts (76%) had advanced stage (III/IV) and 20 pts (36%) had high-risk FLIPI score. GELF criteria were met in 37 pts (67%). Following staging procedures, 49 pts (89%) received treatment with diverse regimens, while 6 pts (11%) were managed expectantly. Pathological uptake was detected for all FDG PET-CT scans. The median SUV_{max} of most FDG avid lesions was 9.04 (range, 2.8-37.5). However SUV_{max} was not correlated with the histological grade. PET identified a higher number of nodal areas than CT scan in 29 pts (53%). Furthermore, 25 pts (45%) showed "new" extranodal lesions not seen on CT. Overall, PET revealed 71 additional nodal areas (+31%) and 28 extranodal lesions not visualized by CT (+127%).. Bone marrow involvement was reported in 19 pts on PET, compared to only one case on CT. The uptake pattern was focal in 12 cases (9 multiple, 3 single) and diffuse in 7 cases. In 5 cases (4 focal, 1 diffuse) BMB showed no infiltration. No guided biopsies were performed to explore these discordant cases. However, focal lesions resolved after treatment in all pts, supporting the presence of disease pretherapy. The sensitivity, specificity, positive and negative preditive value of FDG PET-CT with respect to BMB was 50.0%, 81.5%, 73.7% and 61.1% respectively. PET also detected five splenic involvements not seen on CT. Among 13 pts initially classified as localized stage (I/II) by conventional staging, 5 pts (representing 38%) were found to have stage IV using FDG PET-CT, due to bone lesions on PET. Overall, the incorporation of PET led to a change in Ann Arbor stage in 8 pts (14%), all of them were up-staged. As anticipated, the FLIPI distribution was modified when PET was taken into account: five pts (9%) were re-classified as high risk group on the basis of higher number of nodal areas and/or more advanced stage on PET.
Summary/Conclusions: FDG PET-CT identifies a greater extent of nodal and extranodal disease in FL compared with conventional CT, accounting for an improved stage accuracy. The addition of PET leads to stage migration in a proportion of pts, where it has the potential to modify therapy decisions. Whether this additional information provided by PET improves prognostic stratification remains to be determined.

E1160

RITUXIMAB MAINTENANCE IMPROVED PROGRESSION-FREE SURVIVAL AND REDUCED THE INCIDENCE OF TRANSFORMATION IN PATIENTS WITH FOLLICULAR LYMPHOMA
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Background: Follicular lymphoma (FL) is incurable disease even in rituximab era. Continuous exposure by anti-CD20 antibody after the induction therapy was considered to improve prognosis of patients with FL. Several phase II and III trials demonstrated that progression-free survival (PFS) is upgraded by rituximab maintenance. Transformed FL is known as rare but has a negative impact on prognosis of FL patients. It has not been known the relationship between rituximab maintenance and the incident rate of transformation.
Aims: We analyzed our data to clarify the efficacy and safety of rituximab maintenance in FL patients and to verify whether rituximab maintenance is associated with the incidence of transformed FL.
Methods: We retrospectively analyzed patients aged over 18 who were diagnosed with previous untreated FL grade 1, 2, and 3a in single cancer institute hospital from 2005. Chemo-sensitive patients had given four weekly rituximab infusions every 6 months for two years if we had informed consent. Basically, R-CVP was used except when FL had a feature of (i) bulky disease (≥7 cm), (ii) maximum of standardized uptake value ≥10 measured by PET scan, and (iii) clinical aggressive manifestations. The primary efficacy endpoint is the incidence of transformation during rituximab maintenance phase. Secondary endpoints are the 5-year PFS, time to next treatment (TNT), and overall survival (OS) and adverse events (AEs) during maintenance phase. Estimates of survival were analyzed by using log rank test and by Cox hazard model. Analysis of contingency table was done by Fisher's exact test.
Results: A total of 217 patients achieved at least stable disease by R-CVP/R-CHOP. Overall 217, 162 received maintenance group and 49 were observed after induction therapy. Baseline characteristics of both groups were similar except R-CHOP ratio. At the median follow-up of 68.4 months, 5-year PFS was 78.4% (95%CI: 70.0-84.9) in the maintenance group and 38.8% (95%CI: 16.2-43.3) in the observation group (P<0.0001). Five-year TNT was 70.6% (95%CI: 71.2-86.0) in the maintenance group and 47.6% (95%CI: 22.9-50.9) in the observation group (P<0.0001). The 5-year OS was 95.3% (95%CI: 89.5-97.9) in the maintenance group and 94.8% (95%CI: 71.2-92.7) in the observation group (P=0.96). Backward stepwise selection method in Cox hazard model demonstrated four factors that had a positive impact on PFS independently: rituximab maintenance (HR: 0.34, 95%CI: 0.19-0.61; P<0.01); non-bulky disease (HR: 0.41, 95%CI: 0.22-0.76; P<0.01); no transformation (HR: 0.41, 95%CI: 0.22-0.65; P<0.01); and β_2 microglobulin<2 (HR: 0.33, 95%CI: 0.19-0.59; P<0.01). On the other hand, 27 patients experienced transformation after R-CVP/R-CHOP. Of 27, 7 (4.7%) in the maintenance group and 16 (28%) in the observation group were transformed (Figure, P<0.01), whereas 7 (3.4%) in the R-CVP group and 16 (6.6%) in the R-CHOP group were transformed (P<0.01). Additionally, rituximab maintenance reduced the incidence of transformed FL regardless of induction therapy. In the R-CVP group, the incidence of transformation was 0.67% in the maintenance arm *versus* 4% in the observation arm, (P<0.01), while in R-CHOP group, that was 10.7% in the maintenance arm *versus* 17.9% in the observation arm (P=0.23). The most common AE was infusion reaction (13%). One patient quit maintenance at the first cycles due to severe infusion reaction.

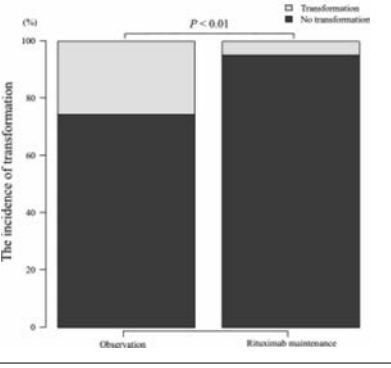

Figure 1. Rituximab maintenance reduced the incidence of transformed FL.

Summary/Conclusions: Rituximab maintenance was beneficial for only PFS at the median follow-up of 5 years. However, rituximab maintenance diminished the incidence of transformed FL significantly and tolerate.

E1161

CLONE-SPECIFIC IGH PLASMIDS AS AN ALTERNATIVE STANDARD FOR ASSESSMENT OF MINIMAL RESIDUAL DISEASE BY REAL-TIME QUANTITATIVE PCR IN FOLLICULAR LYMPHOMA
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Background: Development of novel treatment options, led to improved clinical outcome in follicular lymphoma (FL). Additionally sensitive diagnostic tools

have been established, able to detect low amounts of malignant cells, so-called minimal residual disease (MRD). Real-time quantitative pcr (RQ-PCR) using the t(14;18) as a molecular target is beeing broadly applied in FL. In cases with no PCR-detectable t(14;18), clonal rearrangements of the immunoglobulin genes (IGH) can be used, limited by the required information of lymphoma infiltration in the diagnostic sample, which is not regularly available due to nodal disease character. Cloned IGH-plasmids as an external standard for RQ-PCR could be a useful alternative.

Aims: This study investigated, whether RQ-PCR using clone-specific IGH-plasmids as standards for quantitative MRD-assessment in FL, is an accurate, reproducible and feasible alternative if no t(14;18) is available as a PCR-target.

Methods: IGH-rearrangements of a b-cell-lymphoma cell-line and 24 FL patients, amplified by FR1-IGH-PCR, were sequenced for identification of the clone-specific VDJ-region. Allel specific oligonucleotides were designed to the CDR3- and the FR3-region as reverse/ forward primers for RQ-PCR. Antisense consensus probes fitting to the FR3-region were used if possible, otherwise ASO-probes were designed. The IGH-rearrangements were cloned into plasmids and serial dilutions were performed for the cell-line and each patient to serve as external standards for RQ-PCR. Initially a serial dilution of cell-line DNA containing known IGH-copies was quantified by the plasmid-standard. Subsequently amplification characteristics of the IGH-plasmids of 24 FL patients were compared to each other and to relative genomic DNA (gDNA)-standards. Finally MRD-levels of follow-up samples, determined by using IGH-plasmid- and t(14;18)-cell-line- or IGH-gDNA-standards were compared.

Results: IGH-copies of a cell-line sample could be quantified accurately using IGH-plasmid-standards (*median deviation*: 6,9%, *SD*: 3,9%, *corr. coefficient*: 0,999). IGH-plasmid-standard-curves of the 24 patients indicated high conformity referring to slope (*median*: -3,39; *SD*: 0,08), correllation coefficient (*median*: 0,998; *SD*: 0,001), efficacy (*median*: 0,97; *SD*: 0,03), sensitivity (*median*: 10^{-5}) and specificity (*median*: $5x10^{-5}$). Compared to gDNA- plasmid-standards showed comparable sensitivity (*median*: 10^{-5} *vs* $1,25*x10^{-5}$; *SD*: $1,2x10^{-5}$ *vs* $2,69x10^{-5}$) and a superior specificity (*median*: $5x10^{-5}$ *vs* $1,41x10^{-4}$; *SD*: $2,5x10^{-5}$ *vs* $2,19x10^{-4}$). Moreover a significant superiority of the plasmid-standards was seen regarding to slope (*median*: -3,39 *vs* -3,67; *SD*: 0,08 *vs* 0,18; *p*: 0,008), correlation coefficient (*median*: 0,998 *vs* 0,990; *SD*: 0,001 *vs* 0,011; *p*: 0,009), and efficiency (*median*: 0,97 *vs* 0,87; *SD*: 0,03 *vs* 0,06; *p*: 0,008). In a final step patient follow-up samples were quantified by different strategies, using clone-specific IGH-plasmids *vs* IGH-gDNA or t(14;18)-cell-line-DNA respectively (total of 126 comparisons). In 92/126 (73,0%) of these comparisons Δmrd-level was<0,5 log-steps, in 21/126 (16,7%) within 0,5-1 log-steps, in 4/126 (3,2%) within 1-2 log-steps. Only in 9/126 (7,2%) cases Δmrd-level was >2 log-steps.

Summary/Conclusions: Quantitative MRD-assessment in FL using clone-/patient-specific IGH-plasmids as external standards for RQ-PCR, represents an accurate, reproducible and feasible strategy and can therefore be used in cases with no pcr-detectable t(14;18).

E1162

IS LIMITED STAGE FOLLICULAR LYMPHOMA (FL) A TRULY CURABLE DISEASE? A RETROSPECTIVE ANALYSIS ON 79 PATIENTS

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Background: Limited stage I/II FL is considered potentially curable in contrast to advanced stage (III/IV) disease. The most widely accepted treatment strategy is local radiotherapy (RT), occasionally followed by rituximab (R) monotherapy. However, studies specifically focusing on localized FL are limited.

Aims: To study the clinical and laboratory characteristics of limited stage FL and explore long-term outcomes.

Methods: We retrospectively studied all patients with FL diagnosed and treated in a single University Hematology Unit between 2001 and 2015 and stage I/II cases were recorded.

Results: Among 200 consecutive patients (median follow-up 76 months), 79 patients with limited stages were identified: 55 stage I and 24 stage II. Treatment differed according to histologic grade (p=0.002): RT±R was used in 53% of grade 1/2 histologies, whereas R-CHOP was administered in 54% of grade 3 cases. 5-and 10-year progression free survival (PFS) was 61% and 45%, respectively, while the corresponding values for overall survival (OS) were 94% and 90%. A plateau in the PFS curve was observed beyond 8 years. Disease bulk was identified as a borderline significant factor for PFS (p=0.09): Patients with bulky disease had a 5- and 10-year PFS of 43% and 29% *vs* 65% and 49% for those with non-bulky disease. The comparison of limited *vs* advanced disease stage revealed no significant differences regarding histologic grade, gender, age and bulk. FLIPI differed significantly, as expected (p<0.0001): 91% of limited stage patients had a low FLIPI score, whereas 78% of advanced stage ones had intermediate and high FLIPI scores. LDH was elevated in 5.3% of localized and 17.5% of advanced disease (p=0.01). There was a trend for higher Ki-67 in limited stages (p=0.07). Treatment also differed significantly, as expected (p<0.0001): 44% of limited stage patients were treated with RT±R *vs* 0.8% of advanced stage ones, whereas systemic chemotherapy was used in 53% and 92% of localized and advanced stage FL patients, respectively. Despite treatment heterogeneity, PFS and OS did not differ significantly between limited and advanced stage disease. However, there was a trend for superior OS in localized disease patients beyond 5 years (figure).

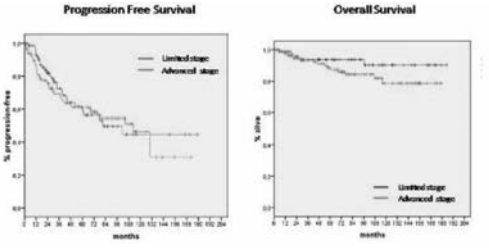

Figure 1.

Summary/Conclusions: Although limited stage FL is considered as a potentially curable disease, the present retrospective analysis revealed a relapsing pattern, similar to the one observed in advanced disease for the initial years of follow-up. However, beyond 8 years, survival curves are suggestive of a plateau, a finding that should be further confirmed. OS is excellent with 90% of the patients being alive at 10 years. The optimal treatment for this group of patients needs further investigation.

E1163

SINGLE CENTER EXPERIENCE OF 90Y-IBRITUMOMAB TIUXETAN IN THE OLDER POPULATION WITH NON-HODGKIN LYMPHOMA

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Background: ^{90}Y-Ibritumomab tiuxetan (^{90}Y-IT) as radioimmunotherapy in the treatment of B-cell non-Hodgkin lymphoma (NHL) has been approved for consolidation and salvage treatment of follicular and indolent lymphoma. It has also been studied in the treatment of diffuse large B-cell and mantle cell lymphoma. Toxicities primarily include myelosuppression with a risk of treatment-associated myelodysplastic syndrome (MDS).

Aims: To evaluate the toxicity and treatment benefit of ^{90}Y-IT administered to patients for consolidation and salvage treatment of non-Hodgkin lymphoma.

Methods: Retrospective cohort analysis of patients with B-cell NHL who received ^{90}Y-IT from 2010 to 2015 at Geisinger Medical Center. ^{90}Y-IT was administered according to recommended dosing guidelines and cytopenia was monitored at routine intervals.

Results: Sixty-five patients with NHL received ^{90}Y-IT at median age of 68 years (range 32-91), 32 (49.2%) patients were male, and 36 (55.8%) were older than 65 years of age. Thirty patients (46.1%) had aggressive NHL and 37 (56.9%) had stage III/IV disease. Twenty-nine (44.6%) patients received >1 line of treatment prior to ^{90}Y-IT therapy and 62 (97%) received Rituximab based regimens. No patient received fludarabine based therapy. Prior to administration of ^{90}Y-IT, the median bone marrow involvement was 0%, median platelet count was 183 x 10^9/L, median hemoglobin count was 12.7 g/dL, and median absolute neutrophil count was 3.8 x 10^9/L. The median follow-up period was 16 months (range 4-68). The overall response rate was 89.2% with a PR-to-CR conversion rate of 36%. The response rate was 54.5% in the salvage setting. The median PFS and OS were 12 and 15 months, respectively. There was no significant difference in PFS and OS between patients younger and older than 65 years of age or between patients in CR and not in CR at the time of ^{90}Y-IT. Patients on Rituximab maintenance after ^{90}Y-IT treatment (n=14, 21.5%) had PFS and OS improvement (p<0.0001), with median PFS and OS of 45.5 and 48 months, respectively. In the population without Rituximab maintenance (n=51, 78.4%), the median PFS and OS were 10 and 13 months respectively. Patients not in CR at the time of ^{90}Y-IT were found to be responsive to Rituximab maintenance: PFS improved from median 10.0 to 48.0 months (p<0.0001) and OS improved from median 14.0 to 53.0 months (p=0.0003). Among patients with grade 3/4 hematologic toxicities, 6 patients required blood transfusion, 4 needed platelet transfusion, and 17 (26.1%) received pegfilgastrim. One patient required hospitalization for febrile neutropenia. Seven patients relapsed after ^{90}Y-IT and one patient (1.5%) developed MDS (del 5q, del 20q) 54 months after treatment.

There were 9 deaths (six were secondary to progressive lymphoma or chemotherapy-related neutropenic sepsis).
Summary/Conclusions: This is one of the largest reported single-center experience of ^{90}Y-IT administered to a predominantly elderly population with B-NHL in the United States. This study demonstrates the safety and feasibility of ^{90}Y-IT in the elderly population with manageable toxicity and similar outcomes between patients younger and older than 65 years of age. Furthermore, treatment with ^{90}Y-IT did not negatively impact continued sensitivity and benefit from Rituximab maintenance.

E1164

DISEASE SURVEILLANCE AND LONG-TERM OUTCOME OF PATIENTS WITH MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA INVOLVING THE STOMACH: A RETROSPECTIVE MULTICENTRE STUDY OF 49 PATIENTS IN FIRST REMISSION

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Background: Gastric MALT lymphoma is a rare subtype of non-Hodgkin lymphoma with an indolent disease course. Treatment options span from simple eradication of Helicobacter Pylori with or without radiotherapy for patients with localized gastric MALT lymphoma to systemic, typically mild chemotherapy regimens and/or rituximab, for patients with non-localized disease. Complete remissions (CR) are often achieved, but relapses occur.
Aims: i) to analyze the relapse patterns and the efficacy of routine gastroscopy for patients with gastric MALT lymphoma in first remission, and ii) report the long-term outcomes of these patients relative to a matched background population.
Methods: We conducted a retrospective study of patients with localized and non-localized gastric MALT lymphoma from six Danish hematology centers between 2000 and 2012. Patients were identified from queries in the Danish Lymphoma registry (LYFO) and consecutive adult patients (>15 years) in CR or CRu were included in the study. Local investigators reviewed medical records and collected information regarding gastroscopic examinations, relapse (including relapse detection method and relapse histology), and cause of death. Gastroscopies were categorized as routine if prescribed to a patient in clinical remissions and clinical if prescribed to a patient with relapse suspicious symptoms/findings. Data collection was compliant with national regulations.
Results: In total, 49 patients with gastric MALT lymphomas stage I-IV and in CR or CRu following first line treatment were included. Median age was 64 years (range 25-87) and male:female ratio was 1.1. During a median FU of 88.8 months, seven patients relapsed and 14 patients died. The five-year PFS and OS estimates were 73% (95% CI 61-88) and 89% (95% CI 80-99), respectively (Figure 1). The OS of a matched background population was not different from the MALT lymphoma (P=0.94, standardized mortality ratio, Figure 1). During follow-up, the 49 patients received 100 routine endoscopies (mean 2 range 0-6) and 15 clinical endoscopies. In four patients (8%), routine gastroscopy detected preclinical MALT relapse or another cancer at an asymptomatic stage (adenocarcinoma). Thus, 4/100 routine endoscopies (4%, 95% CI 1-10%) contributed significantly to detection of relapse or other malignancy. These findings resulted in chemotherapy for 2/4, helicobacter eradication with antibiotics for 1/4 and surgery for 1/4 (adenocarcinoma). A single MALT lymphoma relapse was detected as a result of patient reported symptoms. Thus, 1/15 (7%, 95% CI 0-32%) of the gastroscopies performed in response to clinical symptoms/findings confirmed relapse. A case of adenocarcinoma localized in the stomach was found 28 months after first MALT lymphoma diagnosis, but transformation to high-grade lymphoma was not reported for any patients. Of note, no deaths were related to progressive MALT lymphoma in this cohort of 49 patients.

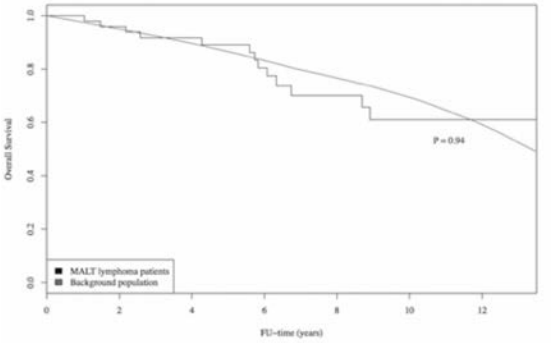

Figure 1.

Summary/Conclusions: This study confirms the very indolent nature of gastric MALT lymphoma with survival not statistically significantly different from the background population. Preclinical relapse detection has not been shown to improve outcome in gastric MALT lymphoma and asymptomatic relapse can be managed with a watch-and-wait strategy in selected patients. In this context, the yield of routine endoscopy is limited. However, one case of adenocarcinoma was detected at an early stage by routine endoscopy.

E1165

PRE-DOSING WITH UNLABELLED ANTIBODY SIGNIFICANTLY INCREASES THE PHARMACOKINETIC EXPOSURE BUT PROTECTS AGAINST MYELOSUPPRESSION OF 177LU-LILOTOMAB IN NON-HODGKIN B-CELL LYMPHOMA PATIENTS

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Background: ^{177}Lu-lilotomab (^{177}Lu-DOTA-HH1; Betalutin^{TM}) is a novel CD37-binding murine IgG_1 antibody (HH1) labelled with the beta-emitter lutetium-177, in a ready-to-use formulation currently in Phase 1/2 clinical development for the treatment of non-Hodgkin`s lymphoma (NHL). Pre-dosing with unlabelled antibody is routinely given with radio-immunotherapies for NHL.
Aims: This pharmacokinetic (PK) sub-study in NHL patients (pts) was designed to determine the PK profile of ^{177}Lu-lilotomab and to compare the PK profile with and without pre-dosing with unlabelled HH1.
Methods: Pts with relapsed incurable indolent NHL and with platelet counts ≥150 x10^9/L were eligible for inclusion in the study. Pts received rituximab (375 mg/m^2) on day 1 and 8 to deplete normal B cells. On day 29 pre-dosing with 50 mg HH1 was administered before the single dose of ^{177}Lu-lilotomab in 9 pts. The remaining 4 pts received ^{177}Lu-lilotomab without HH1 pre-dosing. Activity of ^{177}Lu-lilotomab administered after the pre-dose of HH1 were 10, 15 and 20 MBq/kg *vs* 10 and 15 MBq/kg without HH1. PK samples were collected at 0 and 5 minutes, then after 1, 2, and 20 hours and further 2, 3, 4, 7, 14 and 21 days post-^{177}Lu-lilotomab administration. Urine samples were also collected. Response was assessed by FDG PET/CT scans.
Results: A total of 13 pts, 12 with follicular lymphoma and 1 with mantle cell lymphoma, were enrolled into this sub-study and either received pre-dosing with HH1 (n=9) or without HH1 (n=4). The number of prior therapies ranged from 1 to 7, median body weight was 91.5kg (range: 67-118kg). A total of 153 blood samples were collected. As expected, the median $AUC_{0-\infty}$ increased with increasing activity of ^{177}Lu-lilotomab irrespective of pre-dosing (range: 3830.7 to 15226.48 h*kBq/mL). There was an increase in median activity-adjusted $AUC_{0-\infty}$ (675.51 *vs* 421.11 h*kBq/mL/(MBq/kg); p<0.001) in pts with pre-dosing compared to no HH1 pre-dosing and a decrease in the volume of distribution Vz (10.48L *vs* 17.56L) and clearance rate Cl (126 v 227 mL/h). The median activity-adjusted C_{max} (6.6 *vs* 6.9 kBq/mL/(MBq/kg)) and $T_{1/2}$ (53.3 *vs* 51.6 h) were similar with and without HH1 pre-dosing. The most common toxicities observed were haematological which were all reversible and manageable. With HH1 pre-dosing all Grade 4 thrombocytopenia and neutropenia were reported by pts with a ^{177}Lu-lilotomab $AUC_{0-\infty}$ >13415 h*kBq/mL (n=3), compared with >6147 h*kBq/mL (n=2) without pre-dosing. In addition grade 3 thrombocytopenia was reported by one pt with an AUC of 11730 h*kBq/mL with pre-dosing compared to one pt with an AUC of 4913 h*kBq/mL without HH1. The overall tumor response rate observed in the 12 evaluable pts in the PK sub-study was 67% (one pt had a transformed lesion at 3 months and is excluded from the efficacy analysis). Five pts with pre-dosing achieved CR or PR and had an AUC of >6976 h*kBq/mL. Without HH1 pre-dosing a PR was observed in 3 pts, with AUCs of 3830, 4913 and 6195 h*kBq/mL.
Summary/Conclusions: Pre-dosing with unlabelled antibody (HH1) increases the activity-adjusted AUC and decreases the volume of distribution and clearance rate of ^{177}Lu-lilotomab in NHL pts. Despite the increase in AUC resulting from pre-dosing with HH1 the incidence of haematological AEs was reduced compared with no HH1 pre-dosing, suggesting a protective effect of HH1 against the myelosuppression of ^{177}Lu-lilotomab. Further optimisation of the pre-dosing of ^{177}Lu-lilotomab may lead to greater increases in AUC, and/or improvement in the side-effect profile.

E1166

FOLLICULAR LYMPHOMAS ARE CURABLE DISEASES. DATA FROM A RETROSPECTIVE STUDY ON 133 PATIENTS WITH AT LEAST 10 YEARS OF OBSERVATION

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Background: Follicular lymphomas are usually defined as incurable diseases with a natural history characterized by continuous relapses. This study was launched to evaluate how many patients, after a long observation period, do not relapse or do not experience a new treatment.
Aims: The aim of the study was to identify which clinical characteristics or therapeutical approaches are associated with this cohort of favourable patients.
Methods: All patients with histologically confirmed diagnosis of follicular lymphomas grade I-II or IIIa were selected from our data base starting from January 2000 untill December 2004 in such a way to have at least 10 years of observation for alive patients. We considered patients obtaining at least a partial response and we divided patients in two cohorts, cohort 1 with patients relapsed or progressed and cohort 2 with patients never relapsed or progressed.
Results: One hundred and fourthy-six patients were diagnosed and treated at our Institution. Thirteen patients were excluded from the analysis, 8 lost to the follow-up and 5 did not obtained at least a partial remission. Finally 133 patients were analysed for the study. The median age at diagnosis was 61 years (range 30-87). Stage I-II in 47 patients, III-IV in 86. Bone marrow biopsy was positive in 87 patients, FLIPI 0-1 in 35, FLIPI 2 in 43, FLIPI 3 in 40 and FLIPI 4 in 15 patients. According to treatment 96 patients were treated with antracycline containing regimens, 24 with fludarabine containing regimens and 13 were observed or treated with radiotherapy. Rituximab was used in 92 patients, as sequential treatment in 70 or chemotherapy combined in 22; 41 patients did not use rituximab. We analysed cohort 1 (85 patients) and cohort 2 (48 patients) and the statistically significant differences between the two cohorts were: elderly patients (p 0.05), symptomatic patients (p 0.05), FLIPI and FLIPI2 high score (p 0.005), lack of complete remission (p 0.0000) all observed in cohort 1. The overall survival with a median period of observation of 127 months (range 2-196) was 71%, considering the two groups the overall survival in cohort 1 was 62% with a median of 142 months and it was 94% in cohort 2 with median not reached. In univariate analysis normal value of beta2 microglobulin (p 0.05) and the use of rituximab (p 0.01) were associated with a better overall survival; in multivariate analysis treatment with rituximab manteined a statistically significance.
Summary/Conclusions: In conclusion this retrospective monocentric study confirms that about one third of follicular lymphoma patients could be considered cured particularly if rituximab was used in the treatment. At the present time all patients with follicular lymphoma are treated with combined immuno-chemotherapy, moreover after induction therapy patients are started with manteinance. We can therefore hope for the future in an improvement of survival results.

Infectious diseases, supportive care

E1167

IMPACT OF AZACITIDINE TREATMENT ON INFECTIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA, MYELODYSPLASTIC SYNDROMES AND MYELODYSPLASTIC/MYELOPROLIFERATIVE SYNDROMES

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Background: It has been described in previous studies a benefit on infection rate in azacitidine (AZA) treated patients. However, the characteristics of infectious episodes in patients with AZA or best supportive care (BSC) were not described in detail.
Aims: We decided to analyze the impact of AZA treatment on the risk of infection, the characteristics of the infectious episodes and outcome in our patients with acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative syndromes (SMD/MPS).
Methods: Infections were assessed by reviewing medical records of our department´s database of patients treated with AZA from January 2006 to November 2015. A cohort of matched control patients treated with BSC (two controls for each patient) based on World Health Organization (WHO) subtype, neutrophil count and IPSS-R risk was selected. We took only on account infection´s episodes which required hospitalization.
Results: We included 138 patients, 92 received BSC and 46 treated with AZA at standard schedule (75mg/m^2/day 7 days every 28). The median follow up for both groups was 10 months. In the AZA group, 8/46 patients (17.4%) had<0.5x10^9/L neutrophils at diagnosis and 6/44 (13.6%) at four months, with no significant increase in neutropenia incidence (*p=0.773*). However, In the BSC group, 7/92 (7.6%) patients had<0,5x10^9/L neutrophils at diagnosis and 16/77 (20.8%) at four months, with a significant increase in the incidence of neutropenia. (*p=0.022*). The percentage of patients free of infection in AZA group was higher than BSC group. The median of infectious episodes per year was 1.17 in AZA group (CI 95% 0.44-1.90) and 3.08 in BSC group (CI 95% 1.56-4.60) (*p=0.004*).The days of hospitalization/year due to infection was 20.59 (CI95% 3.91-37.27) in the AZA group and 39.90 (CI 95% 21.63-58.18) in the BSC (*p=0.003*). The cumulative incidence of first infection, with death as competitive risk, was higher in BSC group than in AZA group; with a cumulative incidence at 12 months of 0.40 (CI 0.26-0.54) and 0.54 (CI 0.43-0.63) in the AZA and BSC group respectively (Grays´s test p=0.0195). In the AZA group 4 (18.2%) episodes were fatal and in the BSC group 24 (23.3%) (*P=0.78*). Multivariant logistical regression shows that only AZA treatment (HR 0.25 *p=0.001* CI 0.12-0.55) and absolute neutrophil count (ANC) >0.5X10^9/L (HR 0.28 *p=0.049* CI 0.08–1.03) showed an independent prognostic value. Age, comorbidities and WHO subtype were excluded by the test.

Table 1. Baseline characteristics of patients and number of infections.

	AZA N=46	BSC N=92	P-value
Age in years at diagnosis (median)	72,5	79	<0,001*
WHO subtype, *n*			
MDS/MPS	12	22	
AML	11	21	
MDS : Low / Intermediate / high	23: 5/5/13	49: 10 / 11 / 28	
ANC <1x10^9/L	11 (24)	22 (24)	1**
Hemoglobin (g/L)	92.2	97	0,192*
Platelets (x10^9/L)	67	97	0,157*
Diabetes mellitus (%)	26,1	26,4	1**
Chronic obstructive pulmonary disease (%)	19,6	16,3	0,63**
Number of infections (%)			
0	29 (63)	31 (33,7)	
1	14 (30,4)	41 (44,6)	0,0018***
=2	3 (6,5)	20 (21,7)	

* U-Mann Whitney test. ** Fisher´s test. *** Chi squared test.

Summary/Conclusions: Azacitidine treated patients had less risk of infection, showed with cumulative incidence, less infectious episodes per year and less days of hospitalization per year. However, the outcome of infection episodes was similar in both groups. The higher incidence of infections in BSC group could be explained by the mayor progression to severe neutropenia.

E1168

CLINIC RISK FACTORS OF REFRACTORY CMV REACTIVATION FOLLOWING ALLOGENEIC AHSCT: A SINGLE-CENTER STUDY IN CHINA

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Background: Cytomegalovirus (CMV) reactivation remains the main cause of viral complications after allogeneic hematopoietic stem cell transplantation

(aHSCT). Especially, CMV with gene mutations against antiviral drugs could lead to high mortality rate.
Aims: The purpose of this study is to explore the risk factors and outcome for CMV reactivation and CMV refractory to antiviral chemotherapy after aHSCT.
Methods: CMV reactivation occurred in 282 of 685 (41.2%) patients treated with myeloablative conditioning regimen. Among the patients with CMV reactivation, 84 of 282 (29.8%) cases developed refractory CMV reactivation (RCR), and 37 of 282 (13.1%) cases progressed into CMV diseases.
Results: Patients with RCR have a higher cumulative incidence of CMV diseases (26.2% *versus* 7.6%, *P*<0.001). Seventy nine of 84 cases (94.0%) developed RCR before 100 days after aHSCT (5 cases with PP65 detection only). The copy number of CMV-DNA more than doubled compared to its initial baseline in 42 of 74 (56.8%) cases after 2 weeks of antiviral therapy, whereas 32 cases did not have a double increase above baseline. CMV disease developed in 15 of 42 (35.7%) and 3 of 32 (9.4%) (*P*=0.011) cases in these two groups. The copy number of CMV-DNA increased at least 5 times above baseline in 24 of 74 (32.4%) patients, and among these 24 patients, 8 of 24 (33.3%) cases developed CMV disease. Among patients who do not have a 5-fold increase of CMV-DNA copy number, 10 of 50 (20.0%) patients developed CMV disease (*P*=0.232). Both univariate and multivariate analysis demonstrated that the risk of CMV reactivation and RCR increased in patients from HLA-haploidentical donor and matched unrelated donor, or without using peripheral blood (PB) as stem cell source. The multivariate analysis revealed that patients who developed acute graft-*versus*-host disease (aGVHD) ≥grade 2 have increased risk of developing CMV reactivation, whereas RCR occurred more frequently in those with total body irradiation-containing regimens and a high dosage methylprednisolone (MP) for aGVHD treatment. The prevalence of RCR is three and six times higher in patients who received MP 1-2mg/kg daily(*P*=0.024) and ≥2mg/kg daily(*P*=0.001), with an odds ratio (OR) of 2.74 and 6.03 respectively.
Summary/Conclusions: High dosage corticosteroids treatment is associated with incidence of RCR during the early phase after aHSCT.

E1169

INFECTIOUS CHARACTERISTICS AND LONG-TERM OUTCOME IN OBESE PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: Obese patients were reported to have poorer prognosis in pediatric acute lymphoblastic leukemia (ALL), however, the association with infection during chemotherapy was seldom reported.
Aims: To evaluate the difference of infectious characteristics and long-term outcome between obese and non-obese patients.
Methods: Total 252 newly diagnosed pediatric ALL patients from 1997 to 2012 at National Taiwan University Hospital treated by Taiwan Pediatric Oncology Group (TPOG) protocol were retrospectively reviewed. The fever event was classified into fever of unknown focus, clinically documented infection and microbiologically documented infection (MDI). Infectious sites were recorded by location: upper respiratory (EENT), lower respiratory (LRI), blood stream (BSI), genitourinary tract (GU), gastrointestinal tract, skin or soft tissue, central nervous system and others. Multivariate analysis for risk factors of infectious complications was evaluated by Poisson regression with incidence rate ratio (IRR) and 95% confidence interval (CI).

Table 1. Subgroup Univariate Poisson Regression Analysis in Obese *vs* Non-obese Patients.

		MDI			GU			LRI-MDI	
Variables	IRR	95% CI	*p*-value	IRR	95% CI	*p*-value	IRR	95% CI	*p*-value
Gender									
Male	0.846	0.594-1.206	0.355	1.947	0.958-3.956	0.066	0.790	0.242-2.581	0.696
Female	1.683	1.321-2.145	<0.001	2.053	1.353-3.113	0.001	2.543	1.424-4.543	0.002
Age (years)									
<5	1.223	0.904-1.654	0.191	2.903	1.630-5.168	<0.001	3.728	2.073-6.705	<0.001
5-10	2.046	1.480-2.827	<0.001	2.764	1.474-5.183	0.002	1.102	0.323-3.759	0.877
>10	0.948	0.613-1.467	0.811	1.213	0.619-2.376	0.573	-	-	-*
Risk Group									
SR	1.365	0.908-2.054	0.135	4.013	1.894-8.503	<0.001	3.330	1.494-7.426	0.003
HR	1.563	1.174-2.080	0.002	2.462	1.383-4.384	0.002	0.990	0.415-2.361	0.982
VHR	1.016	0.706-1.462	0.932	1.401	0.774-2.533	0.265	3.537	1.223-10.225	0.020

**No case in this subgroup. LRI-MDI, lower respiratory infection with pathogen; SR, standard risk; HR, high risk; VHR, very high risk.*

Results: Total 219 patients (86.9%) had fever with mean 2.74 episodes per person. Obese patients (n=38) harbored more MDI, GU and LRI with pathogen infections than non-obese patients (IRR=1.349, 2.228 and 2.014; 95% CI=1.109-1.642, 1.558-3.187 and 1.22-3.326; and *p*=0.003,<0.001 and 0.006, respectively). Subgroup analysis revealed the IRR in obese patients was higher in female and younger age patients (Table). The pathogen characteristics in obese patients included more Gram negative pathogen both in blood stream (29% *vs* 14%, *p*=0.029) and GU tract (32% *vs* 10%, *p*=0.001). *Klebsiella* spp. bacteremia was higher in obese patients (11% *vs* 3%, *p*=0.047). Overall prognosis analysis showed obese patients had more relapsed rate (42.1 *vs* 22.4%, *p*=0.012) but the mortality rate was not different (36.8 *vs* 23.4%, *p*=0.063). The 10-year event-free survival was also lower in obese patients (54±8% vs.72±3%, *p*=0.002, figure).

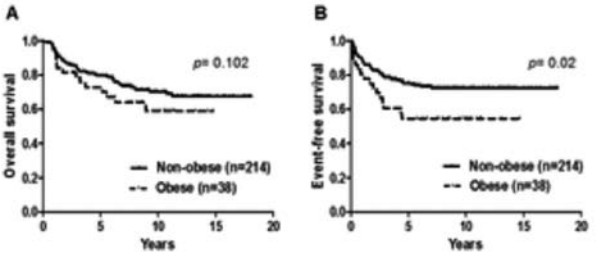

Figure 1.

Summary/Conclusions: Obesity was a potential risk factor in infectious complications in pediatric acute lymphoblastic leukemia patients, especially in microbiologically documented infection and genitourinary tract infection.

E1170

ANTIBIOTICS *VERSUS* G-CSFS *VERSUS* A COMBINATION OF BOTH TO PREVENT INFECTIOUS COMPLICATIONS AND DEATHS IN CANCER PATIENTS RECEIVING CHEMOTHERAPY: PRELIMINARY RESULTS OF A NETWORK META-ANALYSIS

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Background: Cancer patients receiving chemotherapy are at an increased risk for infectious complications, which represent the most relevant treatment-related causes of death (2 to 21%). The risk of infection is dependent on the duration and intensity of treatment-induced neutropenia and often results in a reduction of the dose of chemotherapy. Uncertainty exists on the achievable degree of infection prevention and whether granulocyte colony stimulating factors (G-CSF), antibiotics or both should be administered.
Aims: The aims of this systematic review and network meta-analysis are to evaluate the benefits and risks of infection prophylaxis with antibiotics, G-CSF, or a combination of both in cancer patients receiving chemotherapy at risk for febrile neutropenia.
Methods: We developed sensitive search strategies for CENTRAL, MEDLINE, and conference proceedings (search date 12/2015). Study selection and data extraction:Randomized controlled trials evaluating infection prophylaxis with antibiotics, G-CSF or both in cancer patients at risk for febrile neutropenia. Only trials were included which clearly stated whether all patients received antibiotics or G-CSF in addition to the evaluated prophylaxis. Two authors independently assessed studies for eligibility, extracted data and assessed quality of trials. The primary outcome was mortality during the study. Secondary outcomes included infection related mortality, severe infection rate, rate of febrile neutropenia and adverse events. Data synthesis: Effect measures were odds ratios (OR). Direct comparisons within trials were combined with indirect evidence by using a Bayesian random-effects model. This project was funded by the Federal Ministry of Education and Research, grant number: 01KG1209.

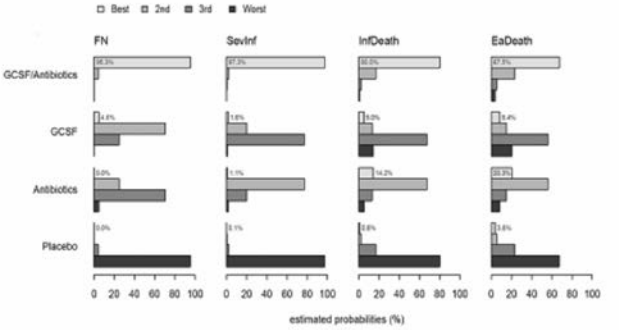

Figure 1.

Results: A total of 63 studies were identified, including 11838 patients. Only one study with 40 patients carried out a direct comparison of G-CSF *versus* antibiotics. Of all studies comparing antibiotics to no antibiotics, only 2 were from 2010 or later. The results of the direct comparisons found an advantage in terms of reduced rate of febrile neutropenia for those trials evaluating G-

CSF, both in the studies using G-CSF *versus* placebo (OR 0.37; 95% CI 0.12 to 0.86) and in those evaluating G-CSF plus antibiotics *versus* antibiotics alone (OR 0.45; 95% CI 0.24 to 0.74). However, there is no evidence for a difference for the outcomes mortality during the study, infection-related mortality and severe infection rate between the various preventive options. Network meta-analyses show that the combination of G-CSF and antibiotics has the highest probability to be the best for all the observed outcomes (see figure 1). Between-trial heterogeneity was considerable for the outcomes febrile neutropenia and severe infections, potentially due to various definitions (tau 1.08 and 0.97, respectively) and negligible for the other two outcomes (tau 0.42 and 0.18 respectively). The endpoint adverse events was very rarely and inconsistently reported, so no overall statement is possible.
Summary/Conclusions: The currently available direct evidence does not show any difference in terms of mortality for the various prophylactic options. The evidence from the network meta-analyses shows an advantage if G-CSF is administered in conjunction with antibiotics compared to placebo, antibiotics or G-CSF alone. Most evidence on antibiotic prophylaxis stems from studies older than 10 years, *i.e.* before the ongoing pandemic of multi-resistant gram-negatives and vancomycin-resistant enterococci. Further studies with clearly defined inclusion and exclusion criteria (risk of febrile neutropenia) and endpoints (e.g. definition of febrile neutropenia) are necessary in order to properly compare the various prophylactic options and to obtain valid results.

E1171

METRONOMIC FIRST-LINE ANTIBIOTICS FOR FEBRILE NEUTROPENIA-IMPACT ON ECOLOGY AND MORTALITY IN PATIENTS TREATED FOR HAEMATOLOGIC MALIGNANCIES

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Background: Rising antibiotic resistance threatens to limit our choices for empirical and definitive treatment of febrile neutropenia (FN). Reports suggest that increasing antibiotic heterogeneity might be associated with a decline in resistance. In our hospital's Haematology unit, cefepime (CEF) was recommended as the first-line empirical antibiotic in FN. Rising drug resistance and the potential usefulness of increasing antibiotic diversity led us to re-consider our approach.
Aims: To introduce antibiotic heterogeneity by alternating use of first-line antibiotics and to determine the impact of this on ecology and mortality of patients treated for haematological malignancies.
Methods: From mid-2013, a new FN guideline was implemented. Neutropenic patients who developed fever on odd dates of the month would be given Piperacillin Tazobactam (PTZ) and on even dates, a combination of Cefepime and Amikacin, post blood cultures. This is termed as "metronomic 1st line antibiotics". If fever persists after 48 hours, physicians could continue on the same antibiotics, or add vancomycin or switch to meropenem (MER). To assess the impact of this change, we collected the following data for the years 2012 and 2014: rates of bacteremia (including rates of bacteremia caused by carbapenem-resistant organisms), Methicillin Resistant Staphilococcus Aureus (MRSA) acquisition, Vancomycin Resistant Enterococcus (VRE) acquisition, mortality. We also calculated the defined daily doses (DDD) of CEF, PTZ, and MER for 2012 and 2014. This study was approved by the Institution Review Board.
Results: In 2012, 1183 patients were admitted 2012 times; in 2014, 1112 patients were admitted 1843 times. Males formed 54.5% and 55.7% of the patients in 2012 and 2014 respectively. The following were similar ($p>0.5$) in the 2 years: mean and median ages of the patients, number of new patients with acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL) who started on chemotherapy, spread of discharge diagnoses (AML, ALL, myeloma, lymphoma). Rate (DDD/1000 days) of CEF use fell ($p<0.001$) and rate of PTZ use rose ($p<0.001$) in 2014. Rate of MER use was unchanged. Bacteremia rates were unchanged. Rates of acquisition of MRSA and VRE were unchanged. Rates of CEF-resistant, and of PTZ-resistant E. coli, Klebsiella spp, and P. aeruginosa isolated from blood cultures were similar in the 2 years. The rate of carbapenem-resistant P. aeruginosa was 12.7% in 2012 and 17.5% in 2014 ($p=0.6$). Mortality among bacteremic patients was 24% in 2012, 14% in 2014 ($p=0.001$).
Summary/Conclusions: We conclude that "metronomic 1st line antibiotics" introduced an element of antibiotic heterogeneity. This was not associated with an increase in the rate of isolation of resistant organisms. It is possible that the increased use of PTZ was associated with a lower mortality.

E1172

ORAL GENTAMICIN THERAPY FOR CARBAPENEM-RESISTENT KLEBSIELLA PNEUMONIAE INFECTIONS IN HEMATOLOGIC PATIENTS: A SINGLE CENTER EXPERIENCE

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Background: The worldwide spread of carbapenem-resistant *Klebsiella pneumoniae* (KPC-Kp) is nowadays a health threat worldwide. KPC-*Kp* mortality infections range from 18 to 48% and depend on the type of therapy administered; mono *vs* combo therapy. This result may be due to delayed time to active therapy, pharmacologic limitations of available treatment options, and the fact that patients with KPC-Kp infections tend to be critically ill. Patients with hematologic malignancies and hematopoietic stem cell transplant recipients are more vulnerable to Carbapenemase-resistant *Enterobacteriaceae* (CRE) because of chemotherapy-induced gastrointestinal mucositis, prolonged hospitalizations and neutropenia, and frequent use of broad-spectrum antibacterial agents. Mortality in these patients might be as high as 85%.
Aims: The aim of our study was to test he efficacy of oral gentamicin in gut decontamination from KPC-Kp in hematologic patients, in view of immunosuppressive therapy, stem cell transplantation or to prevent sepsis after chemotherapy.
Methods: We performed a prospective study in an Italian Hematology Unit to quantify the efficacy of gentamicin gut decontamination from KPC-Kp in hematologic patient with and without concomitant infection. We considered eligible all patient with rectal swab culture positive for KPC-Kp with hematologic disease and those patients were administered oral gentamicin (80 mg four times daily) for 7 to 25 days, having stopped the therapy three days after the proved decontamination. Rectal swab cultures were performed at the time of hospital admission and every 3 days thereafter.
Results: Fourteen patients with a hematologic disease and concomitant *KPC-Kp* positive rectal swab culture were enrolled in the study; only one was resistant to gentamycin. The patients were 5 female and 9 male, with a mean age of 68.5 years (range 39-80 years). Concomitant systemic associated therapy (CSAT) was administered to 4/14 patients due to systemic infections. The enrolled patients were treated with oral gentamicin for 7 to 25 days and the overall decontamination rate in the entire study population administered oral gentamicin was 71% (10/14). Only two patients took gentamicin for more than 15 days. Four patients were not decolonized by gentamicin oral therapy: one of these was the patient harbouring gentamicin-resistant strain and 3 patients have CSAT. One out of this 4 patients died for KPC-Kp sepsis. At 1 month of follow-up, no one KPC-Kp re-infection was documented. The decontamination rate was 90% (9/10) in patients receiving oral gentamicin only, *versus* 25% (1/4) in those also treated with CSAT. No new gentamicin-resistant KPC-Kp strain was isolated during oral gentamycin therapy.
Summary/Conclusions: The KPC-Kp infection are associated with high mortality and morbidity, especially in Intensive Care Units and hematologic patients; prevention of KPC-Kp infection is the first provision to implement, followed by the screening for gut colonization and eventually gut decolonization. In our experience the decontaminations rate in the entire population was 71% and 90% considering the patients receiving oral gentamicin only. Although the small number of patients analyzed in this study, our results were similar to others suggesting that the selective pressure of CSAT on gut microbiota may favor the persistent carriage of KPC-Kp, thereby contributing on the higher failure rates observed *versus* monotherapy. Therefore, the selective oral decontamination therapy of KPC-Kp with oral gentamicin is safe and possibly effective.

E1173

CLINICAL SIGNIFICANCE OF SERUM HEME OXYGENASE 1 IN PATIENTS WITH SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare, threatening-life clinical syndrome, caused by cytokines storm due to provoked, uncontrolled proliferation and over activation of lymphocytes and macrophages. sHLH in adults is an etiologically heterogeneous entity that has been associated with infections, autoimmune disorders, and haematological malignancies, mainly with non-hodgkin lymphoma. Clinically, these disorders feature fever, hepatosplenomegaly, severe cytopenias, activated macrophages in hematopoietic organs. Biochemical abnormalities include hypofibrinogenemia, hypertriglyceridemia, and increased levels of soluble interleukin-2 receptor (sCD25), serum ferritin(SF), and a large number of inflammatory other cytokines. Heme oxygenase 1 (HO-1) is an inducible stress-response enzyme and it is also an rate-limitting enzyme that degrades heme to biliverdin, carbon monoxide (CO) and free iron in mammalian cells. Biliverdin and CO gain particular interest because of mediating most of anti-inflammatory, antioxidant and anti-apoptotic effects of HO-1. HO-1 can be induced by various stressors such as oxidative stress, inflammatory cytokine, heavy metals and ultraviolet irradiation. HO-1 is constitutively expressed in peripheral monocytes and organ-localized macrophages such as hepatic kupffer cells and splenic macrophages. These facts suggest that HO-1 plays a key role in regulation of the inflammatory response in physiological and pathological conditions. Recently, there are accumulating evidences that increased serum HO-1 level is related to macrophage activation. Herein, we measured serum HO-1 levels in sHLH patients and ana-

lyzed the relationship of serum HO-1 with other biological markers of sHLH. The results show that serum HO-1 is a novel marker to evaluating severity and activity of sHLH. In addition, serum HO-1 may be an indicator using in differentiating autoimmune-associated hemophagocytic lymphohistiocytosis (AHLH) from other underline etiologies of sHLH.
Aims: To investigate the levels and clinical significance of heme oxygenase 1 (HO-1) in patients with secondary hemophagocytic lymphohistiocytosis (sHLH).
Methods: Serum HO-1 levels were determined by using enzyme linked immunosorbent assays (ELISA) in 43 sHLH patients and 12 healthy contrlols. Clinically relevant laboratory values, including white blood cell (WBC), hemoglobin (HB), platelet (PLT), serum ferritin (SF), albuminin (ALB), alanine transaminase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), fibrinogen (FIB), blood sedimentation rate (ESR), triglyceride (TG), soluble interleukin-2 receptor (sCD25) and C reactive protein (CRP) were collected.
Results: (1) Serum HO-1 levels of the newly diagnosed group were found to be significantly higher than that of the healthy control group (P =0.001). (2) Serum HO-1 levels of the newly diagnosed AHLH (autoimmune-associated HLH) were significantly higher than those in patients with IHLH (infection-associated HLH), LHLH (lymphoma-associated HLH) and healthy controls (P<0.05); (3) The serum HO-1 levels of the clinical remission group were significantly lower than that of the newly diagnosed group (P<0.05);(4) Serum HO-1 levels in newly diagnosed sHLH patients positively correlated with SF (r=0.582, P<0.001), and negatively with ALB (r=-0.308, P=0.045); (5) The receiver operating characteristic curves (ROC) for serum HO-1 levels of patients with sHLH and healthy controls produced a cutoff value at 520.4ng/mL, with its sensitivity and specificity being 90.7% and 100%, respectively. In addition, an optimal cutoff value for HO-1 was 2922.3ng/mL, and Its sensitivity and specificity were 100% and 99.2%, in patients with AHLH and non-AHLH (LHLH+IHLH), separately. Another ROC for SF levels of patients with AHLH and non-AHLH(IHLH+LHLH) yielded an optimal cutoff value of 1189.0 ng/mL and its sensitivity and specificity were 85.7% and 55.5%, respectively.
Summary/Conclusions: Serum HO-1 levels are clinically significant in disease diagnosis, differential diagnosis, evaluating disease activity and treatment outcomes in patients with sHLH.

E1174

RESPIRATORY VIRAL INFECTIONS IN HEMATOLOGIC PATIENTS IN OUR ENVIRONMENT. A RETROSPECTIVE STUDY

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Background: Respiratory viral infections are an important cause of morbidity and mortality in immunocompromised hosts. Hematology patients are especially susceptible due to their underlying disease and the treatments they receive. Rhinovirus, Influenza, Parainfluenza and RSV are some of the most frequent agents. Molecular diagnostic techniques have improved over time, providing fast and accurate results.
Aims: Analize the characteristics of viral respiratory infections in our patients.
Methods: A retrospective, descriptive study including 237 hematologic patients from the University Hospital Complex of Vigo with suspected respiratory viral infection for a period of two years (May 2013-May 2015). We collected 401 PCR samples of respiratory viruses in nasopharyngeal swab and BAL, focusing on the following data: demographic characteristics, underlying disease, comorbidities, symptoms, diagnostic tests, treatment and clinical evolution.
Results: 122 male patients and 115 female, with a median age of 64 years old (range 4-89). 88 underwent HSCT-46 autologous, 42 allogeneic (35 HLA identical, 6 haploidentical and 1 cord blood). 61'5% of the samples were positive for one or more respiratory viruses (63'9% in transplant patients *vs* 59'8% in non-transplant). 89'4% were positive in nasopharyngeal samples, 4'7% in BAL and 5'9% in both. Causal agents are shown in Table 2. 17% of samples were positive for more than one virus, and 15'4% had microbiologic evidence of infection by a different type of microorganism. 30'5% of the infections were considered nosocomial. Concerning those with an infection acquired in the community, 59'6% patients required hospitalization due to the respiratory illness. The symptoms referred by our patients are shown in table 1. 61'4% of the demonstrated viral infections were associated with lymphopenia. Hypogammaglobulinemia was observed in 57%. 30'3% presented comorbidities associated with an increased risk of respiratory infection (underlying heart or lung disease, smoking, HIV positivity, DM or other tumors). Radiologic findings: 50'8% showed a normal X-ray chest image or nonspecific findings, 11'4% pneumonic consolidation and 6'5% signs of atypical infection. 42 patients underwent a bronchoscopy. 12 obtained concordant results with nasopharyngeal samples. 10 patients with a negative nasopharyngeal swab had a viral identification in the BAL. We had an isolated case with different results in each sample (a RSV was found in the nasopharyngeal swab and a coronavirus in the BAL). In 8 patients we found other microorganisms (bacteria, fungus). Influenza virus infections were treated with oseltamivir. CMV infections in transplant recipients received immunoglobulins and ganciclovir. 28% of the RSV were treated with immunoglobulins and ribavirin. In most patients the outcome was good. 5 patients died, in 4 of them the respiratory infection was the main cause of death.

Table 1.

Fever	56%
Cough	71'5%
Rhinorrhea/nasal congestion	49%
Sore throat	13'4%
Dyspnea	8'5%
Pleuritic chest pain	2'4%

Table 2.

Non-transplant patients		Transplant patients	
Rhinovirus	33.1%	Rhinovirus	17%
Parainfluenza	13.9%	Parainfluenza	13.8%
Influenza A	11.9%	RSV	8.5%
RSV	8.6%	Influenza A	8.5%
Influenza B	6.6%	Coronavirus	6.4%
Bocavirus	4%	CMV	6.4%
Coronavirus	4%	Metapneumovirus	5.3%
Enterovirus	2.6%	Influenza B	4.3%
Metapneumovirus	2%	Bocavirus	2.1%
Adenovirus	1.3%	Adenovirus	1.1%
CMV	0.6%	Coinfection by two or more viruses	26.6%
Coinfection by two or more viruses	11.4%		

Summary/Conclusions: Our study confirms that viral respiratory infections are a common cause of morbidity in hematologic patients, even though related mortality was low. The most common symptoms were those of upper airway involvement. 44% of the patients remained afebrile during the course of infection. The presence of other comorbidities, lymphopenia and/or hypogammaglobulinemia seemed to be related with these infections. Although we didn't find an increased rate of infection in transplant recipient patients, it seems that several microorganisms coinfections are more common in this group. Molecular techniques like PCR have replaced previous diagnostic procedures (like virus cultures), demonstrating a higher sensibility. Given the high rate of nosocomial infections, we would like to highlight the importance of vaccinating staff and family, and isolation measures in patients with suspected respiratory infections transmitted by droplets or contact.

E1175

PHASE 1 SUMMARY OF ANF-RHO, A NOVEL PEG-MODIFIED FILGRASTIM INVESTIGATIONAL PRODUCT WITH SUPERIOR PK/PD PROPERTIES THAT MAY PROVIDE IMPROVED CONTROL OF NEUTROPENIA DURING DOSE-DENSE CHEMOTHERAPY

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Background: Anti-Neutropenia Factor - Rho (ANF-RHO) is a new longer acting granulocyte-colony stimulating factor (G-CSF) consisting of a novel pegylated version of recombinant human G-CSF protein. ANF-RHO has distinct biophysical and biological properties that produce an improved pharmacokinetic (PK) and pharmacodynamic (PD) profile as compared to either filgrastim (Neupogen®) or PEGfilgrastim (Neulasta®). *ANF-RHO is not a biosimilar drug product.*
Aims: A Phase 1 clinical study was conducted in healthy volunteers to assess safety and tolerability of ANF-RHO as well as its PK and PD profile.
Methods: The ANF-RHO cohort dosage levels ranged from 5 to50 µg/kg and were compared against both active (PEGfilgrastim) and placebo (saline) comparators. Subcutaneous, single dose treatment with ANF-RHO in ascending doses or PEGfilgrastim at the standard of care dosage (fixed, 6mg) were compared in a randomized, controlled, double-blind study that included peripheral blood absolute neutrophil counts (ANC) and CD34+ stem cell analytical assessments.
Results: Phase 1 clinical safety results were unremarkable, with no severe adverse events in any cohorts. The ANF-RHO PK/PD results in this Phase 1 study were similar to preclinical findings. PK and PD results (ANC and CD34+) were markedly prolonged in the ANF-RHO treatment groups even at the lowest dose. Mean ANC counts for all ANF-RHO treated subjects showed Cmax at 6-7 days, in contrast to 1-2 days for PEGfilgrastim treated subjects. The peak blood levels of ANF-RHO were significantly lower than PEGfilgrastim at all levels tested. Moreover, assessment of the ANC - AUC revealed that ANF-RHO at10 µg/kg was equivalent to PEGfilgrastim at 100 µg/kg, demonstrating an approximately 10-fold potency improvement over PEGfilgrastim in healthy volunteers, with a longer duration of effect of almost two weeks. Peripheral blood CD34+ levels also yielded similar results. ANF-RHO-induced neutrophil counts increased in a stable and prolonged manner following treatment, followed by a slow gradual decline, in contrast to PEGfilgrastim that showed a rapid ANC spike (2 days) and decrease to baseline within 7 days following administration.

Summary/Conclusions: The unique PK/PD of ANF-RHO suggests that a significantly lower dosage may achieve sustained neutrophil levels sufficient to mitigate neutropenia - specifically during the high-risk 7-day period following dose-dense myelosuppressive chemotherapy. Additionally, the sustained and elevated CD34+ counts suggest ANF-RHO may also have applications in stem cell mobilization. The lower effective dosages would be anticipated to reduce the incidence of leukocytosis. Collectively, the increased potency and prolonged pharmacodynamics of ANF-RHO should provide more effective management of hematological malignancies when treating patients at high risk for neutropenia and difficult to mobilize patients or when performing dose-intensification in advanced stage cancer patients.

E1176

INVASIVE PULMONARY ASPERGILLOSIS IN ALLOGENEIC BONE MARROW RECIPIENTS WITH B- THALASSAEMIA MAJOR OR SICKLE CELL DISEASE: INCIDENCE AND RISK FACTORS

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Background: Invasive Pulmonary Aspergillosis (IPA) is a devastating opportunistic infection and remains a significant cause of morbidity and mortality in allogeneic Haematopoietic Stem Cell Transplantation (HSCT) recipients. IPA has been well characterized in adults and in the setting of oncological transplant. No data are available regarding IPA in patients with β- globin gene disorders undergoing bone marrow transplant (BMT).
Aims: To evaluate the incidence and the outcome of IPA among BMT recipients with β-Thalassaemia Major or Sicke cell Anaemia (SCA).
Methods: We evaluated the occurrence, the clinical setting and the clinical outcome of IPA in pediatric patients affected by Thalassaemia major or SCA transplanted at our institution.
Results: A total of 292 consecutive patients (median age 11,6, range 1,9-28 years) with β- globin gene disorders who underwent BMT (232 HLA-identical, related donor; 54 haplotype-identical donor and 4 matched, unrelated donor) were studied. Overall, the incidence of proven or probable IPA was 2.73% (8 out of 292 cases). The median time to onset IPA infection after transplantation was 68 days (range, 13-183 days). In particular, in 5 cases (50%) IPA were diagnosed in the late phase after transplant (day >60) and in 3 cases (50%) were diagnosed during the post-BMT neutropenic period before engraftment. All grade of Graft-*versus*-host-disease (GVHD) was present in 4 (50.0%) of 8 patients with IPA, compared with 97 (37,7%) of 284 patients without fungal infection (P=n.s.). Among 8 cases with IPA an alternative donor (matched unrelated or haplotype-identical) was used in 5 patients (62.5%) compared with 55 cases (19.3%) of 284 recipients without IPA (P=0.003). The infection remained confined in the lung in 7 (87.5%) of 8 IPA cases, in 2 cases surgical intervention was adopted in addition to the adequate systemic anti-fungal medical therapy; only in 1 case the infection was multifocal with CSN involvement. The overall mortality rate for IPA was 0.7% (2 of 292 patients) whereas the IPA attributable mortality rate observed in our population was 25% (2 of 8 cases).
Summary/Conclusions: Our data show that in a population affected by β- globin gene disorders who undergoing allogeneic BMT, the IPA rarely develop (2,7%) and the overall mortality (0.7%) and the IPA attributable mortality rate (25%) is markedly lower then the one observed in the setting of haematological malignancies. In our cohort, a significant risk factor for IPA was the alternative donor.

E1177

EARLY AND RAPID INCREMENT OF CRP AND IL-8 LEVELS TOGETHER WITH FASTER AND DEEPER DECLINING OF LEUKOCYTES PREDICT FOR TYPHLITIS DEVELOPMENT IN TREATED AML PATIENTS

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Background: Neutropenic enterocolitis (NE) or typhlitis is an important and severe complication in patients with acute myeloid leukemia (AML) receiving intensive chemotherapy.
Aims: In this study we evaluated serum C-reactive protein (CRP), interleukin (IL)-8, fecal calprotectin, and oral mucositis score as possible time-dependent predictors of developing NE.
Methods: Adult patients with AML treated with intensive chemotherapy (with cytarabine) were included. Serum IL-8 and CRP levels were measured daily during 14 days after cessation of chemotherapy, as well as calprotectin in the stool. Oral mucositis was also scored daily. Furthermore, CT scan of the abdomen at day 21 was performed to objectively define patients with NE.
Results: In total, 34 patients were included and 11 episodes of NE were diagnosed. All patients with NE received conservative treatment and only one patient with NE died because of refractory Enterococcus faecium sepsis. From day 5 onward (after cessation of chemotherapy), median IL-8 and CRP levels increased more rapidly to significantly higher levels in patients with NE, whereas white blood cell (WBC) counts decreased faster to significantly lower levels as compared to the non-NE patients. Oral mucositis score and fecal calprotectin levels failed to be different in both groups. Cytarabine dose was of no influence. As was corrected for bacteraemia, no differences were seen between both groups.
Summary/Conclusions: Pattern recognition of early and rapidly increasing IL-8 and CRP levels with significantly lower WBC counts after cessation of chemotherapy can identify AML patients developing NE. Early recognition of developing NE can potentially decrease the high mortality rate (25-50%) of this complication.

E1178

PRESEPSIN (SOLUBLE CD14 SUBTYPE) IS ELEVATED IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGICAL MALIGNANCY: USEFULNESS AS ONE ANOTHER BIOMAKER

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Background: ebrile neutropenia (FN) is often observed in patients with hematological malignancy (HEM). Most of causes are bacterial infections in these FN, and many cases respond to empirical antibiotic therapy including an anti-pseudomonas b-lactam agent. Otherwise, it is often difficult to specify the focus and pathogen of infection in FN patients. Presepsin (Pre-SEP) is a subtype of soluble CD14, which is a receptor for lipopolysaccharide (LPS)/LPS-binding protein complexes and is expressed on the myelo-monocytic cell surface. Recently, it has been shown to be a useful biomarker for assessing the severity of sepsis and has come into use in the field of critical care medicine. However, little is known about the biological characteristics of Pre-SEP in FN patients.
Aims: To clarify the usefulness of new biomarker Pre-SEP in the setting of neutropenia, we performed analyses to determine the clinical relevance of Pre-SEP in the diagnosis of bacteremia or other infectious complications as a biomarker for FN patients after or during chemotherapy in HEM.
Methods: We measured the plasma concentration of Pre-SEP, procalcitonin (PCT) and C-reactive protein (CRP) at Day0, 2, 4, 7, 14 and 21 after the onset of FN, and compared them to those of non-febrile neutropenic patients. Furthermore we reviewed other clinical data including bacterial examination and clinical courses, and evaluated the utility of Pre-SEP as biomarker of FN.
Results: The 65 hospitalized FN patients with HEM (AML 16, ALL 12, MDS 14, NHL 13, MM 8, and ATL 2) were treated by IDSA guideline and administered antibacterial or antifungal agents, and the clinical data including Pre-SEP were evaluated. Pre-SEP (Day 0, 2, 4, 7, 14 and 21) was elevated (447 pg/ml (141-2204), 494 pg/ml (151-1975), 344 pg/ml (164-1264), 312 pg/ml (116-1512), 319 pg/ml (86-1512), 278 pg/ml (129-1425), p<0.05, respectively, cut off value: 314 pg/ml) compared to those in non-febrile neutropenic patients (196 pg/ml (97.5-453): median (range), respectively). Otherwise, PCT (Day 0, 2, 4, 7, 14 and 21) was also elevated (0.12 ng/ml (0.04-3.40), 0.13 ng/ml (0.05-1.60), 0.11 ng/ml (0.04-3.54), 0.08 ng/ml (0.03-8.21), 0.11 ng/ml (0.02-2.64), 0.36 ng/ml (0.10-7.75), p<0.05, respectively, cut off value: 0.05 ng/ml) compared to those in non-febrile neutropenic patients (0.175 ng/ml (0.06-1.91): median (range), respectively), and CRP (Day 0, 2, 4, 7, 14 and 21) was also elevated (5.07 mg/dl (0.19-27.83), 3.19 mg/dl (0.30-20.54), 2.26 mg/dl (0.15-34.01), 1.33 mg/dl (0.10-29.48), 0.28 mg/dl (0.10-22.49), 0.36 mg/dl (0.10-7.75), p<0.05, respectively, cut off value: 0.3 mg/dl) compared to those in non-febrile neutropenic patients (0.625 mg/dl (0.11-8.72): median (range), respectively). There was no relation between Pre-SEP value and bacterial examination data including blood cultures. Almost FN cases responded empirical antibacterial therapy, and elevated Pre-SEP, PCT and CRP values decreased again accompanied by defervescence. However, some cases of persistent remarkably elevated Pre-SEP cases ware observed, and these cases had very poor prognosis. The 5 of 9 cases with remarkably high Pre-SEP value (>1000 pg/ml) at both day7 and 14, or both day14 and 21 were dead by severe infection related events within 4 weeks, otherwise all other 56 cases than the above recovered from infection events and survived (p<0.05, respectively).
Summary/Conclusions: These data suggested that Pre-SEP is elevated due to active infection events in FN patients in spite of conditions being remarkably decreased neutrophil and monocyte. Pre-SEP is useful as a biomarker of infection in FN patients, as well as PCT or CRP.

E1179

INVASIVE ASPEGILLOSIS IN PATIENTS WITH ACUTE LEUKEMIAS: PROGNOSTIC VALUE OF LABORATORY BIOMARKERS DEFINING HIGH RISK PATIENTS

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Background: Invasive fungal diseases, especially invasive aspergillosis (IA) are the major cause of morbidity and mortality in patients with acute leukemias. Detection of early laboratory biomarkers: galactomannan (GM) and anti-Aspergillus antibodies IgM and IgG (Anti-A IgM, IgG) contributes to early recognition, as well as to the monitoring during aspergillosis treatment.
Aims: The aim of study was to evaluate clinical utility and prognostic value of "non- culture" methods: GM assay and anti-A (IgM and IgG) antibodies for early diagnosis of aspergillosis in patients with acute leukemias.
Methods: The study included 102 patients, 80 pts with AML and 22 pts with ALL (52 male/50 female, median age 51 years, range 19-73 years) hospitalized in the Clinic of Hematology during period of time from January 2012 until December 2015. All pts were treated with intensive chemotherapy according to the regimens recommended for the treatment AML and ALL, consequently developing febrile neutropenia, with median duration of 12 days (7-23days). Serum GM level were measured twice a weekly using the Platelia Aspergillus enzime immunoassay (EIA) kit (Biorad, France), applying the cutoff value of 0,5 as positivity. Anti-A IgM/anti-A IgG were measured using commercial tests (Serion ELISA classis, Virion/Serion, Germany). The concentration of antibodies above 12U/ml was considered as positive finding. In all patients routine radiological (high resolution CT scaning) was performed, as well as the biological monitoring consisted of hematological, biochemical and cytopathological tests. Median time of follow-up was 294 days (range 15-1300 days) and treatment outcome was assesed on the 100. day of follow-up. Statistical analysis was performed using IBM SPSS Statistics version 21.0.
Results: In analyzed group of 102 patients, there were 20 possible, 54 probable and 4 proven cases of IA, while in the last 24 pts were detected only positive GM or anti-A IgM/anti-A IgG. During 100 days of follow-up, 39 patients died while 63 patients had survival longer than 100 days. GM index, as well as the anti-A (IgM and IgG) antibodies, was defined as ratio of total number of positive findings and total number of performed tests. Predictive value of GM index was maintained after Cox regression adjustment. In multivariate analysis, GM index was an independent survival predictor (p=0.012). Anti-A IgM and anti – A IgG were not statisticaly significant predictor of survival (p=0,224 and p=0,987).
Summary/Conclusions: The GM assay is a useful tool for monitoring IA in patients with acute leukemias. Persistent GM positivity is associated with death and treatment failure, whereas a successful outcome is associated with GM negativity. Diagnostic and prognostic value of level of anti-Aspergillus antibodies is not documented and more studies, with more patients, are needed to define better the role of these biomarkers.

E1180

HEPATITIS B IMMUNE STATUS IN CHILDREN TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Hepatitis B virus (HBV) infection occurs worldwide, with the highest HBsAg carrier rates found in developing countries, where infection is expected to occur in infancy and childhood. Immunization with hepatitis B vaccine is the most effective way of preventing HBV infection and its consequences, especially in endemic areas. Hepatitis B vaccines are highly immunogenic, inducing a protective anti-HBs antibody titer (≥10 mIU/mL), in more than 95% of healthy children and young adult. Partial or complete loss of protective antibody titers against vaccine preventable diseases makes leukemic children more susceptible to infections. Hemato-oncological patients are considered at risk for either acute acquired HBV infection or HBV reactivation.
Aims: evaluate the immune status to HB vaccine in children treated for acute lymphoblastic leukemia (ALL) after completion of chemotherapy and determine factors affecting anti-HBs titer in those children.
Methods: We evaluated HBV immune status among 76 children who have been treated for ALL, 43 males and 33 females, their age ranged from 4.9 to 16.3 years with a mean of 11.19 years. They were consecutively recruited from the hematology oncology clinic at Alexandria University Children's Hospital. One hundred healthy children were also included as controls, 51 males and 49 females, age ranged from two to 12 years with a mean of 7 years. All have been previously vaccinated with 3 doses of hepatitis B vaccine at 2, 4 and 6 months of age. Anti-HBs titer was assessed in all leukemic children and controls by ELISA, and concentrations<10.0 mIU/mL were considered negative for anti-HBs (non-immune).
Results: Significantly more leukemic children were non-immune (51.3%) compared to only (15%) in the control group, (p<.0001). Moreover, mean anti-HBs titer in normal children was significantly higher than in leukemic children, (61.57 *vs* 42.79 mIU/mL, p=.038). When comparing immune and non-immune leukemic children according to age at diagnosis of leukemia, it was significantly higher in non-immune than in immune ones, (6±2.9 *vs* 4.6±1.9 respectively, p=.017). Using logistic regression analysis, both previous chemotherapy and age were identified as independent variables affecting immunity to HB vaccine, (p=.032, p=.001 respectively). An equation resulted from this analysis to predict the probability of non-immune status: Y=-3.589+[0.928×disease status]+[0.243×age (years)], where disease status is entered as "0" if normal child and as "1" if child has been previously treated for leukemia. The diagnostic performance of the new equation using Receiver Operating Characteristic (ROC) curve analysis was fair to good tool in discrimination between immune and non-immune children, (AUC=0.782, 95% CI 0.713-0.851, p<.0001). Children with value of ≤0.286 will be considered immune with negative predictive value of 86%. On the other hand, children having a value >0.656, are considered to be non-immune with a positive predictive value of 78.9%.
Summary/Conclusions: Several variables affect immunity of children to HB vaccine. Chemotherapy affects immunity to HB vaccine in children treated for ALL regardless the type of leukemia, the intensity of the protocol used for treatment, and the post chemotherapy interval. A novel equation was developed to predict non-immune state to HBV in children using both his age and his disease status thus help taking a decision concerning revaccination of these patients with minimal costs and time consumption.

E1181

IMPACT OF RESPIRATORY VIRUSES IN IMMUNOCOMPROMISED PEDIATRIC HEMATOLOGY PATIENTS

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Background: Respiratory viral infections are very common in children. Children immunocompromised (IC) by treatment for haematological malignancies or following stem cell transplant (SCT) are at increased risk, although the morbidity and mortality are not well understood.
Aims: To assess the impact of respiratory viruses in IC pediatric hematology patients aged 0-18 analysed by diagnosis, intensity of treatment, rates of admission, Pediatric Intensive Care Unit (PICU) and outcomes, including the effect of influenza H1N1 pandemic (swine flu).
Methods: Retrospective review of PCR positive viral isolates of respiratory secretions of children with hematological diseases or post SCT, sent between Jan 2009-Dec 2014. Review of patient records with regards to concurrent neutropenia, blood culture growth, admission, PICU and outcome.
Results: 952 specimens from respiratory secretions were sent, 465 were positive (48.8%), with total 528 viral isolates (63 specimens had multiple isolates) representing 171 patients. 232/465 (50%) were from nose swabs; 134/465 (29%) were nose and throat swabs; 71 /465 (15%) were throat swabs; 9/465 (1.9%) were from bronchio-alveolar lavage. Patients: 141 acute leukemias (AL), 27 SCT, 3 other haematological diseases. 96 boys, 75 girls. Mean age at diagnosis: 4.9 years. Mean age at sample: 6 years. Viral isolates overall: Of the total 528 viral isolates the majority, 52%, were rhinovirus, 14.2% Respiratory Syncitial Virus (RSV), 6.8% parainfluenza 3, 4.6% adenovirus, 4.6% parainfluenza 1, 4.2% metapneumovirus, 3.6% parainfluenza 4, 3.4% influenza B and 3.4% H1N1. 385/465 (83%) positive swabs were from AL patients (mean age at sample 6 years) 70/465 (15%) from SCT patients (mean age at sample 6.8 years) and 10/465 from patients with other disorders (mean age at sample 1.7 years). Treatment intensity: positive results in AL patients occurred at the following phases of treatment 10/385 induction; 164/385 at other phases of treatment; 211/385 in maintenance. In the UK males with ALL are treated for 3 years (compared with 2 years for girls). Of the total of 211 positive samples taken in maintenance, 42 (20%) were from boys in their 3rd year of treatment and resulted in 24 admissions. H1N1 was solated from 18 patients associated with 14 admissions. 1 PICU and subsequent death. Mean age at sample 8.18. 10 maintenance AL, 6 other phases AL and 2 SCT. (4 mixed isolates: 2 rhinovirus, 1 parainfluenza 1 and 1 RSV.) Overall, 301 admissions at the time of respiratory viral isolate. Of the 528 viral isolates, 64.7% during admission (mean age 5.7 years). 32.6% with concurrent neutropenia and only 1.9% with blood culture growth. 10 patients required PICU during their admission, 8 of which were all/partly attributed to the respiratory virus. 4 patients were in AL induction (1 died with RSV and parainfluenza 4). 1 in AL consolidation. 1 with relapsed ALL who died with rhinovirus and metapneumovirus. 2 were SCT patients (1 died with rhinovirus).
Summary/Conclusions: There was a surprisingly high (48.8%) positive rate in the respiratory secretion samples. 64.7% of positive viral isolates coincided with admission. Respiratory viruses are a cause of significant morbidity, including hospital admission and mortality. 38% of patients admitted to PICU all or partly as a consequence of a respiratory virus; 3 patients died. Rhinovirus, often dismissed as irrelevant, was present in 75% patients admitted to PICU and 33% PICU deaths. Although the data reflects a time of H1N1 outbreak: it was isolated in few patients, however 78% of patients with H1N1 isolate required admission and 1 patient died.

E1182

VORICONAZOLE AS PRIMARY PROPHYLAXIS OF FUNGAL INFECTIONS IN HIGH-RISK HEMATOLOGIC PATIENTS: NO NEED FOR EMPIRICAL ANTI-FUNGAL THERAPY

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Background: Invasive fungal infections (IFI), predominantly aspergillosis and candidiasis, remain an important cause of morbidity and mortality following high-dose chemotherapy and allogeneic stem cell transplantation (alloSCT).
Aims: The primary endpoint of this study was to analysed the efficacy and safety of voriconazole primary antifungal prophylaxis in combination with a pre-emptive antifungal strategy in high-risk hematologic patients.
Methods: This is a retrospective, observational and non-interventional study conducted from March 2009 until February 2012 at the Complejo Hospitalario de Navarra. Patients could receive voriconazole prophylaxis at different periods of time in the course of their disease if they were at high-risk of IFI: following intensive chemotherapy (n=24), allogeneic stem cell transplantation (n=43) or receiving high-dose corticosteroids treatment for chronic graft *versus* host disease (n=11). If patients developed prolonged unresponsive fever after 5 days of broad-spectrum antibacterials and/or galactomannan antigen was positive, a high-resolution computed tomography (CT) was performed. If new pulmonary infiltrates were present, a bronchoscopy with BAL was performed. Only patients with abnormal CT findings, positive fungal isolates on BAL or positive GM antigen started on pre-emptive antifungal treatment and changed their anti-fungal treatment to liposomal amphotericin B or an equinocandin. Otherwise, patients remained on voriconazole prophylaxis despite continuing fever. The cumulative incidence (CI) of proven/probable IFI, pre-emptive antifungal treatment and adverse events were estimated by Kaplan-Meier survival curves.
Results: A total of 78 prophylactic voriconazole periods were analysed in a total of 44 hematologic patients. Only one patient developed a proven/probable IFI (a breakthrough candidemia) in the allogeneic stem cell transplantation group. No patient developed proven/probable IFI in the chemotherapy neither in the chronic graft *versus* host disease group. The cumulative incidence of pre-emptive antifungal treatment was 12.5% and 12.3% for the chemotherapy and the allogeneic stem cell transplantation respectively. The most frequent adverse event was hepatic toxicity with a cumulative incidence of 10.7%, 25.8% and 27.3% in the chemotherapy, the allogeneic stem cell transplantation and the chronic graft *versus* host disease respectively. No hepatic failure was registered and hepatic toxicity was reversible after drug discontinuation or transient interruption.
Summary/Conclusions: Voriconazole is a safe and an effective antifungal agent for prophylaxis of IFI in high-risk hematologic patients with a success rate of 97.4% provided that frequent liver monitoring and short intermittent interruptions are performed. Voriconazole prophylaxis allows for strategies with no empirical use of systemic anti-fungals.

E1183

RANDOMISED COMPARISON OF PALONOSETRON AND ONDANSETRON BOTH COMBINED WITH DEXAMETHASONE IN PREVENTION OF EMESIS IN LYMPHOMA PATIENTS RECEIVING MODERATELY EMETOGENIC REGIMEN BENDAMUSTINE AND RITUXIMAB

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Background: Palonosetron is a highly selective, second-generation 5-HT3 receptor antagonist with a prolonged half-life (40 hours) which makes it attractive in prevention of delayed nausea and vomiting. Bendamustine, a moderately emetogenic cytostatic drug is becoming increasingly used in treatment of lymphoid malignancies.
Aims: A randomized comparison of palonosetron and ondansetron both combined with dexamethasone in prevention of chemotherapy induced nausea and vomiting (CINV) in adult patients, receiving bendamustine and rituximab chemoimmunotherapy.
Methods: Data of 1st interim analysis are presented. The study included 23 patients with median age of 67 years (range 31 - 82 yrs), 13 males and 10 females. The patients did not received chemotherapy at least 6 months prior to inclusion. Ten patients had follicular lymphoma, 8-chronic lymphocytic leukemia, 6-marginal zone and other types of indolent lymphomas. The BR regimen consisted of rituximab 375 mg/m2 on day 1 and bendamustine 90 mg/m2 on days 1 and 2. Patients were randomly assigned to receive either a single intravenous bolus dose of palonosetron (0, 25 mg) on day 1 or ondansetron (8 mg on days 1 and 2 of each cycle) along with dexamethasone 30 min before administration of chemotherapy. CINV was assessed during 1st cycle of chemotherapy using MASCC Antiemesis Tool. Frequency and severity of acute (within 24 hours) and delayed (24-120 hours) emetogenic reactions were registered.
Results: Eleven patients received palonosetron and 12 ondansetron. Groups were comparable with regard to emetogenic risk factors including sex, age, kinetosis and alcohol use. Complete response (no emetic episodes and no rescue therapy) in acute phase was observed in 100% of patients receiving palonisetron and in 10/12 (83%) of patients receiving ondansetron. Complete response in delayed phase was achieved in all patients in palonosetron group and in 11/12 (92%) in ondansetron group. Complete protection (no emetic episodes, no rescue therapy, and no significant nausea [Likert scale 2 or less]) in acute phase was achieved in 10/11 (91%) patients in palonosetron group and in 5/12 (41%) in ondansetron group. In delayed phase complete protection of CINV was reported by 10/11 (91%) of patients receiving palonosetron and 6/12 (50%) of patients receiving ondansetron.
Summary/Conclusions: The frequency of delayed nausea in patients, receiving bendamustine is probably underestimated. Prevention of bendamustine induced nausea and vomiting with palonosetron merits further investigation.

E1184

EFFICACY AND SAFETY OF MICAFUNGIN ON THE MANAGEMENT OF INVASIVE FUNGAL INFECTIONS AMONG PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AT TERTIARY HOSPITALS IN GREECE- THE ASPIRE STUDY

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Background: Immunocompromised patients, especially those with cancer or underling hematological malignancies and patients who undergo hematopoietic stem cell transplantation (HSCT), are at high risk for invasive fungal infections, which are often life threatening. Echinocandins are recommended as first -line treatment for the management of invasive candidiasis/candidemia from the recent ESCMID guidelines. However, there are relatively limited data for the daily clinical practice regarding the use of micafungin at hematological settings in Greece.
Aims: The aim of the study was to evaluate the efficacy and safety of micafungin administration in standard clinical practice among patients with hematological malignancies, including patients who underwent HSCT.
Methods: This was a multicenter, non interventional, prospective cohort study conducted in 10 research sites in Greece. Adult hospitalized patients with hematological malignancies, including recipients of HSCT, were enrolled in the study between 29 Jun 2013 and 31 Mar 2014. Eligible patients were intended to receive micafungin which was prescribed as per standard clinical practice according to its label, while their assignment to a particular therapeutic strategy was not decided in advance by the protocol. The observational study period lasted until clinical outcome was recorded. Patients were classified according to their clinical presentation at baseline using the revised EORTC criteria (2008).
Results: A total of 143 patients were enrolled. Of them, 122 patients had a hematological malignancy (85.3%), 4 underwent HSCT (2.8%) and 17 had hematological malignancy and HSCT (11.9%). Acute myelogenous leukemia (n=58, 40.6%) was the most common hematological disorder, while just over half of these patients were newly diagnosed (51%). Of the 21 patients in the HSCT group, 12 underwent autologous and 9 allogeneic HSCT. On treatment initiation, 78 patients had fever (54.5%) and 85 patients had neutropenia (59.4%) respectively, while 58 patients had previous febrile episodes(40.6%). In addition, 112 patients had received systemic antibiotics (78.3%), 64 patients had received corticosteroids (44.8%) and 69 patients other immunosuppressive agents (48.3%) as prior treatment. At baseline, 74 patients of the study received micafungin as prophylaxis while 69 received treatment for possible (52), probable (12) and proven (5) fungal infection. Micafungin was administered as monotherapy in 97.6% of patients and as first line treatment in 75.5% of patients, while the mean duration of micafungin administration was 14.2 days (14.1 days for prophylaxis and 14.3 days for treatment). The success rates of micafungin were comparable for prophylaxis, possible and proven treatment strategies (90.5% *vs* 96.2% *vs* 100% respectively), while for probable treatment strategy was 75%. It is worth noting that in the latest group, treatment was considered as success in 9 patients, as failure in one patient and in two patients treatment definition as success or failure was not available. Treatment success was defined by the Investigators based on the EORTC/MSG criteria and their judgment as applied for daily clinical decision-making. 26 patients (18.2%) discontinued micafungin mainly due to limited availability of therapy at study sites, patient discharge or safety events not related to the study treatment. In total, 30 adverse events were reported throughout the study, including 8 Serious Adverse Events (deaths) that were not related to micafungin and 22 cases of special situations. One case of lack of efficacy was reported.
Summary/Conclusions: Micafungin was effective and well tolerated in patients with hematological malignancies, including patients who underwent HSCT. To this end, micafungin appears to be a valuable treatment option for the management of fungal infections in hematological settings.

E1185

ANTIRETROVIRAL THERAPY AND CHEMOTHERAPY IN PATIENTS WITH HIV INFECTION AND HAEMATOLOGICAL DISEASE - TOXICITY ASSESSMENT

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Background: Haematological malignancies, especially lymphoproliferative diseases, are a common manifestation of infection with human immunodeficiency virus (HIV). With highly active antiretroviral therapy (HAART) the survival of infected patients with hematologic disease has increased, and in most cases, overlapping the general population. Concomitant use of HAART and chemotherapy (CT) has been advocated for enhancing the immune response.
Aims: Characterization of toxicity associated with the concomitant use of HAART and CT and its impact on prognosis.
Methods: Descriptive, observational, retrospective study to evaluate patients with haematological malignancies associated with HIV infection and under HAART during CT, admitted between January 2011 and August 2015. The data was analysed using SPSS V17.0 program.
Results: We evaluated 24 patients, 19 males. The median age was 45.5 years (27-61). 22 patients had HIV-1, 1 patient HIV-2 and 1 patient co-infected with HIV-1 and HIV-2. All patients were on ARVT, 47.8% with regimens based on two nucleoside reverse transcriptase inhibitors (NRTI) and boosted protease inhibitor (PI/ r) and 34.8% with 2 NRTI-based regimens and integrase inhibitor (II). The most common diagnosis was Burkitt's lymphoma (29.2%), followed by diffuse large B-cell lymphoma (25%) and Hodgkin Disease (16.7%). The CD4 cell count was below 250cells/ mcL in 10 patients and none had negative viral load at the start of CT. We documented 120 complications, most infectious (50.8%). Organ toxicity was revealed in 11 patients, of which 35% had renal and 20% liver toxicity. Of the 7 patients who experienced renal toxicity, 6 were treated with regimens containing tenofovir. Of the 4 patients who experienced liver toxicity, 2 were treated with PI/ r and 2 with II. It was necessary to suspend HAART in 5 patients. The scheme has been changed in two. The median of administered chemotherapy cycles was 4 (1-8) with an overall response rate of 77.8% (n=14), 50% (n=9) complete response, 27.8% (n=5) partial response, 4 patients were refractory, and the remaining (n=6) were not evaluated. The overall survival at 2 years was 36.4% (4/11).
Summary/Conclusions: The use of HAART associated with CT is not absent of toxicities, sometimes forcing its suspension. The new generation of antiretroviral drugs, with less pharmacokinetic interactions with antineoplastic drugs, seems to contribute to better compliance with HAART. The impact of this association should continue to be evaluated in further studies with larger samples.

E1186

RE-EVALUATING THE IMPACT OF INVASIVE ASPERGILLOSIS ON THE PROGNOSIS OF ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Invasive aspergillosis (IA) is a common complication of neutropenic and immunocompromised status occuring during acute myeloid leukemia (AML) management. Its prognosis has long been dismal, causing an important additional mortality in hematology patients. However, both diagnostic procedures and therapeutics have much improved, through galactomannan dosing, high resolution CT-scan and voriconazole avaibility.
Aims: We intended to assess frequency and prognostic impact of IA in a recent cohort of patients receiving high dose chemotherapy for AML.
Methods: We have therefore conducted a monocentric retrospective observational study on all consecutive patients diagnosed with an AML between 2008 and 2012 in Strasbourg University Hospital.
Results: Among 285 patients diagnosed with AML between 2008 and 2012 in our hospital, 154 received an intensive chemotherapy, with at least one induction course. Among them, 46 patients (29,9%) have been diagnosed with IA (2 proven IA, 25 probable IA, 19 possible IA). Twenty-eight (18,2%) of these infections occured following the first induction course (2 proven IA, 17 probable IA, 9 possible IA). Both groups of patients, whether they had an IA or not, had similar hematological prognosis as assessed by ELN scoring. Almost all IA patients (44, *i.e.* 96%) received voriconazole as a treatment. At the closing of our analysis, 17 patients diagnosed with an IA were still alive, with a follow-up ranging from 32 months to 93 months (median 64 months). The overall survival (OS) analysis of these patients showed no significant difference compared to patients without IA (p=0.94). Five-year survival of the two groups was respectively 40,6% and 41%.
Summary/Conclusions: With an incidence of IA close to recent data concerning patients treated for AML, our monocentric retrospective study on long-term survival of AML and IA patients shows no significant difference in terms of overall survival. These encouraging results shall now be confirmed on a larger cohort to conclude that the impact of IA on the prognosis of AML patients has been controlled in the era of diagnostic and therapeutic improvement.

E1187

PRIMARY ANTIFUNGAL PROPHYLAXIS (PAP) WITH MICAFUNGIN AT THE DOSE 50MG PER DAY IN ADULT PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) DURING INDUCTION CHEMOTHERAPY: THE "REAL-LIFE" EVALUATION

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Background: Invasive fungal diseases (IFDs) during treatment of acute leukaemias are associated with a high morbidity and mortality. While in patients with acute myelogenous leukaemia PAP during induction treatment is well established (mainly with posaconazole), standardized PAP in patients with ALL is lacking. The major obstacle represents the risk of interactions of mould active azoles with chemotherapy agents during the intensive treatment of ALL. Thus the choice of PAP in these patients is difficult and they frequently remain without mould active PAP.
Aims: Evaluation of the efficacy and safety of micafungin PAP (dose 50mg/day) in adult patients with ALL during the induction chemotherapy.
Methods: A retrospective analysis of patients with ALL (or T-lymphoblastic lymphoma or Burkitt's leukaemia/lymphoma) treated with intensive ALL (or ALL-like) protocols in 2 tertiary care haematological centres in the Czech Republic receiving PAP with micafungin. Patients received micafungin (50 mg/day) from the start of induction chemotherapy till the neutrophils recovery. EORTC/MSG 2008 criteria for IFD diagnosis were used. CCTCAE v 4.0 criteria were used for evaluation of safety of PAP.
Results: Forty- nine patients received micafungin PAP 50 mg/day during induction chemotherapy of ALL between 2012 and 2015. ALL-CELL 2012 Junior protocol was used in 39 patients, GMALL B-ALL/NHL 2002 protocol in 3 patients and ALL-CELL 2012 elderly or EWALL Elderly protocol in 7 patients. The mean length of micafungin prophylaxis was 22 days (2-80 days). Antifungal prophylaxis failed in 2/49 (4.1%) patients, who developed IFD. Both patients developed proven IFD, no episode of probable IFD occurred. Both patients with proven IFD were successfully treated with combination of antifungal agents. One proven IFD had a form of invasive pulmonary aspergillosis (histologically proven and confirmed by PCR identification, culture negative) and occurred only early after the initiation of PAP (day 4). Second proven IFD represented disseminated *Geotrichum capitatum* infection (blood culture, liver and spleen) with MIC 32 µg/ml for echinocandins. Apart from the 2 IFD episodes, 10/49 (20.4%) patients developed pulmonary infiltrates-3/49 (6.1%) specific for IFD based on EORTC/MSG 2008 criteria (without any microbiological criteria and thus fulfilling criteria for possible IFD) and 7/49 (14.3%) non-specific pulmonary infiltrates.
Empirical antifungal therapy was initiated in 10/49 patients (20.4%). As an empirical therapy micafungin at dose 100mg/day was used in 8/10 patients, voriconazole in 1/10 patients and lipid based amphotericin B in 1/10 patient.
All 49/49 (100%) patients developed liver function test (LFT) elevation at least grade 1-2. However 63% patients had pre-existing liver test elevations. None of these LFT elevations were concluded as probably or definitely associated with micafungin and none required an interruption of micafungin prophylaxis.
Summary/Conclusions: Our real-life data proved efficacy of micafungin 50 mg/day as PAP in this population of patients at high risk of IFD, but also at high risk of drug-drug interactions. Only 2 (4.1%) patients developed breakthrough IFD, however in 1 case in a very early stage of PAP. The elevation of LFTs represented a major limitation of the prophylaxis, but was most likely associated with a therapy of ALL and did not require an interruption of micafungin PAP. Echinocandine-based PAP could represent a safe option for prophylaxis of this difficult to treat population of patients with ALL.

E1188

COMPARISON OF DE-ESCALATION AND NON-DE-ESCALATION EMPIRICAL THERAPY FOR CONTROLLING INFECTION IN PATIENTS WITH SEVERE APLASTIC ANEMIA TREATED WITH ANTITHYMOCYTE GLOBULIN

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Background: Aplastic anemia (AA) is a bone marrow failure syndrome, featuring pancytopenia, characterized by the reduction in hematopoietic stem and

progenitor cells.combined immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine A (CsA) is a standard first-line treatment for SAA.However, neutropenia caused by SAA and the immunosuppressive effect of ATG could increase the risk of infections.
Aims: To compare De-escalation and Non-de-escalation Empirical Therapy for Controlling Infection in Patients with Severe Aplastic Anemia Treated with Antithymocyte Globulin.
Methods: We compared the efficacy of de-escalation and non-de-escalation empirical therapy for controlling microbiological culture-confirmed infections in 87 patients with severe aplastic anemia (SAA) treated with antithymocyte globulin (ATG) from 2006 to 2015 in our center.
Results: The response rate at 7 days and 30 days in de-escalation group was significantly higher than that in non-de-escalation group (60.32% *vs* 25.00%, p=0.003)(79.37% *vs* 58.33%, p=0.047). After anti-infection treatment, more patients' absolute neutrophil count(ANC) increased in de-escalation group than that in non-de-escalation (76.19% *vs* 45.83%, p=0.007), though no significant difference of ANC was found between two groups. Patients in de-escalation group had better survival outcome at 90 days (p=0.003). In de-escalation group, the response rate of patients with granulocyte transfusions at 7 days was higher than that of patients without granulocyte transfusions (78.26% *vs* 50%, p=0.027).
Summary/Conclusions: We concluded that early administration of broad-spectrum antibiotics pending the results of microbiological cultures combined with a commitment to change to narrow-spectrum antibiotics should be recommended for controlling infections in SAA patients treated with ATG and granulocyte transfusions might be an adjunctive therapy to improve the response rate.

E1189

A RETROSPECTIVE ANALYSIS ABOUT STENOTROPHOMONAS MALTOPHILIA SEPSIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCY: A SINGLE CENTER STUDY FOR 12 YEARS

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Background: Febrile neutropenia (FN) is often observed in hematological malignancy. Most of FN cases are infections by various causative agents rarely including antibiotic-resistant bacteria or low virulent bacilli. Stenotrophomonas maltophiria (SM) is a low virulent bacillus widely existing in the environment, a causative agent of opportunistic infection and a rare pathogenic bacteria in FN. Recently, some cases of sepsis by SM were reported in FN with hematological malignancy, and most of them had often very poor prognosis.
Aims: To clarify the prognosis of SM sepsis in FN patients of hematological malignancy, we should perform the surveillance of SM infections sufficiently.
Methods: We reviewed all blood culture data from our single institute from 2004 to 2015, and evaluated retrospectively clinical data of sepsis cases by SM in FN of hematological malignancy.
Results: Total 3490 events of blood cultures (BC) were performed just before the first line antibiotic agent administration was started for FN patients in the Hematology-Oncology unit of our institute from 2004 to 2015. In the 16.4% of these BC events, some kinds of bacteria or fungus were detected. The frequency of BC positive cases for SM were rarely observed in 2.3% of all BC events, and total 15 cases of SM sepsis were diagnosed. The primary diagnosis of hematological disease in those SM sepsis cases were NHL(N=5), AML(N=4), ALL(N=5) and CML(N=1), and almost all of these disease were relapse or refractory cases with many treatments for long duration. SMs detected in 15 cases were widely resistant to many antibiotic agents. Fourteen cases died of MOF, and 9 cases died within 7 days after the finding of BC in spite of intensive care including administration of sensitive antibiotic therapies.
Summary/Conclusions: The prognosis of SM sepsis in patients with hematological disease was extremely poor. Surveillance for SM infection in high-risk patients is essential, and rapid administrations of appropriate antibiotics should be considered.

Myelodysplastic syndromes - Biology

E1190

RIGOSERTIB (RIGO) COMBINED WITH AZACITIDINE (AZA) IN VITRO & CLINICAL: MODULATES EPIGENETIC EVENTS AND OVERCOMES CLINICAL RESISTANCE TO HYPOMETHYLATING AGENTS (HMA) IN MYELODYSPLASTIC SYNDROMES (MDS)

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Background: MDS is a challenging disease to treat due to intricate complexities at the genetic, molecular and epigenetic levels. Epigenetic based therapy with the HMA, AZA is the standard of care for patients (pts) with MDS with higher-risk disease. Treatment alters the natural history of MDS with increased survival, reduced risk of transformation to AML and improved hematopoiesis. However, all pts ultimately fail treatment due to either primary or secondary resistance. Initial results of an ongoing Phase I/II study of AZA combined with RIGO, an allosteric "ras mimetic" that binds to the Ras Binding Domain impacting pathways including ras, raf and PI3 Kinase, in pts with MDS demonstrated an overall response rate of 77%; and 64% in pts following HMA failure (Navada et al ASH 2015).
Aims: 1) To determine the in vitro effects of rigosertib alone and in combination with azacitidine on epigenetic signaling in MDS and AML cell lines and on patient samples treated with the combination *in vivo*.
Methods: We investigated the in vitro effects of RIGO alone or in combination on two cell lines: AML (BW90), MDS (MDS-L) and patient samples on global histone post-translational modifications (PTMs) including including methylation (H3K4me3, H3K4me2, H3K27me3, and H3K27me2) and acetylation (H3K9ac, & H3K18ac) levels.
Results: In vitro effects of RIGO alone or in combination on two cell lines: AML (BW90), MDS (MDS-L) altered global histone post-translational modifications (PTMs) including methylation (H3K4me3, H3K4me2, H3K27me3, and H3K27me2) and acetylation (H3K9ac, & H3K18ac) levels associated with transcriptional activation or repression in both cell lines, but effects were more pronounced in combination with AZA. Furthermore, Q-PCR studies demonstrated that individual treatment of BW90 and MDS-L with RIGO or sequential treatment with AZA (AZA/RIGO or RIGO/AZA) altered the class I, IIa, IIb, and IV histone deacetylases (HDACs), DNA methyl transferases (DNMT1, 3a and 3b) and chromatin remodeler (KDM2a, SET1, JMJD3 and LRWD1) transcript marks in cellular context suggesting that RIGO potentially acts as a chromatin modifying agent (CMA), in both cell lines. Treatment with RIGO either alone or combined with AZA differentially governed cellular proliferation, cell cycle arrest, apoptosis, histone PTMs, altered expression of cell cycle related proteins, and inactivation of the PI3/AKT signaling pathway in a cell specific manner. Similar effects were seen on patient samples. We further investigated the *in vivo* effects of RIGO combined with AZA on pt bone marrow samples obtained prior to and after one cycle of RIGO and AZA. Interestingly, the histone PTMs, HDACs and chromatin remodelers were significantly altered in the pts BM with increased apoptosis after AZA/RIGO treatment.
Summary/Conclusions: RIGO combined with AZA can govern epigenetic mechanisms and overcome clinical resistance to AZA in MDS pts. RIGO potentially functions as a CMA, in combination with AZA and significantly modifies global histone repression and activation marks suggesting that the combination may overcome AZA resistance through epigenetic remodeling of chromatin structure. Additional studies are underway.

E1191

PREVALENCE AND DYNAMICS OF LEUKEMIA-ASSOCIATED MUTATIONS IN ELDERLY INDIVIDUALS WITHOUT HEMATOLOGIC DISORDERS

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Background: Aging of the hematopoietic system is accompanied by an increased incidence of myeloid disorders such as myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Recent data indicates that aberrations in the aged hematopoietic stem cell (HSC) compartment lead to maintenance or expansion of myeloid cells, while the number of lymphoid progenitors stagnates or declines. HSCs may be particularly predisposed to acquiring genetic aberrations since they are the only persistent cells of the blood system. Such mutations may provide enhanced survival, proliferation and self-renewal capabilities, and lead to the propagation of a cell clone.
Aims: Clonal hematopoiesis is frequently observed in elderly people and may represent a premalignant condition. Clonal HSCs acquiring additional mutations may then transform to cancer cells and give rise to a number of myeloid disor-

ders and leukemia. Evidence suggests that genetic alterations found in patients with myeloid malignancies may also be causative for induction of clonal hematopoiesis. Given these circumstances, discrimination between age-associated and leukemia-associated mutations is of major clinical importance, with potential applications in future prevention, therapy and prognosis.
Methods: In order to investigate the prevalence and dynamics of genetic alterations among elderly individuals without hematologic/oncologic disorders, genotyping of the 30 most commonly mutated leukemia-associated genes was performed. For this purpose peripheral blood DNA and buccal cells from a cohort of 50 elderly people (29 women; median age 84 years; range 80-90 years) were collected and analyzed by targeted deep next-generation sequencing (NGS). All mutations were validated in separate runs using genomic DNA isolated from leukocytes and buccal cells to confirm the somatic origin of mutations.
Results: A total of 16 somatic mutations in leukemia-associated genes were identified in 13 of 50 (26%) hematologically normal elderly individuals. One subject presented with two, another subject with three different mutations. Ten of 16 mutations (63%) affected epigenetic modifier genes (*DNMT3A*, n=8; *TET2*, n=1; *IDH2*, n=1). Four somatic mutations affected genes involved in the RNA splicing machinery (*SRSF2*, n=2; *SF3B1*, n=1; *U2AF1*, n=1). Mutations in *TP53* and *NRAS* were identified in two individuals. All but one mutation were missense mutations with cytosine to thymine transitions being the most common base pair change (n=7). Mutations occurred at low variant allele frequencies (VAF) with a median of 11.7% (range: 1.0% to 30.7%) indicating that mutations were presented in only a subset of blood cells. During a 2-year follow-up observation two subjects with mutations in splicing factor genes died, one subject with an *SF3B1* mutation developed pancreatic cancer, the other subject harbored a *U2AF1* mutation and died due to a stroke. All other individuals with mutations are alive without any evidence for a hematologic or oncologic disorder. Mutation kinetics remained virtually stable over two years as measured by targeted NGS (median VAF 13.1%, range 1.0% to 35.3%).
Summary/Conclusions: These findings indicate that the appearance of low-level clones with mutations in epigenetic regulator genes and the RNA splicing machinery is a common age-associated phenomenon which may represent a premalignant condition in the development of hematologic cancers and may also predispose to other aging associated disorders. The question why these subclones are selected in the elderly but apparently enter a stage of clonal size stability without further selection in healthy elderly in contrast to patients with myeloid disorders is of particular interest and needs further investigation.

E1192

FLOW CYTOMETRIC RESPONSE MONITORING IN MDS WITH DEL(5Q) USING A SIMPLE 5-PARAMETER-SCORE-A TWO-CENTER-EXPERIANCE

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Background: Recently, we have established a 5-parameter flow cytometry (FCM)-based score allowing for a precise prediction a deletion (5q) in therapy naïve MDS patients (pts.).
Aims: The aim of this prospective study was to test, whether this FCM-based profiling is at least equal to the cytogenetics/FISH-based del(5q) detection for monitoring response to treatment.
Methods: Overall, 164 FCM investigations were performed in 39 pts. with MDS and del(5q) (IPSS-R very low/low: n=17, int: n=9, high/very high n=13) including 27 pts. with isolated del(5q) or one additional cytogenetic abnormality. The majority of analyses were performed in pts. receiving lenalidomide or azacitidine (n=17 and n=15 pts.), or in pts. receiving chemotherapy and/or allogeneic transplantation (n=3), or growth factors (n=4). The del(5q)-FCM-score includes the following 5 parameters: myeloid progenitors (myPC >2%)=3 points, CD45 MFI ratio (lymphocytes: myPC ≤7.0)=10, SSC ratio (granulocytes: lymphocytes<6.0)=2, CD71 (≤20%) on granulocytes=1.5, and female gender=1.5; a score ≥15.0 indicates the presence of del(5q). A standardized FCM (lyse-stain-wash) and cytogenetics/FISH were performed according to ELN guidelines at the TU of Dresden and VUMC of Amsterdam.
Results: Before therapy, in 56 cytogenetic/FISH analyses in 39 MDS pts. a del(5q) was detectable. In 48/56 (86%) FCM measurements, performed in parallel, a del(5q) could be predicted using the del(5q)-FCM-score. A complete cytogenetic response (CCR) was detectable in 17 pts. (41 different evaluations). Remarkably, all determined del(5q)-FCM-scores matched the above mentioned CCR (sensitivity=100%). Otherwise, in 32 pts. (69 different evaluations) del(5q) was still detectable by cytogenetics/FISH, which was accompanied by a matching del(5q)-FCM-score in 58 measurements (specificity=84%). Next, we focused on those pts. who presented with a typical del(5q)-FCM-score before therapy (32 pts.; 49 measurements; median score=16.5). Again, all 31 cytogenetic evaluations, resulting in a CCR, showed a matching del(5q)-FCM-score (sensitivity=100%; median score=13.0). On the other side, 52 of the 54 analyses (96%) without CCR showed a properly high del(5q)-FCM-score (median score=16.5). Furthermore, we compared cytogenetics/FISH and del(5q)-FCM-score with additional methods for response monitoring as cytomorphology, two well established diagnostic FCM scores: FCSS (flow cytometry scoring system; Wells et al. Blood 2003) and the diagnostic score (Ogata et al. Haematologica 2012), as well as the assessment of hematological improvement (HI). Thus, cytomorphology and FCSS, analyzing dyspoiesis of multiple cell lineages, showed a CR or disappearance of a MDS typical FCSS in only around half of all investigations being in cytogenetic CR (sensitivity: 48% and 58%), but those two methods revealed a high specificity (100% and 96%). On the other side, the analysis of HI was highly sensitive (90%) but not specific (56%). Finally, the use of the 4-parameter Ogata score ended up with a nearly as high sensitivity (90%) and specificity (87%) as cytogenetics/FISH and the del(5q)-FCM-score.
Summary/Conclusions: Flow cytometry-based detection of a del(5q)-specific immunophenotype is feasible and can serve as a rapid tool for response monitoring during treatment with disease-modifying drugs. At the moment, we validate the FCM-scores in a larger multicentric study within the iMDS-Flow working group of the ELNet evaluating if response prediction by FCM might result in a better separation of the patients' survival.

E1193

ABT-199 EFFECTIVELY INDUCES APOPTOSIS IN HIGH-RISK MDS PROGENITOR CELLS IRRESPECTIVE OF PROGNOSTICALLY ADVERSE MUTATIONS OF TP53, EZH2, RUNX1, AND ASXL1

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Background: Myelodysplastic Syndromes (MDS) is a group of heterogeneous diseases with a broad spectrum of clinical outcomes. As we could recently show, the BCL-2 selective inhibitor ABT-199 effectively induces apoptosis in leukemic progenitors, as well as in blast cells of patients with high-risk MDS and secondary acute myeloid leukemia (sAML), while the healthy progenitor cell population was only marginally affected. As current therapeutic strategies such as Azacytidine have only a short response duration alternative regimes are urgently needed. Classification systems such as the International Prognostic Scoring System (IPSS) help to estimate the overall survival and the risk of developing a sAML. Disease-related molecular abnormalities are not incorporated in these scoring systems at the moment. However mutations of TP53, EZH2, RUNX1, and ASXL1 have been shown to identify patients with a shorter survival than predicted by the IPSS.
Aims: We were interested in analyzing whether ABT-199 could overcome resistance to apoptosis in high-risk MDS/sAML despite prognostically adverse mutations in TP53, EZH2, RUNX1, and ASXL1.
Methods: Purified bone marrow mononuclear cells (BMMNC) were treated with 1μM ABT-199 or a soluble control (DMSO) for 72h *in vitro*. Apoptosis was analyzed by flow cytometry after staining for 7-AAD, Annexin V, and CD34 as a progenitor marker. The long-term effect was investigated by colony formation assay. Mutational analysis was performed by Sanger sequencing at a certified laboratory (Munich Leukemia Laboratory). In total, seven healthy bone marrow samples and 38 MDS/sAML bone marrow samples (20 patients with proven wildtype alleles for TP53, EZH2, RUNX1, and ASXL1 as well as 18 patients with mutations in at least one of the indicated genes) were investigated.

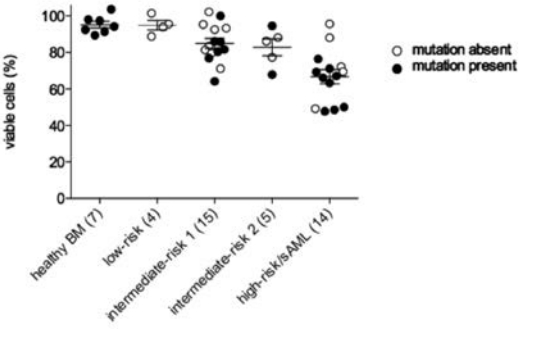

Figure 1. FICOLL-purified CD34+ BMMNC were treated for 72h with 1μM ABT-199 or a vehicle control (DMSO). Apoptosis was measured by flow cytometry. Each circle represents the ratio of viable cells after ABT-199 treatment to viable cells after sole vehicle treatment. Student's t-test showed no significant difference between mutated and not mutated samples within one risk group. When compared with healthy controls, we found that induction of apoptosis was significantly increased in the stem/progenitor population from IPSS-classified patients with intermediate-risk 1 and 2 MDS, high-risk MDS, and sAML.

Results: Prognostically bad molecular genetics including mutations of TP53, EZH2, RUNX1, and ASXL1 did not affect the response to ABT-199 treatment

in MDS progenitor cells. No difference in the induction of apoptosis was seen between mutated and not mutated samples. As expected, the effect of ABT-199 correlated with MDS disease progression to elevated clinical risk groups. In addition, the long-term effect of BCL-2 inhibition could be analyzed in three patients of the high-risk MDS/sAML group with proven mutations by colony formation assays. Here we show that ABT-199 effectively killed colony-forming stem/progenitor cells from patient samples of the high-risk MDS/sAML subgroup.
Summary/Conclusions: We conclude that ABT-199 is a promising drug for patients with high-risk MDS/sAML irrespective of prognostically adverse mutations of TP53, EZH2, RUNX1, and ASXL1.

E1194

DYNAMICS OF GENE EXPRESSION CHANGES IN THE DEVELOPMENT OF MYELODYSPLASTIC SYNDROMES AND EVOLUTION TO ACUTE MYELOID LEUKEMIA

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Background: Myelodysplastic syndromes (MDS) are a group of hematopoietic stem cell disorders at high risk of developing acute myeloid leukemia (AML). Both disorders are characterized by the accumulation of a wide variety of genetic and epigenetic aberrations in hematopoietic progenitor cells, resulting in altered cell growth and differentiation. However, the molecular mechanisms underlying the evolution of MDS to more aggressive stages remain to be discerned.
Aims: To analyze common deregulated genes and gene pathways, which may potentially be associated with the progression of the disease.
Methods: A multi-platform genome-wide expression profiling was carried out in a series of 73 patients with normal cytogenetics and 17 controls with non-malignant disorders. Two platforms for gene expression analysis were used: the Human Exon 1.0 ST (test group) and the Human Genome U133 Plus 2.0 (validation group) (*Affymetrix*). The study was based on the analysis of gene expression changes occurring from non-malignant bone marrow conditions through different stages of MDS and towards AML. Two-steps analysis was carried out: (1) Identifying genes which expression levels evolved following an increasing/decreasing trend during the progression of the disease (Normal, Low-Risk MDS, High-Risk MDS and AML); (2) Identifying common expression patterns among the groups of genes with increasing/decreasing trends. At the end, only the genes that appeared deregulated in common with the two platforms were considered.
Results: This methodological approach allowed us to identify common genes and gene pathways that were progressively up-/down-regulated in the transition from non-malignant bone marrow conditions to early MDS stages (Low-Risk MDS), which also remained progressively deregulated during the progression towards advanced MDS (High-Risk MDS) and AML. These genes were classified in 4 major patterns: Pattern 1, MDS/AML-up, included a set of 83 genes that began to be up-regulated in Low-Risk MDS and remained progressively altered during the progression towards High-Risk MDS, with the maximum in AML (*NPM1*, *MYST1*, *RPL22*, *RPS6*); Pattern 3, AML-up, consisted of 26 progressively up-regulated genes showing the biggest change in their expression level in the transition from High-Risk MDS to AML (*HOXA9*, *MEIS1*, *FLT3*). By contrast, patterns 2 (70 genes) and 4 (32 genes), MDS/AML-down and AML-down, respectively, included those genes that were progressively down-regulated during the evolution of the disease, showing the minimum levels in AML (*CEACAM3*, *CRISP3*, *CAMP*, *MMP9*). These common and dynamically deregulated genes implicated in the progression of the disease were related to key cellular functions of known relevance in MDS. Thus, apoptosis, DNA damage response, ribosome and translation pathways, and chromatin assembly were progressively up-regulated as the disease progressed, since early MDS stages, while the immune response showed an increasing down-regulation. In addition, the transition from advanced MDS to AML would be characterized by a marked up-regulation of cell proliferation and a differentiation arrest.
Summary/Conclusions: The present study demonstrated the presence of a progressive deregulation of several cellular functions, with common deregulated genes, in the transition from non-malignant bone marrow conditions through early and advanced MDS to AML. This evolution seems to occur in an orchestrated way, involving common deregulated functional pathways.

E1195

BONE HOMEOSTASIS IS SIGNIFICANTLY ALTERED IN A MYELODYSPLASTIC SYNDROME MOUSE MODEL

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Background: Myelodysplastic syndromes (MDS) are diseases of the elderly characterized by an ineffective hematopoiesis leading to cytopenias. Increasing evidence suggests a role of the bone microenvironment in influencing the development and progression of MDS. However, the underlying mechanisms are not fully understood. NUP98-HOXD13 (NHD13) mice develop MDS-like symptoms at an age of 6 months, including anemia and an increased number of blasts in the bone marrow. Thus, it is a suitable model to study bone homeostasis in the setting of MDS.
Aims: Here, we assessed bone homeostasis in NHD13 mice before (2 months of age) and after (6 months of age) the onset of MDS.
Methods: Micro-computed tomography was performed using the femurs of 2- and 6-month-old male NHD13 and wild-type mice. Bone samples were then histologically stained for the osteoclast marker tartrate-resistant acid phosphatase (TRAP). The bone formation rate was assessed using dual calcein labeling. In addition, serum was analyzed by ELISAs to assess the bone turnover markers C-terminal telopeptide of type I collagen (CTX-I) and procollagen type I propeptide (P1NP).
Results: At 2 months of age NHD13 mice had not yet developed any cytopenia. Nevertheless, there was already an apparent alteration of bone homeostasis. The trabecular bone volume (BV/TV) [-26%; $P<.05$] and trabecular number (Tb.N) [-20%; $P<.01$] were reduced in the femurs of NHD13 compared to wild-type mice. Serum markers indicated an increased bone turnover in NHD13 mice [P1NP 4-fold, $P<.01$; +17% CTX). At the age of 6 months the NHD13 mice had developed anemia indicated by a reduced hematocrit, -17%, $P<.001$. BV/TV in these mice was unchanged when compared with those of wild-type mice. Of interest, the number of trabeculae was reduced in the 6-month-old NHD13 mice [-15%; $P<.001$] whereas their thickness was increased [+18%; $P<.01$], suggesting increased osteoblast activity. In fact, histology indicated an increase in the osteoblast surface in the femurs from NHD13 by 10% [$P<.01$] and also the bone formation marker P1NP was elevated in the serum by 54% [$P<.05$] compared to wild-type mice. Although NHD13 mice had a lower osteoclast-covered surface on the bone [-78%; $P<.01$], the bone resorption marker CTX-I was increased by 32% [$P<.05$].
Summary/Conclusions: NHD13 mice show an altered bone homeostasis, which is characterized by a high bone turnover. This is in line with an abnormal interaction of dysplastic hematopoietic cells with the bone compartment.

E1196

GENETIC FACTORS ASSOCIATED WITH EVOLUTION OF MYELODYSPLASTIC SYNDROMES TO SECONDARY CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Chronic myelomonocytic leukemia (CMML) is a subset of myelodysplastic/myeloproliferative neoplasms (MDS/MPN), which are morphologically distinct from myelodysplastic syndromes (MDS). Nevertheless, CMML and MDS share several genetic defects and dysplastic features. Moreover, CMML can evolve from MDS, designated as secondary CMML (sCMML). The molecular characteristics of sCMML, which are distinct from those of primary CMML, remain to be elucidated.

Aims: By comparing the genetic/cytogenetic abnormalities between sCMML and other myeloid malignancies, we clarify the molecular pathogenesis of sCMML.
Methods: A total of 601 cases with different myeloid neoplasms, including *de novo* CMML (n=89), sCMML (n=26), MDS (n=348), and secondary AML (sAML) (n=138) were subjected to the analysis, combining the genotyping data from our cohort and publicly available datasets. Serial samples were available for 7 cases.
Results: In the *de novo* CMML cohort, the most frequently mutated genes included *TET2* (40%), *SRSF2* (30%), *ASXL1* (24%), *RUNX1* (16%), *CBL* (11%), *EZH2* (11%), *ZRSR2* (11%), and *KRAS* (10%), which, combined, accounted for 74% of the CMML cases. In the sCMML cohort, by contrast, *RUNX1* (32%), *TET2* (32%), *ASXL1* (24%), *SF3B1* (24%), and *SETBP1* (20%) represented common genetic alterations, together with -7/del(7q) (43%), complex karyotype (38%), and del(5q) (24%). In multivariate analysis comparing the spectrum of mutations between sCMML and *de novo* CMML, *SF3B1*, and *RUNX1* mutations were enriched in sCMML patients. In patients with sCMML, *SF3B1* mutations supposed to be acquired prior to *RUNX1* mutations based on the comparison of their variant allele frequencies. Most likely, *SF3B1* mutations were acquired as founder events in the MDS phase and *RUNX1* mutations occurred during sCMML evolution. However, *RUNX1* mutations were not always identified in patients with sCMML, suggesting that other causes are also related to sCMML evolution. To further identify genetic lesions specifically associated with CMML evolution, we compared the spectrum of mutations between sCMML and MDS. Multivariate analysis revealed that mutations in *SETBP1*, *RUNX1, TET2*, and *NRAS*, del(5q), and -7/del(7q) were significantly and independently associated with sCMML. Then we divided *de novo* CMML cases into 2 categories; Type-1 with ≥2 of these lesions (n=13) and Type-2 with<2 (n=54). Patients with Type-1 CMML had a significantly shorter overall survival compared to Type-2 disease (HR=3.73, 95%CI 1.78-7.79, P<0.001). Serial sample analysis revealed that mutations in *SETBP1*, *RUNX1*, and *NRAS*, and del(7q) tended to be newly acquired during evolution to sCMML. However, the lesions extracted in the comparison between sCMML and MDS were also detected in our cohort of sAML; -7/del(7q) (23%), del(5q) (20%), mutations in *SETBP1* (4%), *RUNX1* (19%), *TET2* (22%), and *NRAS* (7%). To investigate what lesions are really characteristic to sCMML evolution from MDS, we compared the frequencies of genetic lesions between sCMML and sAML. We found *SF3B1*, *SETBP1*, *NRAS*, and *RUNX1* mutations, as well as complex karyotype, and -7/del(7q) were significantly enriched in sCMML, which largely overlapped with the lesions associated with evolution from MDS to sCMML.

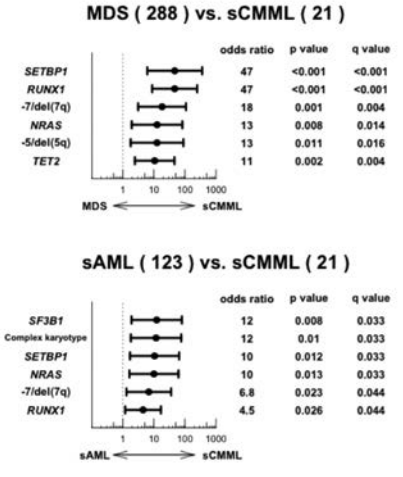

Figure 1.

Summary/Conclusions: In this study, a novel set of lesions associated with sCMML evolution (*SETBP1*, *RUNX1, TET2*, and *NRAS* mutations, del(5q), and -7/del(7q)) was identified and shown to be able to stratify *de novo* CMML into clinically distinct subgroups. By genotyping serial samples and comparing genetic lesions between sCMML and sAML, this set of lesions was thought to be characteristic to sCMML evolution. Our study demonstrated that specific genetic events might play an important role in sCMML evolution from MDS.

E1197

SCAPER AND RPRD1A: TWO NOVEL CANDIDATES IN THE PATHOGENESIS OF FAMILIAL PLATELET DISORDER WITH PROPENSITY TO DEVELOP MYELOID MALIGNANCIES (FPD/AML)

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Background: A patient with acute myeloid leukemia (AML) secondary to MDS and a strong family history of thrombocytopenia and myelodysplastic syndromes was referred to our clinic for evaluation of the possible hereditary nature of myeloid malignancies in the family.
Aims: To identify novel myeloid malignancy-predisposing mutations and contribute to a better understanding of biological processes involved in the development of myelodysplasia, also in cases of sporadic MDS.
Methods: Whole exome sequencing was performed on DNA from peripheral blood of 3 family members with myelodysplasia, one of whom developed AML. Sanger sequencing was used to determine the frequency of the identified mutations in 13 family members as well as 36 sporadic cases of MDS. Corresponding protein expression was evaluated by immunohistochemistry. Structural modelling using Pymol software was performed to estimate the probable impact of mutations on the three-dimensional structure of the corresponding proteins. Using site-directed mutagenesis we have recently created plasmids carrying the identified mutations, which will allow us to further evaluate their functional consequences.
Results: Two confirmed missense mutations were identified by whole exome sequencing. They affect SCAPER (S-phase cyclin A-associated protein in the endoplasmic reticulum) and RPRD1A (regulator of nuclear pre-mRNA domain-containing protein 1A). The SCAPER E342K and RPRD1A I143T mutations were detected by Sanger sequencing in 9 and 10 out of 13 family members, respectively. Molecular modelling showed that both mutations can significantly change the three-dimensional structures of the corresponding proteins. In one of the mutated cases where bone marrow biopsy material was available, both SCAPER and RPRD1A showed high protein expression in the cytoplasm and nucleus. We sequenced the SCAPER and RPRD1A mutation site in 36 patients with sporadic MDS, all of whom showed the wild type sequence. However, when protein expression of SCAPER and RPRD1A was evaluated immunehistochemically on a tissue microarray including core biopsies from 45 patients with MDS, both proteins showed marked heterogeneity of expression level among patients. Lack of RPRD1A expression was associated with significantly shorter overall survival (24 *vs* 50 months, p<0.03, HR=2.2), independent of the revised international prognostic scoring system (IPSS). Patients with high expression of SCAPER showed longer median survival than patients with low expression (50 *vs* 28 months) but the difference did not reach statistical significance. Interestingly, despite strong *inter*individual heterogeneity, there was strong *intra*individual correlation of protein expression between SCAPER and RPRD1A (r=0.818, p<0.0001).
Summary/Conclusions: Molecular modelling convinced us that these two non-conservative mutations cause significant changes in three-dimensional structure of both SCAPER and RPRD1A proteins, which may lead to altered affinity to their main binding partners, *i.e.* Cyclin A and RNA polymerase 2, respectively. Our findings suggest that SCAPER and RPRD1A should be included among the genes to be analysed in cases of familial thrombocytopenia with propensity for myeloid malignancies. Furthermore, strong heterogeneity of SCAPER and RPRD1A protein expression among patients with sporadic MDS, together with the prognostic impact of protein expression, suggests that both genes may play a role in MDS pathogenesis. Mutation analysis covering the full length of SCAPER and RPRD1A genes, as well as analysis of the epigenetic status of both genes may reveal further insight.

E1198

REAL-TIME DEFORMABILITY CYTOMETRY (RT-DC) AS NOVEL DIAGNOSTIC TOOL IN MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a group of disorders, which are often difficult to differentiate from reactive and toxic conditions causing cytopenia. Contractility and cytoskeletal structure of hematopoietic stem cells in MDS are known to differ from the healthy counterparts. Thus, detection of the cells with abnormal mechanical properties could be a promising diagnostic procedure for patients with potential MDS.
Aims: Therefore we aimed to evaluate if real-time deformability cytometry (RT-DC) can be a reliable diagnostic and prognostic tool for MDS patients, as well as to clarify molecular factors, which are associated with differential cell deformability.
Methods: We analyzed MACS-separated CD34+ cells from 19 patients with MDS (median age at BM sampling 65 years [range, 44-87]; m/f=13/6) and 8 age-matched healthy donors (62 years [range, 43-83]; m/f=3/5). Mechanical characterization was carried out using RT-DC at three flow rates according to previously described protocol (Otto O. et al., *Nat Methods*, 2015). With this technology, single cells are flowed through a microfluidic channel constriction and deformed without contact by shear stress. Statistical significance was

assessed by 2-sided Kolmogorov-Smirnov test. Changes in cell cycle were evaluated by flow cytometry using propidium iodide staining. Molecular alterations were evaluated by targeted resequencing on a MiSEQ instrument using an amplicon assay (True Sight Myeloid Panel, Illumina) which covers 54 genes or gene hotspots related to myeloid neoplasms. Cytogenetic analysis was performed in all MDS patients. Overall survival (OS) was estimated using Cox proportionate regression models.
Results: Mean deformability for CD34+ cells of MDS patients was significantly lower than in control cells (0.0241 *vs* 0.0272, accordingly, p=0.018). MDS CD34+ cells underwent significantly less mitosis (2.4% *vs* 17.4%, p=0.002), arresting mostly in G1 phase (91.5% *vs* 66.7%, p<0.001). CD34+ cells from patients with mutations in *RAS*-pathway tended to be more deformable, whereas *RUNX1*-mutated cells appeared stiffer. Cytogenetics had no influence on mechanical properties. The deformability appears to be predictive for the OS starting from time of BM sampling, as 1-year OS in patients with more deformable cells (>median) was 72.9% *vs* 37.0% in patients with less deformable cells.
Summary/Conclusions: RT-DC provides a novel tool to reveal substantial differences in mechanical properties of CD34+ cells of MDS patients compared to healthy donors. This might allow incorporating this technique into diagnostic approaches of MDS patients.

E1199

SPATIAL INTERACTION OF CYTOGENETIC AND MOLECULAR MUTATIONS IN MYELODYSPLASTIC SYNDROMES
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Background: In myelodysplastic syndromes (MDS), several frequent molecular mutations affect the same chromosomal regions as common cytogenetic mutations. These regions are potential targets of total loss of function of cancer-protective genes or could be involved in gain of function of oncogenes. Both alleles could be simultaneously affected by two different mutation types, one allele by a molecular mutation and the second allele by a cytogenetic mutation (deletion, copy number neutral loss of heterozygosity (CN-LOH) and others). Up to now the impact of biallelic mutations composed by a molecular and a cytogenetic mutation on leukemogenesis is unknown in MDS.
Aims: The aim of our study was to identify and to characterize such biallelic mutations (composed by a molecular and a cytogenetic mutation) to delineate the frequency and the potential clinical significance in MDS.
Methods: We included 209 patients in our analysis: 144 proven MDS, 27 suspected MDS with typical genetic aberrations, 9 myelodysplastic/myeloproliferative neoplasms (MSD/MPN), and 29 secondary acute myeloid leukemias (sAML) after MDS. Molecular analysis was done by Sanger sequencing including up to 17 of the most frequently mutated genes in MDS. The basis of cytogenetic analysis was conventional chromosome banding that was complemented by interphase fluorescence in situ hybridization (FISH), multicolor FISH and/or molecular karyotyping (SNP array analysis).
Results: Overall, genetic mutations were detected in 84.7% of patients. Cytogenetics identified mutations in 46.9% and sequencing revealed mutations in 70.8% of patients. In 20 patients (9.6%) a molecular mutation was located in a chromosomal region that was in addition affected by a cytogenetic aberration (assumed to affect the second allele). Such biallelic mutations were more frequent in MDS-RAEB (9/51, 18%), MDS/MPN (2/9, 22%), and sAML (5/29, 17%), but rarer in low-risk MDS (4/93, 4%). The highest frequency of such biallelic mutations was detected in patients with *CBL* mutations: 2/3 *CBL* mutations were located within a CN-LOH of 11q. Furthermore, 4/11 patients with *EZH2* mutations had a cytogenetic 7q aberration (2x deletion, 2x CN-LOH), 4/13 patients with *TP53* mutations additionally showed cytogenetic loss of 17p. We detected two patients with a *SRSF2* mutation and a simultaneous isochromosome iso(17q) that results in gain of 17q. Other molecular mutations involved in such biallelic mutations were: *SRSF2* (1x CN-LOH), *TET2* (2x loss, 1x CN-LOH), *DNMT3A* (2x CN-LOH), and *RUNX1* (2x CN-LOH).
Summary/Conclusions: Biallelic mutations composed by a molecular and a cytogenetic mutation affecting the same chromosomal region were recurrently identified in sAML and MDS/MPN in our study but were detected also in low-risk MDS albeit at lower frequency. Co-occurrence of molecular and cytogenetic lesions affecting e.g. *TP53*/7q, *EZH2*/7q or *CBL*/11q suggests individual pathomechanisms resulting in homozygous loss of function of potential tumor suppressor genes. The co-occurrence of activating *SRSF2* mutations and iso(17q) or CN-LOH of 17q might have an oncogenic effect. Biallelic mutations could be indicative for a regional genetic instability promoting disease progression. According to the two-hit hypothesis such "composed" biallelic mutations might arise stepwise. Therefore, they could refer to previous genetic evolution and consequently to advanced stages of the disease. Sixteen of the 20 biallelic mutations identified in our study affected patients with high-risk MDS or sAML. Thus, patients with biallelic mutations due to a molecular and a cytogenetic mutation in parallel could be at high risk for further progression of the disease.

E1200

MONUCLEAR MYELOID-DERIVED SUPPRESSOR CELLS (MO-MDSC) EXPANSION AND PROGRESSION IN MYELODYSPLASTIC SYNDROMES
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Background: Myeloid Derived Suppressor Cells" (MDSC) are a heterogeneous population of inmature myeloid cells constituent of bone marrow microenvironment, whose expansion hampers the host anti-tumor immune response. They can play a pathogenetic role in the development of ineffective hematopoiesis in myelodysplastic síndromes (MDS), and contribute to progression to acute myeloid leukemia (AML), through inmunotolerance and as a cellular effectors that suppress hematopoiesis. The mechanisms that determine the evolution to AML are not entirely clarified although, a role of the immune response has been suggested. In this sense, the bone marrow microenvironment expansion of suppressor cells of monocytic origin (Mo-MDSC) and T cell inmunosupressive efector (T_{regs}) can contribute to tumor progression.
Aims: Our aim has been to investigate a possible role of the myeloid supressor cells of monocytic origin (CD14+HLA-DR$^{low/-}$) and T_{regs} in the development of ineffective hematopoiesis in myelodysplastic síndromes (MDS), that may contribute to the development and progression to acute myeloid leukemia (AML) of MDS.
Methods: We performed a prospective analysis of a group of 31 patients with MDS and AML evolved from MDS and a control group of 40 healthy subjects. It was analyzed by multiparametric flow cytometry techniques to study the presence of MDSC-Mo (CD14+HLA-DR$^{low/-}$) cells and CD34+blasts, in peripheral blood and bone marrow in both MDS patients and control cases. The monoclonal antibodies marked with their respective fluorochromes were: CD45-V_{500}, CD3-V_{450},. CD4-PER, CD8-APH- 7, CD19-PE-CY7, CD56-PE-CY7, CD127-APC, CD34-APC, CD64-FICT, DR- V_{450}, CD25-PE-CY7, CD14-PE and CD64-FICT and anti DR-V_{450}.The clinical and biological profile of MDS and control group has analyzed with statistical software IBM SPSS.19.
Results: In the 31 peripheral blood samples of patients with MDS and AML evolved from MDS, the WHO-2008 diagnostic classification was: RCUD(4), RCMD(8), RARS and RCMD-RS(2), RAEB-1(3), RAEB-2(6), MMCL-1(5) and AML evolved from MDS (3). We observed a positive correlation between MDSC cells with phenotype CD14$^+$ HLA-DR$^{low/-}$ and CD34+blasts in peripheral blood (p=0,001). In addition, the frequency of expression of CD14$^+$ HLA-DR$^{low/-}$ cells, was significantly elevated in MDS patients as compared to the control cases (26,52+/-5,47 *vs* 6,66+/-1,06; p=0,014). No significant results were obtained in bone marrow for the studied subpopulations. With regard to WHO classification, we observed a higher value of MDSC in RAEB-2 and AML *vs* other MDS, almost statisticaly significant (45,40+/-12,70 *vs* 18,80+/-5,04; p=0.055). The analysis of prognostic scoring system IPSS y IPSS-R shows higher incidence of Mo-MDSC in High risk IPSS (High+Int-2 *vs* Int-1 and Low risk) (59,55+/-14,14 *vs* 15,12+/-3,61; p=0.004) and High risk IPSS-R (Very High, High and Int *vs* Low and very low risk)(37,06+/-7,99 *vs* 9,49+/-4,05; p=0.006). Increase frequency of Mo- MDSCs was also linked to poor and intermediate cytogenetic risks (59,55+/-14,14 *vs* 15,10+/-3,94; Poor and Int *vs* Good and Very good; p=0.006).
Summary/Conclusions: The expansion of cells with MDSCs phenotype of monocytic origin can suppose a higher risk in myelodysplastic syndromes transformation, favouring the immune escape, which may be important for understand the pathogenesis of high-risk MDS and with poor cytogenetic risks. The good correlation between myeloid supressor cells of monocytic origin (CD14+HLA-DR$^{low/-}$) and CD34+blasts in peripheral blood, allows monitoring of patients with MDS.

E1201

ABERRANT METHYLATION OF PROMOTER REGIONS OF SOX7, P15INK4B AND WNT PATHWAY ANTAGONIST GENES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES
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Background: Epigenetic aberrations, including hypermethylation of CpG islands in tumor-suppressor genes, are supposed to be a key mechanism of myelodysplastic syndrome (MDS) development.
Aims: To find out the association between methylation status of *SOX7*, *p15^{INK4b}*, *SFRP1*, *SFRP4* and *SFRP5* genes and some hematological features and overall survival (OS).
Methods: The data of 46 MDS patients with median age of 67.5 years were analyzed. MDS was diagnosed according to WHO classification. Methylation-specific PCR was used to study the methylation status.
Results: Aberrant methylation of ≥1 genes was found in 43 patients (93.5%). The most frequently findings were *SOX7* (84.8%), *SFRP1* (71.7%) and *p15^{INK4b}*

(54.3%) genes methylation. Methylation of 1, 2, 3, 4 and 5 genes simultaneously was detected in 10.9%, 28.3%, 26.1%, 19.6% and 8.7% of patients, respectively. There was no any difference in the number of patients with *SFRP1*, *SFRP4*, *SOX7* and *p15^{INK4b}* methylation in the groups with different bone marrow blasts counts. Methylation of *SFRP5* gene was more frequently seen in patients with refractory anemia with excess of blasts (RAEB): 43.5% *vs* 13.0% in patients without excess of blasts; OR=5.1, 95%CI: 1.2-22.3, *p=0.047*. The patients without excess of blasts were characterized by methylation of 0-1 genes: 26.1% *vs* 8.7% of RAEB patients, although the difference was not significant. At the same time, there was the tendency to increase of the number of cases with 3-5 methylated genes in patients with 10-19% blasts compared to patients with 5-9% blasts. In the whole MDS group, there was no any correlation between the number of methylated genes and patient's age, number of bone marrow blasts or karyotype. Increased number of methylated genes did not influence on the OS.

Summary/Conclusions: MDS progression is associated with enhancement of epigenetic disturbances leading to increase of the number of methylated genes, in particular, *SFRP5*

E1202

IRON OVERLOAD-ASSOCIATED GENETIC INSTABILITY IN MYELODYSPLASTIC SYNDROME

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Background: Not only clinical experience, but also numerous experimental and animal-modell studies demonstrated that iron overload (IO) negatively impacts on survival outcomes in patients suffering from myelodysplastic syndromes (MDS). However, the molecular mechanisms of IO induced genetic damage have neither been sufficiently demonstrated nor been fully understood.
Aims: We aimed to investigate the association between IO, measured as serum ferritin (SF) as suitable surrogate marker, and genetic instability, considered to be a main factor responsible for MDS progression to secondary acute myeloid leukemia (sAML).
Methods: We included 55 patients (median age 74 years, 62% males). The majority (n=50) had different MDS subtypes, 5 patients had myelodysplastic/myeloproliferative neoplasms (MSD/MPN). The patients were divided into two subgroups with normal and elevated SF levels, using 275 µg/l as cut-off for elevated SF. 26 patients showed normal SF levels (median: 65 µg/l, range: 8-256 µg/l), while 29 patients showed elevated SF levels (median: 1150 µg/l, range: 283-3872 µg/l). Various parameters for genetic instability were analyzed and statistically compared between the two subgroups with normal and elevated SF: a) molecular karyotyping (SNP array analysis) to determine the total genomic alterations (TGA) size, b) immunofluorescent determination of γH2AX-foci for quantifying DNA double-strand breaks (DSB) in CD34+ peripheral blood (PB) cells, c) telomere length (TL) of PB granulocytes and lymphocytes, and d) plasma nitric oxide metabolites as markers for oxidative stress.
Results: Subsequent analyses revealed a positive correlation of SF levels and bone marrow blast counts. Thus, all further analyses were adjusted by a logistic regression model for unevenly distributed blast counts. PB nitric oxide metabolites did not correlate with SF level (N=33 patients with available data). Iron overload measured by SF showed a significant correlation with the number of γH2AX-foci per CD34+ PB cell (r=0.481, p=0.039). The median number of γH2AX-foci per CD34+ PB cells was significantly highlower in 8 patients with normal SF as compared to 10 patients with elevated SF level (1.9 *vs* 5.7 γH2AX-foci/CD34+ cell (adjusted p=0.050). TGA size was positively correlating with SF levels (r=0.397, p=0.026) and with marrow blast counts (r=0.381, p=0.077). TGA size was higher in 28 patients with elevated SF (median: 34 Mbp, range: 0-248 Mbp) as compared to 23 patients with normal SF (median: 0 Mbp, range: 0-155 Mbp, adjusted p>0.5). Telomere length and IO showed a significant negative correlation (r=-0.497, p=0.002). TL in granulocytes was significantly reduced in 13 patients with elevated SF (median: -1.61 kb, range: -4.06-1.31) as compared to 13 patients with normal SF levels (median: 0.48 kb, range: -3.13-5.32 kb, adjusted p=0.024). In contrast, lymphocyte TL was not influenced by the SF level.
Summary/Conclusions: In this study, IO was significantly associated with numerous markers of genetic instability. Elevated SF levels were promoting the TGA size, spontaneous nuclear damage assessed by γH2AX-foci and replicative stress (TL) in granulocytes representing the myeloid compartment, whereas the lymphocyte compartment remained uninfluenced by IO. These findings further support the assumption of IO being closely related to leukemic transformation in MDS. Our results contribute to explain the association of IO and disease progression found in several studies. Whether these results may have consequences for diagnostics and therapeutic decision-making in patients with MDS remains to be further investigated.

E1203

CONVENTIONAL CYTOGENETICS, ACGH, AND PCR AS INTEGRATED WORKUP FOR A CORRECT DIAGNOSIS OF MDS

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Background: Conventional cytogenetics continues to have a fundamental role in the classification and risk scoring of myelodysplastic syndromes (MDS). Nevertheless, non-informative karyotypes represent up to 20% of cases. Some different molecular methods, such as FISH, aCGH or mutational analysis, could detect new abnormalities and improve the correct subtyping of MDS patients.
Aims: The aim of the study was to adopt an integrated diagnostic work-up for better characterizing MDS patients.
Methods: We analyzed 61 patients (71% male, with a median age of 74 years). According to the WHO classification, 33% were affected by multilineage refractory cytopenias, 12% by refractory anemia, 15% by refractory anemia with ring sideroblasts, 21% by refractory anemia with excess of blasts, 3% by 5q- syndrome, and 12% by chronic myelo-monocytic leukemia; 67% of patients were at low/intermediate-1 IPSS risk. All patients were assessed by conventional cytogenetics, FISH for chromosomes 5, 7, 8, PDGFRalpha and beta rearrangements, aCGH, and PCR for ASXL1, EZH2, TP53, and TET2 mutations. WT1 and RPS14 gene expression levels were also measured by quantitative PCR.
Results: In our series, conventional cytogenetics analysis failed in only 12% of cases: indeed, the sampling for this analysis was the first one during the bone marrow aspiration. Overall, 39% of patients showed at least one chromosomal aberration, with complex karyotypes in 7% of cases. FISH allowed to correctly classify two cases as affected by the 5q- syndrome and one as affected by deletion of chromosome 7; two patients carried PDGFRbeta rearrangement; these abnormalities had not been detected by the conventional cytogenetics.
The aGCH allowed to detect chromosomal aberrations in 38% of cases: aCGH detected 10 "new" mutated cases in respect of the conventional cytogenetics, including alterations of the ETV6 and GATA2 genes; After the mutational analyses, 28% of patients resulted mutated, with highest frequency for TP53 (mutated in the 16% of the overall series). Eight of these TP53-mutated patients showed normal karyotype, and resulted wild-type by FISH and aCGH. Four low/intermediate-1 risk patients (8%) showed the ASXL1 gene mutation. Two cases showed the TET2 mutation. The statistical analysis confirmed the prognostic role of poor cytogenetics either on overall survival (OS) or progression-free-survival (PFS). Also deletions detected by aCGH resulted to play a negative prognostic impact on OS. WT1 and RPS14 gene expression was assessed: over-expression of WT1 was found in one third of all patients, while 70% showed RPS14 values lower than those measured in healthy donors. Statistical analysis showed that the WT1 over-expression had statistical significance on survival, also in multivariate analysis.
Summary/Conclusions: Our study supports the feasibility and the utility of the introduction in the routinary workup of MDS of FISH and aCGH, in order to better stratify MDS patients and correctly design *ab initio* a patient-tailored treatment.

E1204

CLONAL DYNAMICS OF TWO DISTINCT CLONES IN LEUKEMIC TRANSFORMATION FROM MDS WITH ISOLATED DEL(5Q) HARBORING TP53 MUTATION

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Background: Although myelodysplastic syndromes (MDS) with isolated del(5q) represents a favorable prognosis, the presence of TP53 mutation has been associated with transformation to secondary AML (sAML) accompanied by the emergence of a complex karyotype (CK). The demethylating agent azacitidine (AZA) is currently the standard therapy for patients with high-risk MDS. However, some reports showed AZA treatment cannot improve survival in high-risk

MDS and sAML patients with TP53 mutations, in spite of response to AZA. The molecular mechanisms in the acquisition of resistance to AZA in TP53 mutated cases has not been completely understood.
Aims: To clarify the molecular mechanisms underlying disease progression and the acquisition of chemo-resistance in MDS, we delineated clonal evolution during disease course using high throughput sequencing of serial samples.
Methods: The 77-year-old female patient was diagnosed with MDS with isolated del(5q). She then received lenalidomide (LEN), but progressed to sAML with CK involving -7. She received AZA with LEN and then achieved temporarily marrow CR (blast<5%). Although she received same therapies, she relapsed and died of progressive leukemia. Genomic DNA was collected at four stages (RAEB-1, sAML, remission, and relapse) on informed consent. We performed whole exome sequencing (WES) and PCR amplicon based target deep sequencing by NGS (Illumina HiSeq 2500). Copy number alterations (CNA) were analyzed based on the read number and allelic imbalances were estimated from allele frequency of heterozygous SNPs.
Results: The mean coverage of WES and target deep sequencing was 209 times and 67073 times, respectively. We detected 15 somatic single nucleotide variants (SNV) and 2 frameshift/insertions in 17 genes. We identified the TP53 Y102C and NF1 T676fs as putative driver mutations. As the TP53 Y102C was the most abundant mutation identified in all stages, could represent the founding mutation. On the other hand, NF1 T676fs was rapidly enriched in the relapse. Next we delineated a model of clonal evolution using the proportion of SNV and CNA. Although CK with -7 was acquired during disease progression in this case, interestingly, deleted allele of chromosome 7 was different between sAML and relapse phase. In other words, two distinct clones characterized by deletion of different allele (-7A/-7B) existed after sAML, and clonal competition occurred during disease progression and having therapy.(Figure) The sAML stage was characterized by a founding clone harboring TP53 mutation and del(5q), major clone containing -7A and sub-clone with -7B. del(11p) was acquired in -7A clone and became dominant, but in remission the clone with del(11p) was extinct. On the other hand, clonal evolution took place on -7B clone, which acquired NF1 mutation, del(17p) encompassing TP53, and del(17q) including NF1. Finally, the evolved clone from -7B obtained chemo-resistance and contributed to relapse and also -7A clone flared up with deleted NF1.

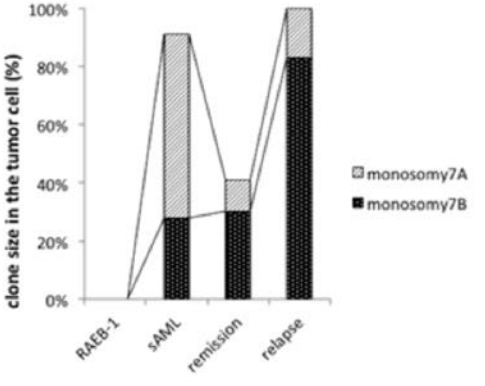

Figure 1. The proportion of -7 on each allele durng disease course.

Summary/Conclusions: To analysis serial samples during disease progression discloses that two distinct clone harboring chromosomal aberration on each allele competed with each other by selective pressure of therapy. These observations uncovered the complicated clonal architecture with the accumulations of genetic abnormality.

E1205

THE DNA METHYLATION INHIBITORS AZACITIDINE AND DECITABINE ACTIVATE UNIQUE AND COMMON TRANSCRIPTIONAL PATHWAYS THAT INCLUDE POTENTIAL NOVEL MARKERS OF RESPONSE

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Background: Myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) patients have poor tolerability of standard chemotherapies and an unmet need for novel therapeutic approaches. Epigenetic silencing of genes can occur via hypermethylation or the recruitment of histone deacetylases (HDACs), with these changes being reversible making them prime targets for therapy. Agents that inhibit DNA methylation have proven successful in the treatment of haematological malignancies and have shown promise by increasing the tolerability for MDS and AML patients, however the underlying mechanism of action remains to be fully elucidated.
Aims: In this report, we have studied the molecular and cellular consequences of treating AML and MDS derived cell lines with Azacitidine as a single agent as well as performing a small meta-analysis comparing Azacitidine an Decitabine gene expression with the intention of finding potential rational combination partners.
Methods: LINE-1 pyrosequencing was used to measure global methylation levels and western blotting used as a measure of protein expression. Cytospins were performed and slides were stained with Wright-Giemsa to assess the morphological effect on OCI-AML3 and SKM-1 cells. Gene expression profiling was performed using Affymetrix U133 plus 2.0 arrays and results were analysed using Partek Genomics Suite. CHI3L1 methylation was determined using pyrosequencing with primers designed against the promoter, intron and exon. Survival analysis examined TGCA dataset of 183 clinically annotated adult cases of *de novo* AML, whilst the local dataset consisted of 228 clinically annotated cases of MDS and AML.
Results: Azacitidine decreased viability and LINE-1 DNA methylation with a concomitant reduction in DNMT1 protein expression. Morphological assessment of treated OCI-AML3 and SKM-1 cells displayed an increase the proportion of macrophage cells-indicative of an induction of differentiation. Gene expression profiling using Affymetrix U133 plus 2.0 arrays identified 173 differentially expressed probesets following treatment with pathway analysis using DAVID identifying the immune system and an inflammatory response, which was also observed with network analysis using STRING. Overlap with Decitabine gene expression analysis highlighted 138 probesets that were common to both drugs and supported the role of the immune system in response to demethylating agents including S100A8/A9, HLA and CHI3L1. CHI3L1 was up-regulated with both agents and an increase in gene expression correlated to a loss of promoter, intron and exon DNA methylation. Survival analysis in locally and publically available datasets of stratified AML patients recognised that CHI3L1 could be used as a potential marker of response in patients with good cytogenetics (p=<0.02) compared to those in other subtypes (intermediate - ns; poor-ns).
Summary/Conclusions: These analyses give further insight into the mechanism of action of both Azacitidine and Decitabine in AML/MDS cell lines by identifying molecular gene and pathway targets that may be primed for novel therapeutic combinations for the treatment of elderly patients with AML or high risk MDS.

E1206

INTEGRATED ANALYSIS OF BOTH BIOLOGICAL AND MOLECULAR EFFECTS OF THE EPIGENETIC MODIFYING AGENT ROMIDEPSIN IN MDS/AML

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Background: Myelodysplastic syndrome (MDS) patients have poor tolerability of standard chemotherapies and an unmet need for novel therapeutic approaches. Epigenetic silencing of genes can occur via the recruitment of histone deacetylases (HDACs) with these changes being reversible, making them prime targets for therapy. Agents that inhibit HDACs (HDACi) have proven successful in certain malignancies and have shown promise by increasing the tolerability for MDS patients
Aims: In this report, we aimed to study the molecular and cellular consequences of treating AML and MDS derived cell lines with Romidepsin as a single agent, which could ultimately provide a rationale for combination therapy.
Methods: Cell Titre Glo® was used to measure viability, alongside HDAC Glo® assay to quantitate HDAC activity. Western blotting was performed to determine protein expression levels and flow cytometry using propidium iodide staining was used to measure cell cycle. Affymetrix U133 plus 2.0 arrays were used for gene expression analysis in conjunction with DAVID and STRING for pathway and network analysis. The Illumina Next-Seq 500 was used in-house to perform ChIP-Seq experiments.
Results: Preliminary analysis identified a candidate dose, time and cell line to be carried forward for gene expression profiling and ChIP-Seq analysis based on the ability of Romidepsin to affect proliferation, HDAC activity, acetylation and cell cycle. Microarray analysis indicated that 484 probesets were significantly (p<0.05) up-regulated compared to only 3 down-regulated after 24 hour treatment with 1.5 nM Romidepsin. Pathway and network analysis, using STRING and DAVID identified that ROS, inflammation and activation of mitochondrial stress pathways were associated with Romidepsin treatment. Matched samples from Romidepsin treated, and untreated SKM-1 cells were used for ChIP-Seq analysis of H3K9ac, H3K9me and H3K9me3 chromatin marks which provided information on positional enrichment and enabled an integrated transcriptomic-epigenetic mapping approach, thus identifying specific Romidepsin-induced gene-expression networks. Overlap of differential enrichment of each of the aforementioned marks with the microarray data has highlighted that increases in acetylation share the highest degree of overlap with gene expression.
Summary/Conclusions: These analyses give further insight into the mode of action of Romidepsin by identifying molecular gene and pathway targets that

may be primed for novel therapeutic combinations for the treatment of elderly patients with AML or high risk MDS.

E1207

THERAPY RELATED MYELOID NEOPLASM FOLLOWING RADIOTHERAPY HAS HIGHER INCIDENCE OF POOR RISK CYTOGENETICS AND ARE ASSOCIATED WITH POOR SURVIVAL COMPARED TO DE NOVO MDS CASES

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Background: Therapy-related myeloid neoplasms (T-MN) are well-known complication of prior cytotoxic/radiation therapy for malignancies and auto-immune diseases and are associated with poor survival compared to de novo MDS cases;most probably due to higher rate of poor cytogenetic in T-MN cases. Poor prognosis of T-MN cases following prior cytotoxic chemotherapy (CT) exposure is well established, however, there is ongoing debate about the prognosis of radiotherapy (RT) only associated T-MN cases.
Aims: This study compares the prognosis of T-MN cases following CT, RT and de novo MDS cases enrolled in the South Australian MDS (SA-MDS) Registry.
Methods: Demographic, clinical, cytogenetic and laboratory data on 744 de-novo and 148 T-MN patients were analysed. Survivals between the groups were compared by using log-rank test and were plotted using Kaplan Meier curve. Categorical variables were compared by using Chi-square test.

Table 1. Distribution of cytogenetic categories in de-novo &T-MN patients according to IPSS, R-IPSS and prior treatment type.

IPSS cytogenetic category	CT±RT N=112	RT N=34	De novo N=741	R-IPSS cytogenetic category	CT±RT N=112	RT N=34	De novo N=741
Good	28(25%)	15(44%)	498(67%)	Very Good	2(2%)	1(3%)	30(4%)
Intermediate	17(15%)	4(12%)	111(15%)	Good	26(23%)	14(41%)	474(64%)
Poor	58(52%)	12(35%)	95(13%)	Intermediate	22(20%)	4(12%)	103(14%)
Not available	9(8%)	3(9%)	37(5%)	Poor	25(22%)	3(9%)	31(4%)
				Very Poor	28(25%)	9(26%)	66(9%)
				Not available	9(8%)	3(9%)	37(5%)

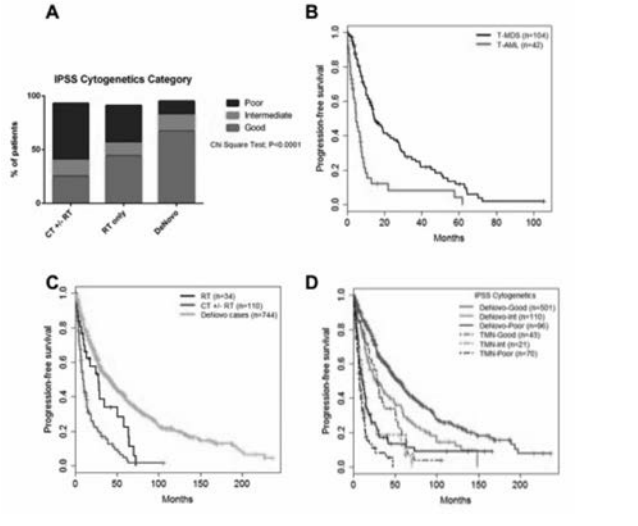

Figure 1.

Results: Median age of T-MN and de novo MDS was 71 (20-91) and 73 years (19-98). Most frequent primary diseases (PD) were lymphoproliferative diseases (n=56, 38%) and prostate cancer (n=22, 15%). Sixty-four (43%) patients had received CT only, 34 (23%) patients RT only, and 48 (32%) patients received both CT and RT for PD. 29(20%) patients received autologous stem cell transplant for their PD. During follow up, 42(28%) patients developed T-AML, while 104 (70%) developed T-MDS. The median time between cytotoxic treatment and t-MN diagnosis was 38.5months for RT only (range 0.9-123) and 31.9months (range 0.2-216.2) for CT(±RT) patients, respectively. As shown in table 1 and Fig1A, poor risk cytogenetic cases were significantly higher in T-MN cases treated with CT compared to cases treated with RT alone and de novo MDS cases (52% *vs* 25% *vs* 13% respectively; P<0.0001). Similarly, 47% CT(±RT)patients had R-IPSS defined poor and very poor cytogenetics compared to 35% of RT only and 13% of de-novo MDS/AML patients (P=<0.0001). Overall, 40(27%) of T-MN patients received disease modifying therapy (Induction CT, Hypomethylating agents, low dose Ara-C, and Allogeneic transplant). Within the T-MN group (144 patients), T-MDS patients had significantly better median survival than T-AML (14.7 *vs* 5.0 months, P<0.0001)(Fig 1B). T-MN patients who received prior RT only had worse median survival than de-novo patients but better than T-MN patients receiving prior CT (±RT) (9.7 *vs* 27.9 *vs* 41.3 months; p=0.001)(Fig 1C). Further, within the individual IPSS cytogenetic risk category, T-MN consistently had poor median survival than de-novo patients (Fig.1D) regardless of the type of prior therapy(p=0.001).
Summary/Conclusions: T-MN patients receiving prior CT(± RT) have poorer survival compared to RT only & de novo MDS patients. Contrary to the published literature (Nardi *et al* JCO 2012), our data suggest that the incidence of adverse cytogenetics is higher in T-MN patients who received prior RT compared to de-novo patients. T-MN patients have consistently poor survival than de-novo patients even when matched for IPSS cytogenetic categories.

E1208

POLY [ADP-RIBOSE] POLYMERASE 1 (PARP-1) EXPRESSION IS CORRELATED TO THE TYPE OF MYELODYSPLASTIC SYNDROME ACCORDING TO BOTH WHO CLASSIFICATION AND IPSS SCORE

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Background: Poly [ADP-ribose] polymerase 1 (PARP-1) has a central role in the repair of single-stranded DNA breaks, thus protecting the cell from genomic instability. Overexpression of PARP1 may lead a cell to apoptotic or necrotic death, thus defining the cell's fate. Genetic defects are important in the pathogenesis of myelodysplastic syndromes (MDS) and the role of PARP1 in the apoptotic pathways seems to be promising, since new agents targeting PARP1 are available. PARP1 expression has never been studied in MDS.
Aims: Our aim was to detect PARP1 mRNA in bone marrow samples of patients with MDS and investigate its correlations to the hematologic and prognostic characteristics of the patients.
Methods: Bone marrow samples were collected from patients with MDS. Quantification of PARP1 mRNA was performed by a SYBR-green based PCR, performed on BIORADCFX96 (BIORAD) and the results were expressed in correlation to those of the housekeeping gene of beta actin. Statistical correlations were carried out using IBM SPSS statistics, version 19.0.
Results: Fifty three (53) patients with MDS were included in the study. The basic demographic and hematologic characteristics of the patients are shown in the Table. The vast majority of the patients (92.5%) were treatment naïve. The median PARP1 mRNA levels were 0.0259 (range 0.0003-0.6410) and showed a statistically significant correlation to the type of MDS (according to the WHO classification) (p=0.016) and to the IPSS score (p=0.001) (detailed results in Table). The lowest levels were observed in patients with RA (0.0053 *vs* 0.1477 for non-RA patients, p= 0.016), as well as in patients with low and intermediate 1 IPSS score (0.0107 *vs* 0.1831, p=0.01) that had almost 20 times lower PARP1 mRNA levels than patients with intermediate 2 and high risk MDS.

Table 1. Patients' characteristics and results.

Characteristic	Result		
Number of patients, N (%)	53 (100)		
Sex (Male to female ratio)	1.41		
Age (years), median (range)	76 (45 – 91)		
Previous treatment, N (%)*	4 (7.5)	PARP1 mRNA, median (range)	p†
MDS type (WHO classification), N (%)			0.016
RA	14 (23.0)	0.0053 (0.0010-0.5250)	
RARS	2 (3.3)	0.1546 (0.0003-0.3089) ‡	
RCMD	11 (18.0)	0.0167 (0.0007-0.2226)	
RAEB-1	17 (27.9)	0.2097 (0.0047-0.6410)	
RAEB-2	9 (14.8)	0.1477 (0.0060-0.5901)	
IPSS, N (%)			0.001
Low	20 (32.8)	0.0062 (0.0007-0.5250)	
Intermediate 1	16 (26.2)	0.1639 (0.0003-0.6410)	
Intermediate 2	11 (18.0)	0.2097 (0.0061-0.3184)	
High	6 (9.8)	0.0910 (0.0135-0.5901)	
Previous treatment, N (%)*	4 (7.5)		

** hypomethylating agent*
† Independent Samples Kruskal-Wallis Test, 2-sided p
‡ based on only 2 samples

Summary/Conclusions: The correlation of higher levels of PARP1 mRNA with higher risk MDS has never been reported in the past. This correlation, if confirmed by larger studies, may render PARP1 a prognostic factor for patients with MDS. Moreover, this result can lay the basis for the design of clinical trials evaluating the use of PARP1 inhibitors in patients with higher risk MDS.

E1209

NEXT GENERATION SEQUENCING IN HIGH RISK MYELODYSPLASTIC SYNDROMES AND SECONDARY ACUTE MYELOID LEUKEMIA PATIENTS TREATED WITH AZACITIDINE ACCORDING TO HIGH RISK MDS 2009 PROTOCOL FROM CETLAM GROUP

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Background: Risk-adapted treatment strategy is currently used in myelodysplastic syndromes (MDS). Hypomethylating agents have introduced a new perspective in the therapeutic approach of higher risk MDS, azanucleosides being the standard of care for most patients with this disease. The application of next generation sequencing (NGS) technologies to study MDS has identified several recurrently mutated genes involved in RNA splicing, DNA methylation, chromatin modification, transcription, DNA repair control, cohesin function, RAS pathway and DNA replication. Even though there is a significant overlap between the commonly mutated genes in MDS with those found in AML, mutation status is not widely used to select MDS treatment.
Aims: The aim of this study is to identify the mutational status at diagnosis of MDS and secondary AML (sAML) patients treated with azacitidine to evaluate if it could help to discriminate which patients will respond from those who will not and its correlation with clinic-biological data.
Methods: A preliminary prospective study was performed on 39 patients with MDS and sAML (14 RAEB-2, 9 RAEB-1, 5 RCMD, 1 RCMD-RS, 1 RARS and 9 sAML). Genomic DNA was obtained from bone marrow at diagnosis. SeqCap EZ and KAPA Library Preparation Kit (Roche) reagents have been used to generate libraries and enrich DNA of 83 genes implicated in myeloid neoplasm. The customized panel has been analyzed in MiSeq Illumina platform with 150bp paired-end reads. NGS data were analyzed using Illumina MiSeq Reporter and Variant Studio softwares. In 21 patients a CD3+ matched control study was done, in order to distinguish between somatic and germline variants. Data from treatment response and overall survival (OS) has been collected from all patients.
Results: The mean depth of targeted resequencing per base was 779-fold. After filtering all the variations obtained for quality, biological consequence and discard the known SNPs, we obtained 116 variations with a mean of 3 variants per sample. The average of alterations detected in each cytological category can be observed in Table 1.

Table 1. Average of abnormalities according to cytological category.

	Nº patients	Average of alterations per patient (range)
sAML	9	3.56 (1-7)
RAEB-2	14	3 (0-6)
RAEB-1	9	2.78 (1-5)
RCMD	5	2.2 (1-4)
RCMD-RS	1	5
RARS	1	1

The most frequent altered genes have been *TP53* (48.7%), *TET2* (20.5%) and *DNMT3A* (20.5%). Statistical analysis confirmed that *TP53* alterations are associated with complex karyotype (p<0.001) and with the very high IPSS-R risk category (p=0.002). On the other hand, mutations of RNA splicing machinery (*SRSF2*, *U2AF1, SF3B1* or *ZRSR2*) and variations in cohesion complex (*STAG2, RAD21* or *SMC3*) are associated with non-complex karyotype (p=0.022 and p=0.02) and alterations in *SRSF2* are rarely detected in very high IPSS-R risk category (p=0.007). In our series we have not observed the impact of *TET2* mutations in treatment response. Survival analysis showed that the presence of variants in *TP53* correlated with shorter OS (9.2 *vs* 19.2; p=0.008). Alterations in *SRSF2* and mutations in cohesion complex are associated with longer OS (median not reached *vs* 10.4; p=0.025 and median not reached *vs* 10.4; p=0.048).
Summary/Conclusions: NGS technique is a good tool to study mutational profile in MDS and sAML. Patients with sAML and RAEB-2 present more variations than patients with RAEB-1 or RCMD. The most affected genes match with those described in the literature for high risk MDS. Alterations in *TP53* and *SRSF2* genes seem to be good markers to predict the outcome.
Acknowledgements: Instituto de Salud Carlos III, Spain (PI 11/02519).

E1210

MOLECULAR ALTERATIONS ASSOCIATED WITH PROGRESSION OF MYELODYSPLASTIC SYNDROMES TO SECONDARY CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Myelodysplastic syndromes (MDS) are heterogeneous disorders characterized by ineffective hematopoiesis and risk of progression to acute myeloid leukemia. However, MDS may progress to clearly myeloproliferative neoplasms. Some reports have demonstrated that *de novo* MDS patients can transform to secondary chronic myelomonocytic leukemia (CMML) independently of the presence of relative monocytosis (>10%) at initial diagnosis of MDS (Rigolin et al, Haematologica 1997; Wang et al, Am J Clin Pathol 2006). Clinical parameters and cytogenetic alterations failed to correlate with probability to develop this type of progression.
Aims: The knowledge of the mechanisms at the basis of proliferative-non leukemic progression could help define the prognosis of this group of MDS patients. We postulated that specific acquired somatic mutations could be associated with the transformation from MDS to secondary CMML.
Methods: Seven IPSS lower risk MDS patients (pts) that progressed to CMML were included in the study. The mutational profile was characterized using a TruSeq Custom Amplicon panel (Illumina) targeting the 57 recurrently mutated genes in myeloid malignancies. Around 250 ng of genomic DNA extracted from mononuclear cells obtained at diagnosis (MDS phase) and at progression (CMML phase) were used to prepare sequencing libraries following the Illumina standard protocol. Samples were run on an Illumina MiSeq and variants were annotated by ANNOVAR. Detected variants were distilled on the basis of their exonic function, allele frequency, the presence in variants databases, and several prediction scores.
Results: At diagnosis, 2 pts had RA and 6 pts had RCMD and at progression 5 pts presented a CMML-1 and 2 pts a CMML-2 (mean time to progression: 20 months). The total WBC count in the CMML phase was significantly higher than that of the MDS phase (mean, 24.4×10^9/L *vs* 4.04×10^9/L; *P*= 0.03). The mean hemoglobin level, platelet count, and blast count were not different between the MDS and CMML phases. In both phases, 3 pts presented a normal karyotype, 1 case a monosomy 7, 1 patient a trisomy 8 and 1 patient a trisomy 14. Only 1 patient presented a new trisomy 8 at progression. The most frequently mutated genes at MDS diagnosis were *TET2* and *ASXL1* (mutated in 7 and 6 pts, respectively). Only two pts presented new mutations in the progression CMML sample: one patient in the *SETBP1* gene and the other one in the *DNMT3A* gene. The mutational profiling of these 7 MDS pts at diagnosis (group 1) was compared with that of a second cohort of 40 low-intermediate1 MDS pts (group 2) that did not progress (mean follow up: 25 months) (Figure 1). The number of mutations was higher in group 1 (100% pts presented >3 mutations) than group 2 (80% of pts presented 0-2 mutations). The most commonly mutated genes differed between the two groups: *ASXL1* (100%) and *TET2* (86%) in group 1, while in group 2 were *SF3B1* (25%) and *TET2* (22%). In order to study the role of *ASXL1* and *TET2* mutations in the progression we compared variant allele frequency (VAF) data: VAF of *ASXL1* was significantly higher in CMML than in MDS phase (38 *vs* 21; *P*=0.02), while VAF of *TET2* did not shown differences.

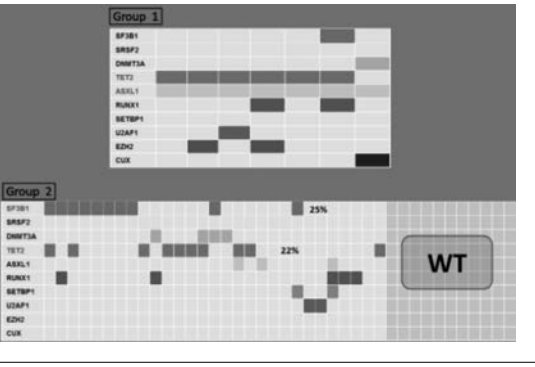

Figure 1.

Summary/Conclusions: This analysis of the rare type of progression from frank low risk MDS to MDS/MPN suggests that number of somatic mutations and presence of *ASXL1* mutations during the MDS phase is an important factor correlated with/predictive of proliferative-non leukemic progression. Increase in VAF of *ASXL1* seems also important in determining progression.

Myelodysplastic syndromes – Clinical

E1211

COST CHANGES ASSOCIATED WITH ACHIEVING TRANSFUSION INDEPENDENCE (TI) IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Background: Given the bone marrow failure characteristic of the disease, patients with MDS are often dependent upon red blood cell (RBC) transfusions. Guidelines from the European Society of Medical Oncology note that chronic RBC transfusions are associated with chronic anemia, leading to excess morbidity, and cannot completely resolve impaired quality of life. This transfusion dependence (TD) has been shown to be a risk factor for progression, and achieving TI is an important treatment objective.
Aims: To evaluate MDS cost patterns associated with initial periods in which patients were TD, and subsequent periods of TI.
Methods: Patients were identified from a large US claims database (2008–2013) by ICD-9 codes for MDS; the index date was the earlier of ≥1 inpatient or ≥2 outpatient claims. Patients with medical and pharmacy (Rx) coverage for ≥12 months pre- and ≥6 months post-index were included. TD was defined as ≥2 consecutive 8-week periods with ≥1 transfusion each and no interim 56-day period without transfusion; TI was defined as 8 subsequent transfusion-free weeks. Patients with high-risk MDS or acute myeloid leukemia at diagnosis were excluded. TD patients were followed until they no longer met TD criteria or end of data. Patients who subsequently became TI were followed until they returned to TD or end of data. Total costs of care were based on paid claims and reported for 24 months post-onset of TD or TI.
Results: 13,741 MDS patients met the inclusion criteria regardless of transfusion needs; 2,645 (19%) were TD and 1,378 (52%) subsequently became TI. Median TD duration was 7.5 months. Age, prior treatment, baseline anemia, del(5q) mutation, and comorbidities were similar for TD patients who did and did not subsequently achieve TI. The average total cost of care (medical+Rx) was lower for TI than for TD patients ($8,138 *vs* $16,640 per patient-month) (Figure 1). TI patients had 54% lower monthly medical costs ($7,004 *vs* $15,289) largely due to differences in inpatient costs ($2,353 *vs* $7,056). Monthly Rx costs for TI patients were 16% lower than for TD patients ($1,134 *vs* $1,351). Over time, monthly costs for TI patients declined 50% from initial levels; TD patient costs declined 27%.

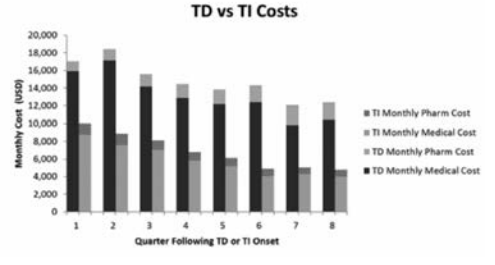

Figure 1.

Summary/Conclusions: Patients who are TD incur significant total costs of ≥$16,000/month. For patients who subsequently achieved TI, monthly costs were halved from TD costs and continued to decline over time. This suggests that treatment of TD patients offers the potential for a return to TI, resulting in economic benefit as well as clinical improvement.

E1212

FERRITINE LEVEL AND PERFORMANCE STATUS ARE SIGNIFICATIVE PROGNOSTIC FACTORS FOR OVERALL SURVIVAL IN LOWER-RISK MDS PATIENTS GRUPO ARGENTINO DE ESTUDIO DE LOS SINDROMES MIELODISPLASICOS

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Background: Myelodysplastic syndromes (MSD) are a heterogeneous group of clonal haematopoietic stem cell malignancies characterized by defects in haematopoietic cell maduration/differentiation. According to the original IPSS, the majority of patients are classified within low or intermediate-I (lower) risk with a survival of >3.5 years. However, a large diversity exists within these patients varying in several characteristics at diagnosis. Up to 10% evolve to AML and 50% succumb as a result of the severity of cytopenias. The WPSS, MD Anderson (MDA) and IPSS-R recognized some IPSS limitations. The use of these systems has improved survival prediction but their usefulness is not clear in lower risk patients. Also, the inclusion of non-classical variables, as ferritine level or performance status, is also in debate.
Aims: To analyze the usefulness of risk stratification to predict survival and evolution to AML in lower risk patients. And, to test whether non classical variables are independent prognostic variables in these patients.
Methods: This is a retrospective study of 566 patients from the *Registro Argentino de Enfermedades Hematologicas (RAEH)*, *Sociedad Argentina de Hematologia*, diagnosed between 2007 and 2015, last up-date Jan 2016. Out of the registry, 328 (57.9%) were selected and evaluated according to the IPSS criteria, excluding those with secondary MDS and with proliferative CMML. WPSS (both the one including transfusion dependence and with hemoglobin levels according to gender), MDA and the specific for Low Risk (MDA-LR), and IPSS-R systems were applied. Univariate (Kaplan-Meier and Long-rank) and multivariate (Cox regression) analysis were performed to evaluate survival and evolution to AML.
Results: The selected 328 lower risk patients showed a median age of 72.5 years, 54.6% were male and 17.7% showed a performance status ≥2. With a median follow-up of 25.4 months, 86 (26.2%) died and 38 (11.6%) evolved to AML. Gender, ferritine level (<170, 170-350, >350 mg/ML) and all variables grouped according to different evaluated systems were significant predictive variables for prognosis. Table 1 shows the distribution of patients according to all evaluated systems and their respective median survival and time to evolution to AML. It also depicts that all systems were useful to predict outcome. The IPSS-R sustained its independence for both survival [p<0.001, exp(B) 1.676] and evolution to AML [p<0.001, exp(B) 2.544]. Also the classic MDA was useful for survival [p<0.001, exp(B) 1.987] and both WPSS for evolution to AML [p<0.001 and 0.02, exp(B) 2.281 and 0.554, respectively including either trf or Hb]. As the IPSS-R was the only system that sustained its independence to predict both survival and evolution to AML, all variables not included were analyzed in a multivariate model. Performance status and ferritine level were significative predictive parameters in the model to predict survival [p<0.001 and p=0.012, exp(B) 1.807 and 1.455]. However, any variable showed an independent prognostic impact for evolution to AML besides the IPSS-R.

Table 1. Univariate analysis of variables of prognostic factors for survival and evolution to AML.

Variables	Patients (%)	Survival			Evolution to AML		
		4 (yr)	Median (mo)	*Log Rank*	4 yr (%)	25% (mo)	*Log Rank*
IPSS							
Good	169	77.3	105.5	21,082	6.0	116.1	22,944
Intermediate-1	157	47.0	43.8		28.5	26.3	
IPSS-R							
Very Low	116	91.9	NR	52,104	5.6	NR	75,986
Low	151	58.0	NR		16.1	NR	
Intermediate	39	48.7	33.7		35.1	11.6	
High	13	10.4	17.3		84.2	9.5	
WPSS-Trf							
Very Low + Low#	7+81	80.9	NA	29,221	1.4	NR	39,708
Intermediate	128	61.7	NA		14.8	NR	
High + Very High#	51+3	27.9	33.7		50.6	12.0	
WPSS-Hb							
Very Low	27	72.4	NR	34,766	0.0	NR	25,025
Low	143	76.1	NR		9.4	NR	
Intermediate	79	33.7	35.6		31.8	42.9	
High + Very High#	32	41	17.8		39.9	17.6	
MDA							
Good	95	79.8	NR	83,971	5.3	NR	28,447
Intermediate-1	152	73.0	105.5		13.6	116.1	
Intermediate-2	58	26.5	33.1		35.6	17.6	
High	17	15.8	11.8		61.1	25.8	
MDA-LR							
Good	67	79.1	NR	83,659	3.5	NR	25,935
Intermediate	210	66.2	105.5		15.7	116.1	
High	44	30.6	19.5		44.4	13.8	

NR: not reached; mo: months; all univariate p-values not explicitly stated are *p<0.001*, # group of risk with less of 10 patients were joined for statistic purpose IPSS: International Prognostic Scoring System according to P. Greenberg *et al.*, 1997. IPSS-R: revised IPSS according to P. Greenberg *et al.*, 2012.

Summary/Conclusions: WPSS, MDA, MDA-LR and IPSS-R systems allowed us to identify subgroups of patients (11.5%-26.2%) that shift to worse prognosis categories in lower risk-IPSS patients. IPSS-R sustained its independence to predict both survival and evolution to AML, while the MDA did it for survival and the WPSS for leukemic evolution. Performance status and ferritine level were significative independent prognostic factors to predict survival along with the IPSS-R in lower risk patients.

E1213

CHARACTERIZATION OF TREATMENT PATTERNS AND OUTCOMES IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: ANALYSIS OF UNITED STATES COMMERCIAL CLAIMS DATABASE

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Background: Myelodysplastic syndromes (MDS) constitute a heterogeneous form of blood cancer that primarily affects the elderly, and are characterized by anemia and other cytopenias as well as a high risk of transformation to acute myeloid leukemia (AML). Treatment, which is selected based on the International Prognostic Scoring System (IPSS), presence of cytopenia, and the presence of 5q deletion (del[5q] MDS), may include supportive care (eg, whole-blood and/or platelet transfusion), erythropoiesis-stimulating agents (ESA), hypomethylating agents, and lenalidomide for del(5q) MDS.
Aims: To characterize the burden of disease, treatment patterns, and outcomes of MDS patients using a large US database.
Methods: Optum's integrated claims and Electronic Medical Record database ("Optum Database") was retrospectively analyzed to identify adult patients with an index diagnosis of MDS between 2006 and 2014; to ensure index diagnosis, only patients with 365 days of retrospective data preceding the index diagnosis were included. Patients with baseline AML and patients younger than 18 years of age were excluded. MDS in patients was categorized as low-grade (ICD 9 code 238.72: Refractory anemia [RA], Refractory anemia with excess blasts-1 [RAEB-1], Refractory anemia with ringed sideroblasts [RARS], Refractory cytopenia with multilineage dysplasia [RCMD], Refractory cytopenia with multilineage dysplasia and ringed sideroblasts [RCMD-RS]), high-grade (ICD 9 code 238.73, which includes Refractory Anemia with Excess Blasts [RAEB] II), del(5q) (ICD 9 code: 238.74), or unspecified (ICD 9 code: 238.75) based on the earliest recorded ICD-9 diagnosis code based on French-American-British classification. Demographics and clinical outcomes were analyzed by descriptive summary. Treatment patterns were summarized by grouping treatments as "lenalidomide," "azacitidine," "decitabine," "imatinib," "chemotherapies," "ESA," and "others." Kaplan-Meier survival analysis was performed to define progression to AML.
Results: Of 10,465 MDS patients in the Optum Database, 8493 met the inclusion criteria and were evaluated over a median follow-up of 2.3 years; MDS was categorized as low-grade in 2136 (25.2%), high-grade in 367 (4.3%), del(5q) in 198 (2.3%), and unspecified in 5792 (68.2%) patients. Across all MDS categories, mean age at diagnosis was 70 to 72 years, 77% of patients were aged ≥65 years, roughly half were male, and the majority were white and non-Hispanic. In the overall cohort, 40% received ≥1 prescribed agent (46% in low-grade, 61% in high-grade, 42% in del[5q], and 36% in unspecified MDS). The most commonly used regimens were epoetin-alfa or darbepoetin in both low-grade and unspecified, hypomethylating agents (decitabine or azacitidine) in high-grade, and lenalidomide in del(5q) MDS (Table). In the overall cohort, 500 (5.9%) MDS patients progressed to AML, including 103 (4.8%) patients with low-grade and 78 (21.3%) with high-grade MDS. In the overall cohort, 38% of patients who received ≥1 regimen and 13% of patients without prescribed treatment went on to have a blood transfusion (Table).

Table 1. Demographics and treatment of patients with MDS in optum database.

Demographics	Low-grade MDS (ICD 9 code: 238.72) n = 2136	High-grade MDS (ICD 9 code: 238.73) n = 367	Del(5q) MDS (ICD 9 code: 238.74) n = 198	Unspecified MDS (ICD 9 code: 238.75) n = 5792
Age[a]				
≥ 65 years, %	75	76	75	77
Mean (SD)	71 (13)	71 (12)	70 (13)	72 (12)
Sex, %				
Male	51.7	58.0	48.0	53.1
Female	48.0	42.0	52.0	46.8
Distribution of regimens across all lines of treatment[b,c] %				
Lenalidomide	4.8	9.3	37.9	8.6
Azacitidine	10.8	35.2	10.2	12.8
Decitabine	3.8	15.0	2.3	4.5
ESA	73.4	24.9	41.2	61.7
RBC transfusion[c]				
Received RBC transfusion, %	23	39	27	22
Progression to AML				
Patients progressing to AML, %	4.8	21.3	6.1	5.3
Median days to progression from index MDS diagnosis	349.0	205.5	418.0	302.0

[a]Proportion of total number of patients within each risk category.
[b]Proportion of the total number of regimens within each risk category.
[c]Regimens containing listed agents (may reflect use as monotherapy or in combination regimens).
[d]Transfusion analysis for the interval of 30 days prior to index MDS diagnosis to end of follow-up.
ESA, erythropoiesis-stimulating agents darbopoietin or epoietin alfa; RBC, red blood cell; SD, standard deviation.

Summary/Conclusions: Despite the variety of agents available, a significant proportion of MDS patients across all categories did not receive any treatment, and many received only supportive care. Rates of transformation to AML and transfusion dependence are consistent with published estimates.

E1214

MINIMAL RESIDUAL DISEASE MONITORING AND PREEMPTIVE IMMUNOTHERAPY IN MYELODYSPLASTIC SYNDROME AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is one of the most effective treatments for high-risk myelodysplastic syndromes (MDS). Although allogeneic HSCT has advanced significantly, post-transplant relapse remains an important cause of transplant failure. The presence of minimal residual disease (MRD) after HSCT can indicate impending relapse.
Aims: This study investigated the efficacy of MRD monitoring and MRD-directed preemptive immunotherapy in high-risk MDS patients who received allogeneic HSCT.
Methods: MRD assessment consisted of Wilms' tumor gene 1 (WT1) detection with PCR and leukemia-associated immunophenotypic pattern examination with multiparameter flow cytometry (FCM).
Results: Post-HSCT, 31 patients were positive for WT1, and 8, for FCM; positivity for WT1 (18.6% *vs* 6.1%, *P*=0.040) or FCM (62.5% *vs* 3.6%, *P*<0.001) indicated a higher 2-year relapse rate. Twenty-one patients met our combined criteria for MRD, and the presence of MRD was associated with a higher 2-year relapse rate (27.3% *vs* 4.5%, *P*=0.003). Thirty-one patients showed positive results for preferentially expressed antigen of melanoma (PRAME) after HSCT, which indicated a higher 2-year relapse rate (19.3% *vs* 6.2%, *P*=0.035). In patients positive for both PRAME and MRD, the relapse rate was 60% despite preemptive immunotherapy. Multivariate analysis confirmed the association between the increased relapse rate and positivity for both PRAME and MRD (hazard ratio=42.8, *P*=0.001).
Summary/Conclusions: MRD monitoring predicted relapse in high-risk MDS post-HSCT patients, and PRAME- and MRD-positive patients did not benefit from preemptive immunotherapy.

E1215

PROGNOSTIC SIGNIFICANT OF ASXL1 MUTATIONS IN MYELODYSPLASTIC SYNDROMES

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Background: Although additional sex comb-like 1 (*ASXL1*) gene mutations have long been reported in Myelodyspastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMML), the prognostic significance has been controversial.
Aims: Perform a meta-analysis to study impact of ASXL1 mutations on patients with MDS and CMML.
Methods: The identified articles were retrieved from some common databases. We extracted hazard ratios (HRs) for overall survival (OS) and leukemic-free survival (LFS) and P value of some clinical parameters, which compared *AXSL1* mutations and those without from the available studies. Each individual HR and P value was used to calculate the pooled HR and P value.
Results: 6 studies covering 1689 patients were selected for this meta-analysis. The pooled HRs for OS and LFS were 1.45(95%CI, 1.24-1.70) and 2.20(95%CI, 1.53-3.17), respectively. When considering CMML patients alone the HR for OS was 1.50(95%CI, 1.18-1.90). Additional, *ASXL1* mutations were more frequently found in male (P=0.008), older (P=0.019) and patients with lower platelets (P=0.009) or hemoglobin level (P=0.0015) and associated with other mutations such as EZH2, IDH1/2, RUNX1 and TET2.
Summary/Conclusions: *ASXL1* mutations were associated with poor prognosis in MDS, which may contribute to risk stratification and prognostic assessment in the disease.

E1216

CIRCULATING CLONAL CELLS ARE PLENTYFUL IN PERIPHERAL BLOOD OF PATIENTS WITH MYELODYSPLASTIC SYNDROME: A COMPARISON OF THE PERCENTAGE OF CLONAL CELLS IN PB AND BM BY FLUORESCENT IN SITU HYBRIDIZATION

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Background: Treatment response of MDS is determined by peripheral blood hemogram, bone marrow blast%, and cytogenetic results in BM. For the determination of treatment response, invasive BM study is required. We questioned whether the malignant cells are circulating or not in PB of patiens with MDS. We compared the percentage of clonal cells determined by fluorescent in situ hybridization (FISH) between PB and BM. If clonal cells are present in PB, monitoring of treatment response could be more easily accessible.
Aims: We aimed to investigate whether interphase fluorescence in situ hybridization (iFISH) clone size in peripheral blood correlate with bone marrow.
Methods: A tailored FISH panel (-5/5q-, -7/7q-, +8, -20/20q-, and +1/1q+) based on reported cytogenetic changes in Korean MDS patients, was performed in 98 patients with MDS at initial diagnosis. We performed corresponding FISH on peripheral blood in 28 patients in whom aberrant FISH results were observed in BM. The FISH probes used in the study were LSI EGR1 SpectrumOrange

(5q31)/D5S23, D5S721 SpectrumGreen (5p15.2), LSI D7S522 SpectrumOrange (7q31)/CEP7 SpectrumGreen, CEP8 SpectrumGreen, LSI D20S108 SpectrumOrange (20q12) and LSI Trisomy 1q (1q25) SpectrumGreen (Abbott,Downers Grove, IL, USA).
Results: Among 98 patients diagnosed with MDS at initial diagnosis, 56.1% (55/98 patients) showed positive result by 5 kinds of FISH probes. In 82.1% of patients (23/28 patients), clonal cells was detectable in PB and the mean percentage was 30.4% (range from 2.5% to 80.0%). The proportion of FISH-positive cells in PB was lower compared with the those in BM, and the mean percent difference between BM and PB was 30.5% (range from -11.2% to 45.0%). The proportion of FISH-positive cells in PB was lower compared with the those in BM, and the mean percent difference between BM and PB was 30.5% (range from -11.2% to 45.0%). However, the correlation of percentage value was high between PB and BM (r2=0.85); trisomy 8 (r2=0.85), 5q deletion (r2=0.85), 1q gain (r2=0.85) and 20q deletion (r2=0.85). In 3 patients with disease progression, percentage of clonal cells showed signficant interval change.
Summary/Conclusions: Our results suggest FISH monitoring of peripheral blood can be used as a potential candidate for monitoring tool of treatment response in MDS.

E1217

TREATMENT-EMERGENT ADVERSE EVENTS IN LENALIDOMIDE-TREATED LOW/INT-1-RISK MYELODYSPLASTIC SYNDROMES PATIENTS WITHOUT DEL(5Q) INELIGIBLE FOR OR REFRACTORY TO ERYTHROPOIESIS STIMULATING AGENTS

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Background: In the phase 3 MDS-005 study of lenalidomide (LEN) in RBC transfusion-dependent lower-risk non-del(5q) myelodysplastic syndromes (MDS) patients (pts) ineligible for or refractory to erythropoiesis-stimulating agents (ESAs), a significantly higher proportion of LEN-treated pts achieved RBC transfusion independence ≥8 weeks *vs* placebo (*P*<0.001).
Aims: To describe the frequency, timing, and management of treatment-emergent adverse events (TEAEs) in pts from MDS-005.
Methods: AEs of all 160 pts randomized to LEN who received ≥1 dose were reported per NCI-CTCAE v3.0.
Results: Median duration of LEN treatment was 164 days (range 7–1,158). Grade (G)1/2 and G3/4 TEAEs were reported in 96.9% and 86.3% of pts, respectively. The most common G3/4 TEAEs were neutropenia (61.9%), thrombocytopenia (35.6%), anemia (5.6%), pneumonia (5.6%); G3/4 deep vein thrombosis (DVT) occurred in 1.9%. G3/4 neutropenia and thrombocytopenia generally occurred in cycles 1–4. The most common G1/2 TEAEs were diarrhea (42.5%), constipation (22.5%), asthenia (21.9%), rash (21.3%), fatigue (20.6%), peripheral edema (20.6%). TEAEs led to 54.4% dose interruptions, 6.3% dose reductions, and 42.5% dose interruptions with subsequent reduction. Median time to first dose interruption or reduction was 57 days (range 6–504). The most common reasons for dose reduction following interruption were neutropenia (25.0%) and thrombocytopenia (16.3%); 1.9% of pts due to rash. TEAEs led to LEN discontinuation in 31.9% of pts; the most common reasons were thrombocytopenia (8.8%), neutropenia (4.4%), DVT (1.9%). In this short follow-up period, no increased incidence of AML or secondary primary malignancies was seen.
Summary/Conclusions: LEN has a predictable and manageable TEAE profile in this pt population. Diarrhea was the most common G1/2 TEAE; G1/2 TEAEs generally did not require dose reduction or discontinuation. The most common G3/4 TEAEs were neutropenia and thrombocytopenia; these occurred early and, in the majority of pts, reverted with dose reductions or interruptions, avoiding the need for discontinuation. The timing and characterization of TEAEs highlights the importance of frequent monitoring at the start of therapy.

E1218

IMPACT OF PREVIOUS TRANSFUSION AND COMORBIDITIES IN THE IPSS-R FOR OVERALL SURVIVAL

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Background: Myelodisplastic sindromes(MDS)are heterogeneus clonal hematological disorders, with different clinical outcome.The revised international prognostic score system(IPSS-R)showed improvement prognostic power as compared to IPSS. Disease and patient related factors not considered in IPSS-R are emerging as relevant for prognosis of individual patients with MDS.
Aims: This is a retropective cohort study to validate the IPSS-R and evaluate the impact of new variables in a MDS patients serie, wondering if adds any prognostic survival prediction at diagnosis.
Methods: Data from 339 patients diagnosed with MDS in 11 hospitals sited in Galicia, Spain from January 1,2007 to december 31,2011. End of the follow-up time was december 18,2012. The patients were registered in the central area citogenetic database and were reclassified according to 2008 WHO Criteria. Data of 30 patients with secondary MDS were included and CMML or AML diagnosys were excluded. Patients treatment was heterogeneous (conservative, supportive treatment, chemotherapy, IMIDs, hypometilant agents, BMT and others). The study was approved by the regional ethical committee.
Results: Population: 44% females and 56% males. Median age: 78 years (range 29-100). Median follow-up time: 1,7 years. Median survival time: 3,5 years. According to 2008 WHO classification: 14% CRDU, 11% RARS, 31% RCMD, 8% RCDMSA, 8% MDS(del5q), 12% RAEB-1, 12% RAEB-2,1% U-MDS. Included 1% fibrotic and 2% hipoplastic MDS. According IPSS, patients were classified as low risk: 43%, (median survival-MS-: 5 years), intermediate-1:37% (MS: 3,2 years), intermediate-2:16% and high risk: 4% (MS:5, 3.2, 1 and 0.3 years respectively). According IPSS-R: low IPSS risk patients were shifted to very low,low and intermediate risk (43%,53% and 4%. MS not reached, 4.4 and 3.6 years respectively).Intermediate-1 patients spread into very low(5%), low(54%),intermediate(31%), high, and very high risk (8% and 1%, MS:1 and 0.6 year). Intermediate-2 IPSS patients were classified into intermediate (26%), high (46%) and very high(28%) risk.High risk IPSS patients were staged in very high IPSS-R risk. The 3 and 5 year ROC survival curves and AUC was better for the IPSS-R than the IPSS(p<0.001)but not at 1 year. We studied 4 new variables at diagnosis to improve the survival prognostic IPSS-R prediction. Ferritin and LDH serum levels, comorbidities (measured by MDS-CI index)and previous RBC transfusión (at least two RBC in the 4 previous months).Univariate analisys not showed statistical significance for the serum LDH.The multivariate analisys didn´t it for serum ferritin levels.Data available for 327 patients for previous transfusion showed 46 individuals transfused (14%).Risk stratification according MDS-CI: 339 patients: 41% low risk,43% intermediate risk,16% high risk. The most frecuent observed comorbidities were: cardiac disease (35%), other tumour (15%) pulmonary (14%), renal (9%), hepatic (2%). ROC curves and AUC at 1,3 and 5 years showed better survival prediction power for IPSS-R if we added the two last variables individually and increase if we add the two variables simultaneously(p<0.001).

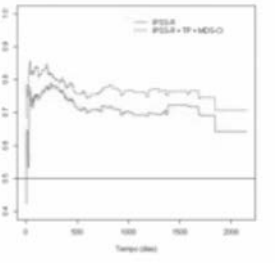

Figure 1.

Summary/Conclusions: IPSS-R provides pronostic power for OS compared to IPSS but not includes emerging relevant clinical events. Comorbidities exert a negative impact in life expectancy on MDS patients.Intrinsic MDS characterystics like anemia can worse patients sickness.Previous transfusion can reflect more aggressive disease or late diagnosis.Late iron develop has a negative impact in morbidity/mortality.Our study showed that adds the two variables improve the IPSS-R prognostic power for OS and helps in clinical decisión making.

E1219

ANALYSIS OF P53 EXPRESSION BY IMMUNOHISTOCHEMISTRY AS AN ADDITIONAL PROGNOSTIC TOOL IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background: Somatic mutations in *TP53* have been shown to be associated with aggressive disease, resistance to therapy and poor survival in many cancers including haematological neoplasms. The incidence of *TP53* mutations in Myelodysplastic Syndromes (MDS) is low (5-15%) compared to other malignancies but may be associated with adverse outcomes. Consistent correlations of up to 96% between *TP53* mutation by conventional sequencing and demonstration of p53 expression by immunohistochemistry (IHC) has been demonstrated by prior studies.
Aims: We assessed p53 expression by IHC on marrow trephine samples of 246 MDS patients to correlate the p53 expression with their clinical characteristics and outcomes.
Methods: IHC for p53 expression on marrow trephines of 246 MDS patients diagnosed between 2003 and 2014 in a single centre were analysed using DO-7 monoclonal antibody. Clinical information and overall survival including transformation to acute myeloid leukaemia (AML) were obtained from clinical notes and patient information systems. P53 expression was determined by assessing 1000 haematopoietic cells under high magnification and scored using a Modified Quick Scoring System.
Results: Thirty nine patients (16%) were positive for p53 expression and 207 patients (84%) were negative. P53 positive patients showed greater degree of peripheral cytopenias and higher marrow blasts. None of the patients with WHO subgroups of RARS, RCMD-RS or MDS 5q syndromes were p53 positive. P53 expression was positive in 5% of RA, 5% of RCMD, 18% of RAEB-1, 8% of RAEB-2 and 15% of MDS/MPN including CMML. P53 expression was significantly higher (51%) in patients with t-MDS (N=39). P53 positive patients showed a higher incidence of complex karyotype with ≥3 abnormalities (58%) and single/double abnormalities (40%). Only 7% of p53 positive patients had a normal karyotype. This is reflected by a higher proportion of patients with Int-2 and high risk IPSS (57% *vs* 14%) and with high and very high IPSS-R (53% *vs* 14%) in p53 positive patients compared to p53 negative patients. The median overall survival of p53 positive patients was 14 months compared to 33 months in p53 negative patients (p=0.000). The rate of AML transformation was 16% in p53 positive patients compared to 8% in p53 negative patients and the time to development of AML from MDS was shorter in p53 positive patients (9 months *vs* 20 months).

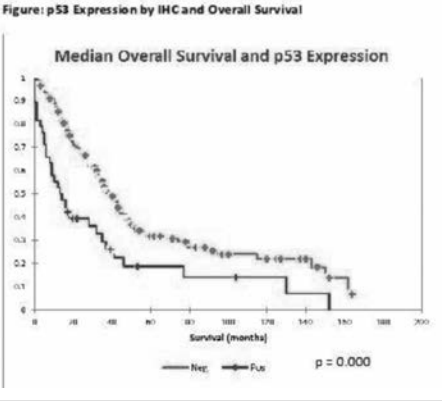

Figure 1.

Summary/Conclusions: Positive p53 expression of 16% in the MDS patients in this study reflected the incidence of p53 mutation published by prior studies. Positive p53 expression by IHC correlates with higher blast count, adverse WHO subgroup, complex karyotype, higher IPSS and IPSS-R, poor overall survival and increased risk of AML transformation. Analysis of p53 expression by IHC is a reproducible, rapid and inexpensive method and is a useful adjunctive prognostic tool in clinical practice.

E1220

A PROGNOSTIC SCORING MODEL FOR PATIENTS TREATED WITH AZACITIDINE FOR MYELODYSPLASTIC SYNDROMES/ACUTE MYELOID LEUKEMIAS

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Background: Azacitidine (AZA) is widely used in clinical practice for patients with higher-risk myelodysplastic syndromes (MDS), as well as a proportion of patients with acute myeloid leukemia (AML). However, the predictive factors of a poor outcome in AZA-treated patients with MDS or AML have not been fully evaluated.
Aims: To clarify the risk factors (RFs) for patient survival, we retrospectively analyzed data of patients treated with AZA for MDS/AML.
Methods: We analyzed the clinical backgrounds, treatments, responses (hematological improvement: HI), survival, and prognostic factors of 31 patients with MDS/AML (MDS=24, AML with myelodysplasia-related changes=7) who were treated with AZA at our institute from 2011 to 2015. Expression of p53 protein in bone marrow cells (BMCs) was assessed immunohistochemically using a monoclonal mouse anti-human p53 protein antibody (Clone DO-7); p53 protein overexpression was defined as positivity in >50% of immunoreactive blasts. Overall survival (OS) was estimated from AZA treatment initiation via Kaplan-Meier analysis and compared with a log-rank test. RFs associated with OS were evaluated using a univariate (chi-squared test) or multivariate analysis (Cox proportional hazards model).
Results: The patients included 24 men and 7 women with a median age of 73 (range: 44-89) years. One, 10, 9, and 11 patients belonged to the low, intermediate (int)-1, int-2, and high-risk International Prognostic Scoring System (IPSS) groups, respectively; 2, 11, 6, and 12 belonged to the low, intermediate, high (H) or very high (VH) revised IPSS (IPSS-R) groups, respectively. Complex karyotypes (CKs) and p53 protein overexpression were observed in BMCs from 9 and 7 patients, respectively. Patients received a median of 6 (range: 1-43) AZA cycles at a median interval of 35 (range: 28-56) days. Eighteen (58%) patients exhibited HI, and 14 (45%) died from disease progression. The estimated 4-year OS of all patients was 36.4%. RFs associated with OS in a univariate analysis were CKs (p=0.0079), p53 protein overexpression in BMCs (p=0.0309), high C-reactive protein level (p=0.0018), serum ferritin >500 ng/ml (p=0.0291), no HI (p=0.0018), blood transfusion dependency (p=0.0039), and IPSS-R H/VH risk group (p=0.0484). Multivariate analysis identified p53 protein overexpression in BMCs (hazard ratio [HR]=10.2, p=0.0366) and serum ferritin >500 ng/ml (HR=10.1, p=0.0113) as independent RFs for OS. Two OS risk groups were defined according to scores from these risk factors: low risk (0) and high risk (1-2). The OS curves of AZA-treated patients with MDS/AML were significantly stratified into 2 risk groups using our scoring model (p<0.0001, Figure 1).

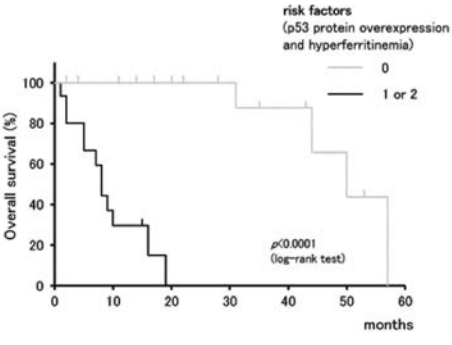

Figure 1. Overall survival for patients with MDS/AML treated with azacitidine according to our prognostic scoring model.

Summary/Conclusions: We demonstrated clear OS stratification of AZA-treated patients with MDS/AML into 2 risk groups according to our proposed scoring model using two risk factors, p53 protein overexpression and hyperferritinemia. Although further verification via prospective analysis is needed, these findings may provide valuable information for prognostic predictions of AZA-treated patients with MDS/AML.

E1221

THE IMPORTANCE OF IMMUNOPHENOTYPING OF HEMOPOIETIC PRECURSORS IN THE DIAGNOSIS OF CHILDHOOD MDS

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Background: immunophenotyping of BM hemopoietic precursors has been recognized as useful ancillary technique to confirm the diagnosis of adult MDS. In children, few data about its utility are available.
Aims: to analyze retrospectively the immunophenotypic features of bone marrow (BM) cells at diagnosis of patients registered in the Brazilian Pediatric Cooperative Group of Myelodysplastic Syndromes (2012-2015).
Methods: diagnosis of the patients was based on clinical data, peripheral blood (PB) counts, BM cytology and histology and cytogenetics. Deficiency anemias, autoimmune diseases and viral infections were excluded, as well as patients with Down's syndrome. Classification was made by the WHO Pediatric Classification. Four pediatric deficiency anemias were used as controls. Immunophenotyping was made by an 8 color platform to evaluate myelomonocytic maturation and progenitor populations according to the recommendations of the European LeukemiaNet.
Results: 32 patients (RCC=6 cases; RAEB/-t=13 cases; JMML=13 cases) and 4 controls (deficiency anemias) were analyzed. Median age: controls=20 months, RCC=66 months, RAEB/-t=68 months and JMML=29 months. The

median number of abnormalities in myeloid maturation was 1.5 (0-4); 3 (0-6) and 3 (1-5) respectively. The median number of abnormalities in $CD34^+$ cells were 2 (1-3); 2.5 (1-6) and 3 (1-5) respectively. So, the total number of phenotypic alterations was 4 (1-6); 3 (2-11) and 6 (2-9) respectively. The percentage of $CD34^+/CD117^+/CD13^+$ cells was 0.5% (0.1-2.8); 3.7% (0.5-8.6) and 4.2% (0.3-10.1) respectively, compared to 1.1% (0.7-1.6) of the control cases. Aberrant cross-lineage antigen expressions in myeloid progenitors were found in 63% of the cases of JMML, in 45% of RAEB/RAEB-t but in none of the RCCs. Hematogones type I ($CD34^+/CD19^+/CD10^+$) were decreased in all groups: 0.28% (0-2.4); 0.5% (0-6.4) and 0.03% (0-0.6) respectively, compared with the controls: 2.1% (0.9-3.2). The same occurred in%$CD34^-/CD19^+/CD10^+$ cells: 1.6% (0-15.5); 0.8% (0-6.2) and 0.02% (0.-2.1) respectively, compared with the controls: 12.0 (3.9-12.9). T lymphocytes were markedly decreased only in patients with JMML. In a discriminant analysis, a model containing age of the patient, percentage of $CD34^+/CD117^+/CD13^+$ cells, hematogones type I, T lymphocytes and total number of phenotypic alterations were able to classify correctly 81% of the patients.

Summary/Conclusions: in childhood MDS, alterations in myeloid precursors and $CD34^+$ myeloid progenitors presented similar changes as in adult MDS. However, the alterations in B cell precursors and T lymphocytes were more pronounced, even in very young children. Phenotypic abnormalities in JMML were more similar to those of RAEB, regardless of the number of BM blasts counted in cytology or any feature of PB counts.

E1222

FAVORABLE OUTCOMES WITH TUMOR BURDEN REDUCTION WITH HYPOMETHYLATING AGENTS BEFORE ALLOGENEIC HEMATOPOIETIC CELL TRANSPLNATATION FOR PATIENTS WITH HIGHER RISK MYELODYSPLASTIC SYNDROME

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Background: The use of hypomythylating agents (HMA) have improved the outcomes of myelodysplastic syndrome (MDS) in terms of hematologic improvement and long-term survival. HMA treatment while awaiting allogeneic hematopoietic cell transplantation (allo-HCT) or as a purpose of cytoreduction for higher risk MDS can be an attractive option with the hope of successful cytoreduction before allo-SCT. However, the role of induction response or cytoreduction for the patients with higher risk myelodysplastic syndrome (MDS) is not yet clearly defined.

Aims: In this sutdy, the clinical significance of tumor burden and transition of IPSS risk group for transplant eligible patients with higher risk MDS were evaluated.

Methods: The data of 79 transplant eligible patients (<65 years old) diagnosed with higher-risk MDS from Jan 1992 to Mar 2013 and received HMA as frontline therapy were retrospectively analyzed. To evaluate the effects of the tumor burden on treatment outcomes, treatment response (responder *vs* non-responder), IPSS risk group after HMA treatment, transition of IPSS risk group, and blast percentage in bone marrow after HMA treatment were evaluated.

Results: Among 79 patients, 30 patients (38.0%) performed allo-HCT (HCT group) and 49 patiens (62.0%) treated with HMA without allo-HCT (non-HCT group). Median follow-up duration was 778 days (range 143-2921 days) and 375 days (range 7-6561 days) in HCT group and non-HCT group, respectively (p=0.001). Three-year overall survival (OS) rate was significantly higher with HCT group (47.0±12.1%) than non-HCT group (19.6±7.0%, p<0.001), which confirmes the the role of allo-HCT for patients with higher risk MDS. For HCT group, the short duration until allo-HCT showed a better outcomes as regards OS (p=0.035). In the multivariage analysis, blast percentage ≥10% in bone marrow (HR 2.569, 95% CI 1.116-5.916, p=0.027) and IPSS higher risk prior to allo-HCT (HR 5.371, 95% CI 1.886-15.291, p=0.002) were found to be significantly correlated with the OS. However, IPSS transition did not affect the long-term outcomes (HR 0.703, 95% CI 0.079-6.285, p=0.752).

Summary/Conclusions: To predict the clinical outcomes of patients with higher risk MDS, the optimal time for tumor burden evaluation is prior to allo-HCT than at the time of initial diagnosis. For those with lower blasts in the BM or lower IPSS risk group were related with favorable OS. However, as early performance of allo-HCT was associated with favorable OS and IPSS transition did not affect the OS, it may not be resonable to delay allo-HCT to achieve better response to HMA for those who are planning to allo-HCT.

E1223

DISCONTINUATION OF HYPOMETHYLATING AGENT, DOES IT BRING REDUCED SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MYELODYSPLASTIC SYNDROME?

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Background: The myelodysplastic syndromes (MDSs) are a heterogeneous collection of clonal hematopoietic malignancies that primarily affect the elderly and are characterized by bone marrow failure, dysplasia. In previous study, prolonged treatment duration could have contributed to survival benefit. Although treatment with HMA undoubtedly prolongs survival in patients who have poor prognosis, there are still a significant proportion of patients with MDS who do not respond to therapy with HMA and patients who lose response or progress on therapy. However, in clinical practice, it is difficult to continue treatment of HMA because of problems such as toxicities, poor economics, comorbidities and compliance etc. For those reasons, discontinuation of HMA before disease progression often happen in clinical setting.

Aims: So there are some questions about survival in MDS patients treated with HMA in real clinical practice. Are there any differences of survival in patients with MDS received HMA between treatment failure and discontinuation of HMA ?. And what factors will be helpful to predict survival in both patients group ?.

Methods: The medical records of 246 patients were collected retrospectively from fourteen university hospitals in South Korea between January 2001 and October 2013. All included patients had been newly diagnosed to MDS and they were treated with HMA as front line therapy and they were treated continuously at least 4 cycles. Patients divided two groups into treatment failure group who discontinued HMA treatment due to disease progression and discontinuation of HMA group who stopped HMA treatment because of other cause without disease progression. The treatment free survival (TFS) was defined duration from the end date of HMA therapy to the date of disease progression, relapse, or death from any causes.

Results: The median age of the patients was 68 years. (range 24-92 years) and the male to female ratio was 1.9:1.0. The percentage of lower than 5% of bone marrow myeloblast was 37.9% in treatment failure group, 62.1% in discontinuation group (p=0.015). Patients who found lower risk IPSS or WPSS were documented in discontinuation group more than those in treatment failure group (p=0.050 or 0.005). The median TFS was 4.7 months (range 2.4-7.0) and the median OS was 34.8 months (range 28.4-41.2). In multivariate analysis, treatment failure of HMA is independent risk factor for shorter TFS (RR:5.996, 95% C.I. 3.684-9.757, p<0.001). Age of more than 65 years, bone marrow myeloblast of more than 5%, poor cytogenetics, platelet counts of less than 50 x 10^3/uL, higher risk of WPSS and IPSS-R, median number of HMA cycles of less than 7 cycles and treatment failure of HMA were independent risk factors for shorter OS. Especially, The 3-year TFS and OS were 0.0% *vs* 40.7%, (p<0.001) and 29.7% *vs* 60.2% (p<0.001) in treatment failure group *vs* discontinuation of HMA group, respectively. However, in univariate and multivariate analysis, higher risk of WPSS was independent risk factor for shorter TFS and higher risk of WPSS and median number of HMA of less than 7 cycles were independent risk factors for shorter OS in only discontinuation of HMA group.

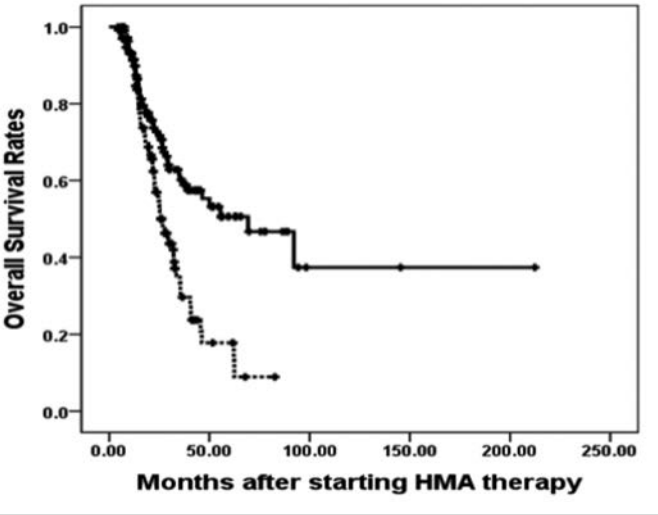

Figure 1.

Summary/Conclusions: Patients who discontinued HMA without disease progression showed prolonged survival than those who failed HMA treatment in real clinical practice. Especially, lower risk of WPSS and more number of performed cycles of HMA may be helpful to predict TFS and OS in patients who discontinued HMA because of other causes such as toxicities, economics or compliance etc.

E1224

HETEROGENEITY IN CYTOGENETIC AND CLINICAL FEATURES OF PATIENTS WITH MYELODYSPLASTIC SYNDROME AND DELETION 5Q

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Background: Deletion 5q [del(5q)] represents a distinct cytogenetic abnormality in patients with myelodysplastic syndrome (MDS). Although isolated del5q has been linked with favorable prognosis, the role of additional cytogenetic aberrations has not been clearly defined yet.
Aims: We aimed to study patients with MDS del (5q) in terms of cytogenetic and clinical features, as well as treatment outcomes.
Methods: We retrospectively analyzed data from 408 patients (pts) diagnosed with MDS and partial or complete deletion of the long arm of chromosome 5 in our centre from 2007 to 2014. All patients were studied by karyotyping and received standard of care by their treating physicians.
Results: Among 408 MDS pts, del(5q) was detected in 32 (7.8%). Isolated del5q was found in 7/32 (21%). Additional cytogenetic abnormalities were detected in 25/32 pts: chromosomal translocations of 1q, 11q and 20q and deletion of chromosomes 7, 17 and 18. Clinical data were available for 20/32 pts: 17 males: 3 females with a median age of 69.7 at diagnosis. MDS was de novo in 15 pts, post polycythemia vera in 2 pts and treatment-related in 3 pts (post chemotherapy±autologous transplant for Hogdkin lymphoma in 2 pts and for multiple myeloma in 1 pt). At diagnosis, median bone marrow blasts were 6% (range 0-19%). In the peripheral blood, median Hb levels were 8.7g/dl (8-11.7), white blood cell count 4.2K/μl (1.85-2.65) and platelets 131K/μL (26-330). No evidence of thrombocytocis was found. Cytogenetics revealed del(5q) as an isolated abnormality in 3/20 (15%) pts and additional abnormalities in the rest 17/20 (85%) pts. Patients with an isolated del (5q) presented no disease progression and 2/3 are alive in complete remission. One patient with isolated del (5q) succumbed from other causes. According to the IPSS (International Prognostic Scoring System), 6/20 (30%) pts were diagnosed as low and intermediate-1 risk; whereas, 6 pts were classified as intermediate -2 and 8 pts as high-risk. Lenalidomide treatment was initiated in 4 pts among which, 3 had additional cytogenetic abnormalities and classified as intermediate -1 (2 pts) and intermediate -2 (1 pt) risk. Median overall survival in pts treated with lenalidomide was 28 months (3-32) compared to 10 months (1-2) in pts not treated with lenalidomide. Other treatment options were: azacytidine (5 pts classified as high and 2 as intermediate -2 risk), erythropoietin (2 pts classified as intermediate -1 risk), low dose cytarabine (2 pts classified as intermediate -2 and high risk int-2) and hydroxyurea (1 pt classified as high risk). Allogeneic hematopoietic cell transplantation was performed in two pts with intermediate -2 risk according to IPSS.
Summary/Conclusions: Our study confirms that del(5q) as an isolated cytogenetic abnormality is rather rare in patients with MDS compared to del(5q) additional cytogenetic aberrations. Although the majority of additional cytogenetic abnormalities have been traditionally considered non high-risk, our findings link additional cytogenetic abnormalities with higher risk stratification and worse clinical outcomes.

E1225

VALUE OF THE MULTIPARAMETRIC FLOW CYTOMETRY IN LOW RISK MYELODYSPLASTIC SYNDROMES AND TYPE I CHRONIC MYELOMONOCYTIC LEUKEMIA

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Background: Heterogeneity in Low Risk Myelodysplastic Syndromes (MDS) and Type I Chronic Myelomonocytic Leukemia (CMML) is very remarkable and demands a maximum expertise to establish the diagnosis and prognosis. Multiparametric Flow Cytometry (MFC) should allow physicians to systematize and standardize this assessment.
Aims: To analyze, by using MFC, the value of the immunophenotypic changes in Low Risk MDS and type I CMML patients.
Methods: This retrospective study included low risk MDS and type I CMML adult patients (pts) at diagnosis, and used FAB, WHO, and IPSS classifications. An eight-color MFC and a specific MDS panel validated by Euroflow with Infinicyt® program were applied. Granulocytic maturation is classified into 3 groups according to precise stops or blocking maturation observed in myeloblasts (Mbl) (stage 1), in promyelocytes (Pro) and metamyelocytes (MT) (stage 2), and without any blocking or Neutrophil (N) (stage 3):

Stage 1 (Mbl)	Stage 2 (Pro+MT)	Stage 3 (N)
>3,5% or >2,8% + >Stage 2	>48% with < stage I	>58,5% with < stage 2

Blasts (CD34), erythroid, monocyte (Mo), and mast cells count (≥0,04), aberrance and blocking maturation were taken into account. Peripheral cytopenia, cytogenetic, AML progression, and mortality rate were also studied. Informed consent was obtained.
Results: Sixty-six pts with low risk primary MDS (n=49) and CMML (n=17) were assessed. Median age 71 (R 21-89), male/female gender: 37/29, WHO classification: RCMDRS (1), RCMD (37), 5q- (2), RARS (1), RA (4), RAEB-1 (5) and type I CMML (17). AML progression: 15%; mortality rate: 30%, and causes of death: infections (35%), AML (40%), comorbidities (20%) and second tumor (5%). Sixty-two pts were evaluable for blocking granulocytic maturation and were divided into 3 groups: stage 1 (20), stage 2 (16) and stage 3 (26). Stage 1: (20 pts), 31.3% (CD34+ 1.92; range: 0.14-6.59), overall survival: 34 months (median), p=0,001 and, stage 2 and 3 were p=ns. Progression to AML was similar among all groups (p=ns). CMML patients had an increased blocking maturation in promonocyte stage (p=0,05), but not showed difference in mature monocytes (p=ns). Excluding CMML pts, we found that immature monocyte group (<56%) was significantly associated with thrombocytopenia (p=0,038), without any incidence over other cytopenia and cytogenetic abnormalities (p=ns). The overall survival was decreased with mast cells count ≥0.04 (with a median of 34 months; p=0.04), independently of the staging maturation group. Erythroid Aberrancies did not show higher transfusion requirement rate (p=0,5).
Summary/Conclusions: MFC in low risk MDS and type I CMML proved to be objective and reproducible by standards. Immature monocyte in MDS group correlated with thrombocytopenia (p=0,038). Short overall survival was associated with high mast cells count (p=0.04) and early granulocyte stages blocking maturation (p=0,001).

E1226

CLINICAL AND HEMATOLOGICAL FEATURES OF PRIMARY MYELODYSPLASTIC SYNDROMES IN ADULTS AND CHILDREN

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Background: Myelodysplastic syndromes (MDS) are included into a heterogeneous group of clonal blood diseases characterized by peripheral cytopenias, dysplastic features of hematopoietic precursors, progressive deterioration and a high risk of transformation into leukemia. MDS occur in several versions that differ in frequency of appearance, the duration of the course and the probability of transformation into acute leukemia. There are differences in the structure of MDS variants in patients of different age. This is the basis for the discussion of clinical and hematological characteristics of primary MDS and systematization of the results.
Aims: Analysis of the clinical and laboratory characteristics of patients with primary MDS, depending on age.
Methods: The study included 162 (144 adults and 18 children) with primary MDS patients aged from 1.5 to 60 years (median age 49 years, adults, children - 8 years). The material of the study were clinical and medical history, peripheral blood (PC) and bone marrow (BM). Clinical criteria for inclusion of patients in the study: a verified diagnosis of MDS, setting variant of the disease in accordance with the criteria of the WHO classification of myeloid neoplasms (2008), written informed consent for inclusion in the study. The calculations are performed in the R version 3.1.3 statistical package.
Results: Primary MDS have a features of clinical - hematological manifestations in the age groups under 18 years, 18-39 years and 40-60 years, with the result that appears different variants of structure (p=0.028) and by IPSS risk categories (p=0.001). IPSS demonstrates a high prognostic significance in adult patients (p<0.001), in contrast to pediatric MDS (p=0.110). Most cases of MDS in children have been the normocytic anemia (MCV 86,5 (73... 103) fl). While MDS for adults, irrespective of age, a feature of dysplasia is macrocytosis (MCV 101,5 (83.. 123) fl), p<0.001. In children occur the hypocellularity BM in 22.2%. Adult patients have this characteristic in 2.8% of the age group of 18-39 years and there is no group in the 40-60 years of age (p<0.001). Erythroid hyperplasia BM and availability of microforms megakaryocytes BM are frequent signs of MDS in adults (72.2% and 94.4% in the age group 18-39 years, 62% and 92.6% in the age group 40-60 years, respectively). In children occur this feature only 22.2% and 66.7% of cases (p<0,001 and p= 0.002, respectively). Many young granulocytes in BM biopsy (p=0.022) and BM stroma fibrosis (p=0.036) more common in MDS in the age group 40-60 years compared to patients under 18 years and 18-39 years. A normal karyotype was detected in 71.8% of adults and 77.8% of children with MDS. In adult patients with MDS with increasing age decreases the frequency of normal karyotype with an increase in the num-

ber of unbalanced and complex aberrations. Children with MDS were observed with the same frequency the isolated and complex cytogenetic damage.
Summary/Conclusions: Patients with MDS of different age groups (children 18-40 years and 40-60 years) have variable manifestations of MDS, which is determined by the structure of variants, frequency of cytogenetic and dysplastic abnormalities BM cells, the intensity of the progression. Childhood MDS is a separate group of disease with features of clinical and hematological manifestations different from adults, which makes the feasibility of its version in the classification.

E1227

IS IT POSSIBLE TO DIAGNOSE LOW RISK MYELODYSPLASTIC SYNDROME (MDS) BY FLOW CYTOMETRY?
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Background: World Health Organization (WHO) classifies myelodysplastic syndromes due to patolological hallmark of marrow dysplasia. Flow cytometry immunophenotyping has a crucial role in diagnosis and management of hematological malignancies. Ogata et al. demostrated a simple flow cytometry (FCM) score which helped to establish the diagnosis of MDS in patients without specific markers of marrow dysplasia.
Aims: Our aim in this study is to evaluate the patients who had biopsied for diagnosis of MDS in 2015, confirm with pathology and restage them with FCM-score at Ankara University School of Medicine Department of Hematology.
Methods:: From 386 patients presented with cytopenias, 78 (20%) was morphologically diagnosed with MDS. Four parameters were combined in a regression model in FCM score; increased myeloblast-related cluster size, decreased B-progenitor related cluster size, aberrant CD45 expression and reduced granulocyte side scatter. All MDS diagnosed patients were reevaluated with FCM score. The diagnosis of MDS was formulated in the case that the value of the FCM-score was 2 or more. Chi-squared test was used for categorical variables.
Results: Median age of 78 patients was 62 (range, 18-90), 49 (62%) of them was male. Patient characteristics were shown in table. Totally 44 patients (56%) from 78 patients had FCM-score 2 or more. Patients with MDS without specific markers of marrow dysplasia (such as ring sideroblasts and/or clonal chromosomal abnormalities) were 34% of FCM score positive population. In MDS patients high FCM score (3 or 4) was found to be significantly associated with transfusion dependency (P=0.021) and resulting in higher WPSS risk (P=0.002).

Table 1.

Karyotype, n (%)		Treatments, n (%)	
Very good	2 (3%)	None	34 (44%)
Good	48 (62%)	ESA and/or Filgrastim	15 (19%)
Intermediate	13 (17%)	Tabelomid/Danazol	6 (8%)
Poor	8 (10%)	Azacytidine	23 (29%)
Very poor	7 (9%)		
WHO diagnosis classification, n (%)		Response to treatments, n(%)	
RA	26 (33%)	Remission	38(49%)
RARS	7 (9%)	Stable	4 (5%)
RCMD	18 (24%)	Progression	6 (8%)
RAEB-1	7 (9%)		
RAEB-2	20 (26%)		
R-IPSS, (%)		WPSS, (%)	
very low/low/intermediate /high/very high	18%/27%/23%/28% /14%	very low/low/intermediate/high /very high	28%/32%/12%/28%/17%

Summary/Conclusions: FCM score can be a new tool for low risk MDS patients without specific markers of marrow dysplasia.

E1228

CLINICAL CHARACTERISTICS OF PATIENTS WITH MYELOID NEOPLASMS FROM REGIONS CONTAMINATED BY DEPLETED URANIUM: 20 YEAR ANALYSIS
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Background: Depleted uranium (DU), a radioactive heavy metal, is used in military ammunition. It emits alpha particles and has the half life of 4.5 billion years. Studies have shown the potential health risks of contamination by DU by wounding, ingestion, and inhalation. *In vivo* and *in vitro* experiments showed the carcinogenic potential of DU by neoplastic transformation of human and mouse cells, leading to the development of myeloid neoplasms. It is assumed that the DU exposure induces genomic stability leading to carcinogenesis. During the war in Bosnia, DU ammunition was used in several towns, including the town of Hadzici. United Nations have measured the concentrations of depleted uranium and showed a significant increase in the municipality of Hadzici.
Aims: Increased numbers of patients with blood cancers was observed by hematologists in DU-stricken area, so we systematically analyzed hematological patients from this region in the last 20 years, from 01.01.1996.-31.12.2015.
Methods: Two regions were analyzed: DU stricken and a control region. As a control region, patients from a town with the same population number were used. Patient data from 01.01.1996.-31.12.2015 was collected including age at diagnosis, sex, address, blood parameters, cytogenetics, therapy, and survival.
Results: In the 20 year analyzed period from 01.01.1996–31.12.2015, we found 717 patients with hematological conditions (437 from DU-stricken town *vs* 280 from control town). There were 74 patients with myeloid malignancies (54 *vs* 20), 55 patients with non Hodgkin lymphoma (26 *vs* 29), 21 patients with Hodgkin lymphoma (9 *vs* 12), and 6 patients with ALL (4 *vs* 2). Among the myeloid neoplasms, MDS showed a 6 fold change. The median age at diagnosis for MDS patients was 50 *vs* 65. Male to female ratio was MDS 0.5 *vs* 1. Median age at diagnosis was 50 in DU-stricken area *vs* 65 in control region. Patients from the DU area showed the trends towards higher IPSS-R score (50% of patients had intermediate and 17% had very high score) compared to the control and international data.
Summary/Conclusions: Clinical parameters showed more severe course of myeloid malignancies in DU stricken area compared to the control and internatinal data. Further investigation is needed to elucidate the possible causes of stark increase in myeloid neoplasms in DU stricken area.

E1229

CLINICAL AND LABORATORIAL CHARACTERISTICS OF A COHORT OF PATIENTS WITH MYELODYSPLASTIC SYNDROME AND ERYTHROID HYPERPLASIA
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Background: Myelodysplastic syndrome (MDS) is a clonal disease of hematopoietic stem cells, which is a clinically and biologically heterogeneous group of disorders associated with ineffective hematopoiesis, abnormal differentiation, and cytopenias. Approximately one third of MDS patients progress to secondary acute myeloid leukemia (sAML). Erythroid hyperplasia is very common in MDS patients, the proportion of erythroid was frequently more than thirty percent,even fifty percent, compared to ten percent to forty percent in normal bone marrow. The Erythroid hyperplasia is characterized with relatively or absolutely increase in the number of erythroid precursor cells, erythroid maturity curve shifts to the left, increase in early/late erythrocyte ratio, propagation of erythroid blasts and erythroid precursor cells. Erythroid hyperplasia is an important characteristic of MDS, however, there is still a lack of a related study of erythroid hyperplasia's significance in MDS.
Aims: To investigate the clinical and laboratorial characteristics of patients with myelodysplastic Syndrome (MDS) and erythroid hyperplasia.
Methods: We defined MDS patients whose bone marrow was hypercellular, the proportion of erythroid was more than fifty percent, and the ratio of mature erythrocytes and nucleated erythrocytes was no more than 20 as MDS patients with erythroid hyperplasia.(MDS-E). The retrospective analysis comprised 102 patients with MDS-E from the first affiliated hospital of Suzhou university. We analyzed their clinical characteristics, karyotype, and the effect of erythroid hyperplasia on their prognosis.
Results: 48 of 102(47%)MDS-E patients had a variety of cytogenetic abnormalities. The most frequent involved chromosomes were chromosomes 8(39.5% of all abnormal karyotypes), chromosomes 7(23%), followed by chromosomes 1(16.7%) and 20(16.7%). Survival analysis showed that the level of hemoglobin make a influence on prognosis. The overall survival(OS) of MDS-E patients with the level of hemoglobin more than 70 g/L was longer than that of patients whose hemoglobin concentration was less than 70 g/L(*P*<0.001). Hematopoietic stem cell transplantation (HSCT) could improve OS of MDS-E patients(*P*<0.001). In the chemotherapy group, MDS-E patients receiving chemotherapy including Decitabine had superior OS over those who were not treated with Decitabine.(*P*=0.014).
Summary/Conclusions: MDS-E patients have their own unique biological features compared with other MDS patients. The level of hemoglobin concentration and erythroid hyperplasia in bone marrow can affect MDS-E patients' prognosis. The using of Decitabine in chemotherapy and HSCT can improve OS of MDS-E patients.

E1230

TP53 GENE MUTATIONS IMPACT ON OVERALL SURVIVAL OF PATIENTS TREATED WITH LOW-DOSE CLOFARABINE AS A SECOND LINE THERAPY DUE TO THEIR RESISTANCE TO AZACITIDINE

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Background: Patients with higher risk Myelodysplastic Syndromes (IPSS-INT2 and High; HR-MDS) failing treatment with hypomethylating agents (azacitidine and decitabine; HMA) have a very poor outcome with a median survival of 5 months. No standard treatment is defined in this setting. We have recently conducted a dose escalation trial of clofarabine, at a starting daily dose of 5 mg/m2, among patients with HR-MDS or AML evolving from MDS if failing prior azacitidine therapy (NCT01063257; Braun et al., ASH 2011). Among MDS/AML associated mutations, TP53 gene mutations confer a poor patients overall survival and a short response duration to HMA (Takahashi et al., Oncotarget 2016).
Aims: We analyzed these MDS/AML mutations in respect to drug response and overall survival in this second line therapy trial.
Methods: Twenty-seven patients with a median age of 71 years (62-90) were included after informed consent from December 2009 to July 2012. All were due to receive clofarabine at a dose ranging from 5 to 10/m2 either for 5 consecutive days or 5 doses over 10 days, for a maximum of 8 cycles. All study patients were analyzed by next generation sequencing for evidence of mutations in the following genes: TET2, ASXL1, RUNX1, DNMT3a, TP53, IDH1/2, NRAS/KRAS, CBL, ETV6, EZH2, FLT3-ITD, NPM, JAK2, MPL515, PTPN11, SETBP1, SF3B1, SRSF2, U2AF1 and ZRSR2. Gene mutations identified by NGS were checked by Sanger sequencing if necessary.
Results: Twenty patients (74%) had HR-MDS including 1 patient with Chronic Myelomonocytic Leukemia-2 and 7 (26%) patients had AML evolving from MDS (16F/11M). Fifteen (56%) patients had adverse karyotype including 9 patients with complex cytogenetics. Among 8 (29%) patients, a TP53 mutation could be detected, which was isolated in 4 cases and associated with a complex karyotype in 7/8 cases. Distribution of the other gene mutations detected in this cohort were as follows: ASXL1 (26%), DNMT3a (22%), RUNX1 (15%), TET2 (15%), SRSF2 (15%), U2AF1 (11%), SF3B1 (11%), EZH2 (7%), IDH2 (7%), ETV6 (7%), NRAS (3.5%), ZRSR2 (3.5%), FLT3-ITD (3.5%), PTPN11 (3.5%) and SETBP1 (3.5%). Four (15%) patients had no gene mutation detected, 8 (29%) patients had 2 and 6 (22%) patients had at least 3 or more mutations detected. Only the detection of TP53 mutations had a significant impact on overall survival with a median survival of 4.7 *versus* 8.4 months of TP53 mutated patients and unmutated patients respectively (p=0.0022). The two other most frequent mutations of the ASXL1 and DNMT3a genes in this population, did no impact overall survival even among patients unmutated for TP53. As expected, adverse karyotype was significantly associated with shorter overall survival (8 months *versus* 4.4 months; p=0.017). When excluding patiens with TP53 mutations, survival of patients with unfavorable cytogenetics was not significantly shorter when compared to patients with intermediate or favorable cytogenetics (7.2 months *versus* 10.9 months; p=0.31). Eight (32%) of 25 patients evaluable for response achieved response according to IWG 2006 criteria after 1 or 2 cycles of clofarabine (1 CR, 4 mCR+HI, 2 mCR and 1 HI) and 10 (40%) patients had stable disease. Overall survival was 11.1 months (CI 0.68-3.9) *versus* 6.7 months (CI 0.25-1.46) in responders and non-responders to clofarabine respectively (p=0.18). In this phase I low-dose clofarabine trial, TP53 mutations were detected among 7/19 non-responders and 1/8 responding patients (p=0.36) to clofarabine. Interestingly, 6 of the 8 patients mutated for TP53 had obtained a prior response to azacitidine before relapse. Mutations of ASXL1 (p=0.63) and DNMT3a (p=1) had no impact on response to clofarabine.
Summary/Conclusions: Presence of TP53 mutations, detected here in about 50% of patients with an unfavorable karyotype, appears to be the most powerful adverse prognostic factor for overall survival in this MDS/AML population selected by its resistance to azacitidine.

E1231

REPORT ON OUTCOME OF HYPOMETHYLATING THERAPY WITH ANALYSIS ON PROGNOSTIC VALUE OF REVISED-INTERNATIONAL PROGNOSTIC SCORING SYSTEM IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES OF LOWER-RISK BASED ON IPSS

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Background: The outcome of patients with lower risk myelodysplastic syndromes (LR-MDS) by International Prognostic Scoring System (IPSS) is very various and sometimes poorer than expected.
Aims: We retrospectively evaluated the prognostic value of revised-International Prognostic Scoring System (IPSS-R) in accordance with Hypomethylating therapy (HMT) response for precise prognostication.
Methods: The treatment outcome of 236 patients with IPSS LR-MDS who received HMT in the Korean MDS Working Party was retrospectively evaluated. The patients were reclassified to very low/low (VL/L), intermediate (INT), and high (H) risk groups by IPSS-R.
Results: According to the HMT response, 3 year overall survival (OS) did not differ between response group (43.4±0.8%) and stable group (Fig 1. 53.6±0.6%, p=0. 672). Although 25 patients (38.4%) out of 65 responders had a benefit to continued HMT with a median 14 cycles, H risk group was only 2 patients. Among 27 patients (41.5%) progressed to secondary failure, H risk group was most common. Indeed, 42 patients (20.8%) out of 236 patients with LR-MDS had changed to H risk group based on IPSS-R. Median OS was 63.8 months (range, 32.6-95 months) in VL/L, 35 months (range, 24.2-45.9 months) in INT, and 22.3 months (range, 16.6-27.9 months) in H risk group, respectively (Fig-2. p<0.001). Transformation to AML was 8 patients (8.8%) in VL/L, 12 (12.7%) in INT, and 10 (23.8%) in H risk group (p=0.049). On multivariate analysis, the following factors were associated with survival: age ≥65 (HR= 1.731, p=0.023), ECOG ≥2 (HR= 4.997, p<0.001), H risk group by IPSS-R (HR=3.054, 95% CI=1.714-5.441, p<0.001), blast ≥5% (HR= 2.070, p=0.035), P/VP cytogenetic risk by IPSS-R (HR= 4.501, p=0.006) and transformation to AML (HR=2.208, p=0.007).

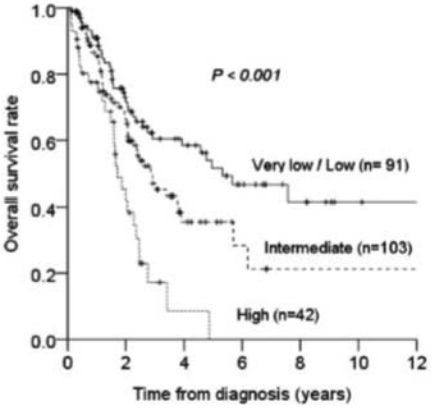

Figure 1.

Summary/Conclusions: If patients with LR-MDS are included in the H risk by IPSS-R, they should be considered early allo-HCT regardless of current benefits from HMT. Prospective study will be needed to establish the therapeutic strategies in MDS patients with Low-risk by IPSS including optimal timing of allogeneic transplantation and the continuing HMT.

E1232

SPRESAS (SPANISH REGISTRY OF ERYTHROPOIETIC STIMULATING AGENTS STUDY) SUBANALYSIS: IMPACT OF COMORBIDITIES AND CENTER LEVEL ON RESPONSE TO ESAS IN LOWER RISK MDS

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Background: Myelodisplastic syndromes (MDS) are heterogeneous diseases in old patients. These patients show very often comorbidities that could decrease the overall survival and the quality of life. Erythropoietic-stimulating agents (ESAs) are the most used treatment for low risk MDS with anemia and are available in almost all centers from the smallest ones to the big reference centers. The impact of the presence of comorbidities and the size of center where the treatment is administered on treatment response has not been still analyzed.
Aims: The main endpoint was to evaluate the impact of comorbidities on the response to ESAs treatment. The secondary endpoint was to evaluate if the size of the hospital where this treatment is administered influences on it.
Methods: Data from 530 patients with low or intermediate-1 risk MDS (according to FAB and WHO criteria) who received treatments with ESAs were recorded in SPRESAS. 458 of these patients had data available at diagnosis of MDS regarding comorbidities. These were classified as relevant (diabetes mellitus, atrial fibrillation, thromboembolic disease and renal impairement) and less rel-

evant (arterial hypertension, others). Data from the size of the center where the treatment was administered were available in all the patients. Centers were distributed in levels according to the number of beds available: centers above 1000 beds were considered "level 1" centers, between 500 and 1000 as "level 2" and below 500 beds as "level 3". Response was evaluated according IWG 2006 criteria.

Results: 370 patients (80.8%) had at least one comorbidity, but when only relevant comorbidities were analyzed, only 252 patients (55.0%) presented at least one comorbidity (table 1). The presence of one comorbidity relevant or not did not show any impact on the overall response to ESAs (HR=1.441; p= 0.138 for one comorbidity; HR=1.278; p=0.223 for one relevant comorbidity). However, curiously, the presence of two or more comorbidities was associated with statistically significant better response to ESAs (68.9% response in patients with two or more comorbidities *vs* 64.2% in patients with less than two comorbidities; HR=3.014; p=0.004), the only difference between both groups was ferritine level while other variables with impact on response were similar in both groups (PB and BM blasts, Hb level, cytogenetics, transfusion dependency, IPSS and EPO level). Additionally, the presence of two or more relevant comorbidities was also associated with a better response (75.2% response in patients with two or more relevant comorbidities *vs* 64.0% in patients with less than two relevant comorbidities; HR= 4.126; p=0.001). These 2 groups were different regarding ferritine and EPO level. When the impact of the different comorbidities was studied, only hypertension showed influence on the overall response rate: patients with hypertension had statistically significant better response to ESAs (72.7%) than those who did not have hypertension (61.5%); p=0.015, both groups had similar characteristics regarding variables with impact on response. Regarding center size, 213 patients (40.2%) were treated in "level 1"; 174 (32.8%) in "level 2" and 143 (27.0%) in "level 3" centers. When the center level was analyzed we could verify that patients who were treated in the smallest "level 3" centers had a statistically significant higher overall response rate to ESAs (81.7%) when compared with "level 2" patients (66.0%) and "level 1" patients (52.8%), HR=3.995; p<0.001; there were less patients in transfusion dependence in "level 3" centers than in the others while other characteristics were similar in the 3 groups. No differences were observed regarding duration of ESAs response (p=0.098).

Table 1. Comorbidities distribution.

COMORBIDITIES	YES	NO
Any comorbidity	370 (80.8%)	88 (19.2%)
Any relevant comorbidity	252 (55.0%)	206 (45.0%)
Two or more comorbidities	228 (49.8%)	230 (50.2%)
Two or more relevant comorbidities	106 (23.1%)	352 (76.9%)
Hypertension	204 (44.5%)	254 (55.6%)
Diabetes mellitus	109 (23.8%)	349 (76.2%)
Thromboembolic disease	26 (5.7%)	432 (94.3%)
Atrial fibrillation	65 (14.2%)	393 (85.8%)
Renal impairment	82 (17.9%)	376 (82.1%)
Concomitant hematologic disease	35 (7.6%)	423 (92.4%)
Other less relevant comorbidities	119 (26.0%)	339 (74.0%)
Other relevant comorbidities	128 (27.9%)	330 (72.1%)

Summary/Conclusions: The present study shows that ESA treatment should not be avoided in patients with lower risk MDS with comorbidities, as response rates are similar to those of patients without. Patients treated in the smallest centers had the best response rates to ESA.

LB2260

IMPACT OF PREVIOUS TRANSFUSION AND COMORBIDITIES IN THE IPSS-R FOR OVERALL SURVIVAL

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Background: Myelodisplastic sindromes(MDS) are heterogeneus clonal hematological disorders, with different clinical outcome. The revised international prognostic score system (IPSS-R) showed improvement prognostic power as compared to IPSS. Disease and patient related factors not considered in IPSS-R are emerging as relevant for prognosis of individual patients with MDS.

Aims: This is a retropective cohort study to validate the IPSS-R and evaluate the impact of new variables in a MDS patients serie, wondering if adds any prognostic survival prediction at diagnosis.

Methods: Data from 339 patients diagnosed with MDS in 11 hospitals located in Galicia, Spain from January 1,2007 to december 31,2011. End of the follow-up time was december 18,2012. The patients were registered in the central area citogenetic database and were reclassified according to 2008 WHO Criteria. Data of 30 patients with secondary MDS were included and CMML or AML diagnosys were excluded. Patients treatment was heterogeneous (conservative,supportive treatment, chemotherapy, IMIDs, hypometilant agents, BMT and others). The study was approved by the regional ethical committee.

Results: Population: 44% females and 56% males. Median age: 78 years (range 29-100). Median follow-up time: 1,7 years. Median survival time: 3,5 years. According to 2008 WHO classification: 14%CRDU, 11%RARS, 31% RCMD, 8% RCDMSA, 8% MDS (del5q), 12% RAEB-1, 12% RAEB-2,1% U-MDS. Included 1% fibrotic and 2% hipoplastic MDS. According IPSS, patients were classified as low risk: 43%,(median survival-MS-: 5 years), intermediate-1:37% (MS:3,2 years), intermediate-2:16% and high risk: 4%(MS:5, 3.2, 1 and 0.3 years respectively). According IPSS-R: low IPSS risk patients were shifted to very low, low and intermediate risk (43%, 53% and 4%. MS not reached, 4.4 and 3.6 years respectively). Intermediate-1 patients spread into very low (5%), low(54%), intermediate(31%), high, and very high risk(8% and 1%, MS:1 and 0.6 year). Intermediate-2 IPSS patients were classified into intermediate (26%), high (46%) and very high(28%)risk. High risk IPSS patients were staged in very high IPSS-R risk. The 3 and 5 year ROC survival curves and AUC was better for the IPSS-R than the IPSS(p<0.001)but not at 1 year. We studied 4 new variables at diagnosis to improve the survival prognostic IPSS-R prediction. Ferritin and LDH serum levels, comorbidities (measured by MDS-CI index)and previous RBC transfusión (at least two RBC in the 4 previous months).Univariate analisys not showed statistical significance for the serum LDH.The multivariate analisys didn´t it for serum ferritin levels.Data available for 327 patients for previous transfusion showed 46 individuals transfused (14%).Risk stratification according MDS-CI: 339 patients:41%low risk,43% intermediate risk,16% high risk. The most frecuent observed comorbidities were: cardiac disease (35%), other tumour (15%) pulmonary (14%), renal (9%), hepatic (2%). ROC curves and AUC at 1,3 and 5 years showed better survival prediction power for IPSS-R if we added the two last variables individually and increase if we add the two variables simultaneously(p<0.001).

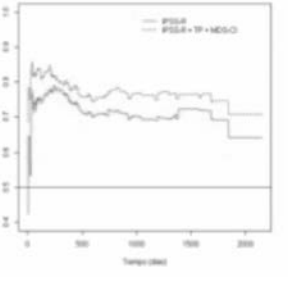

Figure 1.

Summary/Conclusions: IPSS-R provides pronostic power for OS compared to IPSS but not includes emerging relevant clinical events. Comorbidities exert a negative impact in life expectancy on MDS patients.Intrinsic MDS characterystics like anemia can worse patients sickness.Previous transfusion can reflect more aggressive disease or late diagnosis.Late iron develop has a negative impact in morbidity/mortality.Our study showed that adds the two variables improve the IPSS-R prognostic power for OS and helps in clinical decisión making.

LB2261

THE EFFECT OF MESENCHYMAL STEM CELLS DERIVED FROM DIFFERENT SOURCES IN COMBINATION WITH HAPLOIDENTICAL HSCT IN THE TREATMENT OF BONE MARROW TYPE RADIATION SICKNESS

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Background: Mesenchymal stem cells (MSCs) in the microenvironment can come from different origins and exhibit a diversity of properties. However, source-dependent and donor-dependent differences in MSCs properties, including their clinical application are still largely disputed.

Aims: To investigate the effect of the transplantation of mesenchymal stem cells (MSC) derived from different sources in combination with hematopoietic stem cells (HSC) in the treatment of bone marrow type radiation sickness.

Methods: 100 mice (CB6F1(H-2b×d)) were hybrids of C57BL/6 (H-2b) and BABL/C (H-2d), 8 weeks old, weight 18g-22g ,and were divided into 5 groups after 8.5Gy total body irradiation (TBI) of ^{60}Coγray, according to a completely randomized design, irradiation group (n=20), HSC transplantation group (n=20), human umbilical cord blood MSCs (HucbMSCs) and HSC cotransplantation group (n=20), bone marrow MSCs (BMMSCs) and HSC cotransplantation group (n=20), dermal MSCs (DMSCs) and HSC cotransplantation group (n=20). The clinical manifestation of mice were observed after trans-

plantation. The survival rate, pathological changes of BM, and other indicators were tested.
Results: Hair back , decreased activity, diarrhea and weight loss appeared at three days after irradiation. At the 11th day, all mice in irradiation group died while the amount of eating and drinking increased and body weight began to recover in the transplantation group. The clinical manifestation score of GVHD in cotransplantation group was lower than that in single transplantation. At 28th day after irradiation, the survival rate of mice in HucbMSCs group was 95%,which were 85%, 80% and 70% in BMMSCs group, DMSC group and transplantation group respectively. The hematopoietic reconstitution taperoffed in cotransplantation group, transplantation group, HucbMSCs group, BMMSCs group, and DMSCs group. The lowest value of hemogram were appear at 3-7d of WBC, 7-10d of PLT, and 10-14d of RBC after transplantation. The pathological change and the colony formation ability of BM were consistent with the change in hemogram.
Summary/Conclusions: MSCs could promote hematopoietic reconstitution and reduce GVHD effect in bone marrow type radiation sickness. HucbMSCs has better application prospect than others in bone marrow type radiation sickness.

Myeloma and other monoclonal gammopathies - Biology

E1233

IN MULTIPLE MYELOMA THERE ARE TWO SUBSETS OF IMMUNOSUPPRESSIVE POLYMORPHONUCLEAR NEUTROPHILS WITH INCREASED LEVELS OF ARGINASE

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Background: Multiple Myeloma (MM) is a plasma cell malignancy with a well documented immune dysfunction. Development of MM is associated with accumulation of Myeloid-Derived suppressor cells (MDSC), mostly represented by pathologically activated relatively immature polymorphonuclear neutrophils (G-MDSC). Our previous work showed that also mature polymorphonuclear neutrophils (PMN) are activated and immunosuppressive in MM.
Aims: We hypothesized that two populations of polymorphonuclear neutrophils are present in MM, with differential distribution after a Ficoll separation and differential immunosuppressive activity
Methods: Using oligonucleotide microarrays we first evaluated the gene expression profile (GEP) of PMN at the steady state in 10 MM, 5 MGUS and 8 healthy subjects matched for sex and age, identifying Arg-1 as the first gene differentially expressed in MM *versus* healthy PMN. Thus, we validated Arg-1 by RT-PCR, immunohistochemistry and circulating levels in serum by ELISA in a series of 60 newly-diagnosed MM patients, 30 MGUS and 30 healthy subjects in both G-MDSC and PMN. Finally, we tested the immunosuppressive properties of G-MDSC (isolated by CD66b+ immunomagnetic beads in the upper Ficoll layer) and PMN (sorted from the lower Ficoll layer) isolated from the same MGUS or MM patients, through functional assays, based on in vitro co-culture with T-lymphocytes from healthy subjects.
Results: MM-PMN exhibit an increased expression of ARG-1 compared to MGUS and healthy subjects (25.5 *vs* 6.2 *vs* 1 fold changes in gene expression, p=0.003), confirmed by functional assay of enzymatic activity of ARG-1, positively correlated with advanced disease. In MM patients, increased levels of ARG-1 were positively associated with advanced bone disease and unfavourable cytogenetics. Circulating Arg-1 in serum was higher in MM than MGUS and healthy subjects (183.6±21.9 *versus* 88.3±15.6 *versus* 98.8±11.4 ng/ml, p=0.0022). In MM patients there was a progressive increase from ISS stage I through III (p=0.003). Immunostaining showed that Arg-1 was increased in MM *versus* healthy subjects, but higher levels were evident in G-MDSC than PMN. Moreover, only in MM-G-MDSC Arg-1 was evident at the nuclear level, while in PMN Arg-1 had an exclusive cytoplasmic localization. This differential distribution of Arg-1 was functionally evident, since G-MDSC were more immunosuppressive than PMN. Indeed, after 72 hours of co-culture with T-cells obtained from healthy donors in presence of mitogen stimulation (PHA), MM-PMN were able to reduce T-cell proliferation at both tested 1:2 and 1:8 ratios (respectively 38.3±2.6% and 14.3±0.6%, p<0.0001), while MGUS-PMN induced a partial inhibition only at the 1:8 (25.4±4.3%, p=0.002). This effect was partially reverted with the treatment of 200 μM nor-NOHA, an Arg-1 inhibitor, since within first 24 hours T-cell proliferation increased in presence of MM-PMN (14.3±0.6 *versus* 24.5±1.3%, p<0.0001) and MGUS-PMN (25.4±4.3 *versus* 31.6±2.3%, p<0.0001). MM-G-MDSC were more immunosuppressive than their MM-PMN counterpart, since at 72 hours T-cell proliferation was 22.3±1.6% and 8.4±0.5% (p<0.0001) at 1:2 and 1.8 ratio respectively.
Summary/Conclusions: Polymorphonuclear neutrophils in MM are immunosuppressive, but distinguished in two main subpopulations, at different stages of maturation, based on the expression of Arg-1 and grains distribution, to target in order to improve the effects of the immunochemotherapy.

E1234

RESISTANCE TO PROTEASOME INHIBITORS IS MEDIATED BY A OVER ACTIVATION OF THE UPR AND THE DNA DAMAGE RESPONSE IN A PRECLINICAL MODEL OF MM

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Background: MM is still considered incurable partly due to the development of resistance.
Aims: We have developed a cellular model of resistance to proteasome inhibitors to investigate the underlying mechanisms.
Methods: Cells sensitive to proteasome inhibitors (MM1S, MM1R, RPMI-8226) were continuously exposed to increasing concentrations of bortezomib until

development of resistance. Viability was analyzed by MTT. Activity of different subunits of the proteasome was analyzed with Proteasome-Glo™ Assay Kit and presence of unfolded proteins with ProteoStat® Aggresome Detection Kit. Western-Blot, flow cytometry and qRT-PCR were also used.
Results: The development of acquired resistance to bortezomib in three cell lines was assessed by MTT assay with IC50 (nM) of 2.5 *vs* >100; 6.7 *vs* 78.5 and 21.2 *vs* >100 for the sensitive *vs* the resistant counterpart in MM1S, MM1R and RPMI at 24 hours. Interestingly, cells were also resistant to other proteasome inhibitors such as carfilzomib, oprozomib or ixazomib, indicating a class-effect resistance. In absence of bortezomib treatment, resistant cells presented a lower activity of the different subunits of the proteasome compared to the sensitive counterpart by 52% for caspase-like (C-L); 54% for trypsin-like (T-L) and 42% for chemotrypsin-like (CT-L) activities. This was even lower in case of MM1R (26%, 25% and 12%) and RPMI-8226 (21%, 40% and 23%) resistant cells. This inhibition led to an accumulation of unfolded proteins in the resistant MM1S cells as compared with their sensitive counterpart (96% *vs* 45%). Moreover, the concentration of bortezomib required to inhibit the proteasome was around 20-fold higher in the resistant than in the sensitive MM1S cells (x23; x21 and x27 for the C-L, T-L and CT-L activities). Finally, and most importantly, even with doses of bortezomib achieving a complete inhibition of the three subunits, resistant cells were viable, indicating they were not dependent on the proteasome and had developed alternative mechanisms of survival. In this context, resistant cells significantly upregulated the pro-survival chaperone BIP/GRP78, a critical sensor of the ER stress. This upregulation activated the three main arms of the unfolded protein response (UPR): increased levels of p-IRE1, ATF-6 and activation of PERK as observed through phosphorylation of its downstream substrate EIFα. A sequelae of the activation of the UPR is the transcriptional activation of chaperones, and, accordingly, there was increased expression of HSP-40 and, particularly, HSP-70 (but not HSP-90) in resistant cells. The ER stress also induced a DNA damage response with an increase in pH2Ax in resistant cells and a subsequent upregulation of p53 protein levels. Moreover, the increase in p-IRE-1 induced the nuclear translocation of NFkB, where it could exert its pro-survival and transcriptional function. In fact, we also observed an increase in the anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-1 and XIAP, some of them known substrates of NFkB. Interestingly, these resistant cells had an increased sensitivity to Pifithrin, a Hsp-70 and p53 inhibitor (6 hours Pifithrin treatment induced 69% *vs* 26% apoptosis in resistant *vs* sensitive cells).
Summary/Conclusions: We have demonstrated some of the mechanisms associated with resistance to proteasome inhibitors: activation of the UPR (with subsequent upregulation of the chaperone system), DNA damage response and NFkB signaling with increased levels of antiapoptotic Bcl-2 family members.
This work was supported by ISCIII-FIS PI15/00067 and /02156 and GRS 1175/A/15.

E1235

DEREGULATION OF A-TO-I RNA EDITING IS FUNCTIONALLY AND CLINICALLY RELEVANT IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a plasma cell neoplasm that remains high risk and incurable despite its therapeutic advancement. Adenosine-to-Inosine (A-to-I) RNA editing is a highly conserved post-transcriptional mechanism involving sequence alterations at the RNA but not the DNA level, mediated by ADAR family proteins (ADAR1, ADAR2 and ADAR3). RNA editing can result in recoding of the proteome, modification of mRNA stability and alteration in the miRNA biogenesis. In line with its physiological importance, an aberrant RNA editing activity has been implicated in various human cancers. However, the relationship between dysregulated RNA editing and the pathogenesis of MM remains elusive. Importantly, chromosome 1q21 which harbours the ADAR1 locus is amplified in more than 35% of MM, and this chromosomal abnormality is a poor prognostic marker. Considering the co-occupancy of other genes in 1q21, it is unknown if ADAR1 is an important driver gene.
Aims: We aim to define the RNA editome landscape of MM and to characterize the critical function of a disrupted editome in driving myelomagenesis. We would also like to delineate if ADAR1 is a critical gene in 1q21.
Methods: To establish the clinical significance of ADARs in MM, we analysed the publicly available myeloma datasets. Primary patient samples and human myeloma cell lines (HMCLs) were used as our study models. The level of ADARs was modulated through the highly-efficient lentivirus system. Systematic high-throughput RNA-sequencing is employed to investigate the genome-wide RNA editing status before and after ADAR1 knockdown and overexpression. The functional effects of differential ADARs expression were examined through cell viability (CTG), cell cycle (PI staining) and colony formation (Methocult semi-solid medium) assays.
Results: Comparing with normal plasma cells, MM samples showed a severely disrupted ADAR1:ADAR2 expression ratio, in which an overexpression of ADAR1 and downregulation of ADAR2 were observed. In correlation, we identified that there was a close association between 1q21 amplification and ADAR1 expression level, which consistently increases along the disease progression route, *i.e.* from normal plasma to MGUS, SMM and finally MM. Furthermore, analysis based on the disease subtypes revealed that the 4p16 and MAF subtypes (high-risk prognostic groups) displayed significantly higher ADAR1 expression than other groups, signifying its role in conferring an aggressive disease nature. More importantly, patients with high ADAR1 and low ADAR2 expression demonstrated a poorer overall survival. At the physiological level, knocking down and overexpressing ADAR1 in HMCLs affects the global editing events, as detected by RNA-sequencing. Gene specific validation demonstrated a differential degree of A-to-I conversion of bona fide ADAR targets, depending on the ADAR1 expression level. *In vitro* analysis implicated that both ADAR1 and ADAR2 can functionally affect MM growth and survival, whereby, ADAR1 behaves like an oncogene and ADAR2 displayed tumor suppressor properties. When the stable cell lines were treated with velcade, ADAR1-knockdown cells showed increased sensitivity while ADAR2-silenced cells were more resistant to the drug.
Summary/Conclusions: Considering that RNA editing is a widespread occurence in the human genome (85%), its biological significance in MM cannot be undermined. Our study unveils a potential novel role of ADAR-mediated-A-to-I-editing in conferring myelomagenesis. The functional relevance of ADAR1 and ADAR2 denotes that the RNA editing activity is clinically important and may represent an important therapeutic target for MM. Further characterization of the direct mechanisms of A-to-I editing will provide critical knowledge on whether this event would provide an extra layer of epigenetic deregulation to MM, leading to a worse disease prognosis.

E1236

TARGETING PROTEIN KINASE CK1A IN THE BONE MARROW MICROENVIRONMENT: A NEW POSSIBLE THERAPEUTIC APPROACH FOR MULTIPLE MYELOMA THERAPY?

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Background: Multiple myeloma (MM) is an incurable blood tumor arising from plasma cells. This malignancy is characterized by a multistep pathogenesis during which plasma cells progressively accumulate in the bone marrow (BM) and in the late stages of the disease spread to the peripheral blood and other tissues. Contacts between MM cells and the surrounding hematopoietic and non-hematopoietic (stromal) cells activate paracrine and autocrine intercellular loops, which support tumor cell growth and provide a protective *niche* against cytotoxic agents. Despite significant improvements in the overall survival over the last years, patients frequently develop resistance to many chemotherapeutic agents, therefore new curative strategies are urgently needed. Protein Kinase CK1α (CK1α) is a Ser/Thr kinase that we found overexpressed in a fraction of MM patients and cell lines. It is essential for the regulation of BM microenvironment dependent pathways (such as WNT/β-catenin and PI3K/AKT) important for plasma cell survival, suggesting a possible function of CK1α in the microenvironment dependent progression of this disease.
Aims: We aimed at evaluating whether or not CK1α inactivation in MM plasma cells could overcome the BM microenvironment protection, affecting cells growth and proliferation by impinging on survival signaling pathways associated to chemotherapeutic resistance. We also aimed to outline whether CK1α inactivation in the BM microenvironment context, could potentiate the therapeutic effect of drugs approved for the treatment of MM, such as Bortezomib.
Methods: Patient derived plasma cells and the BM microenvironment dependent MM cell lines INA-6 and SaMMi (a patient derived MM cell line, newly generated in our laboratory) were used. A model of BM microenvironment was established colturing MM cells with HS-5 stromal cells or with BM stromal cells (BMSC) from MM patients. RNA interference for CK1α was obtained through the generation of IPTG inducible MM clones. CK1 inhibition was obtained with D4476. Apoptosis was investigated with AnnexinV/PI staining and FACS analysis. CK1α downstream pathways were analyzed by WB.
Results: We found that CK1 inhibition caused apoptosis of MM cell lines and patient derived plasma cells. The presence of a defensive BM microenvironment did not protect MM cells from D4476-induced cell death. These results were confirmed also by RNA interference using siRNAs targeting the α isoform of CK1. We investigated also the effects of CK1α inactivation on the microenvironment-dependent prosurvival pathways WNT/β-catenin and PI3K/AKT and found a proteasome independent reduction of both β-catenin and AKT proteins expression. Furthermore CK1α inhibition enhanced Bortezomib induced cytotoxicity not only on plasma cells grown alone, but also on cells co-cultured with protective stromal cells.
Summary/Conclusions: Our results indicate that by impinging on critical microenvironment dependent survival pathways, CK1α inactivation leads to MM cell apoptosis and empowers Bortezomib induced cytotoxicity, overcoming stromal cell dependent protection and resistance to chemotherapeutic agents. These results might predict a possible use of CK1α inhibitors in the clinical setting as a new therapeutic approach for MM therapy.

E1237

RHOU GTPASE: A NOVEL POTENTIAL TARGET TO DISRUPT MULTIPLE MYELOMA PLASMA CELL INTERACTION WITH PROTECTIVE BONE MARROW NICHES

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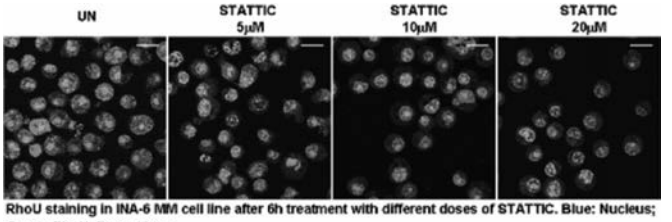
Background: Bone marrow stromal cell (BMSC)-produced soluble factors, like the IL-6 cytokine, may impinge on Multiple Myeloma (MM) intracellular signaling and cytoskeletal properties, protecting it from cytotoxic agents. Rho GTPases, in their active GTP-bound state, interact with effector proteins in order to control cytoskeleton remodeling, cell adhesion and polarization, and other essential processes for cell-cell interaction. The atypical Rho protein Wrch-1/RhoU displays spontaneous activation and is normally expressed at low levels in various tissues and organs. This protein might mediate the effects of the IL6R/STAT3 signaling in inducing filopodium formation and stress fiber dissolution, both critical steps in promoting cell motility. While typical Rho proteins (that share significant sequence homology with RhoU) as Cdc42 and Rac-1 have an established role in cancer, very little is known on RhoU in tumorigenesis, in particular in hematologic malignancies.
Aims: Since the IL6R/STAT3 signaling is of great importance in MM malignancy, we have endeavored to study RhoU expression in normal *versus* MM plasma cells. We also focused on understanding its localization and cellular function in these cells. Lastly, we aimed at unraveling the mechanisms through which RhoU expression is regulated in MM cells in the context of bone marrow (BM) microenvironment.
Methods: Malignant plasma cells were isolated from BM extracts or peripheral blood of MM patients using a magnetic CD138 positive selection kit. Normal B cells were purified using a magnetic human B cell isolation kit. mRNA expression was analyzed by qRT-PCR for MM cell lines, MM patients and normal B cells from healthy donors. Fluorescence microscopy was performed using Zeiss LSM 700 microscope and the following antibodies were used for staining: Phalloidin 594, rabbit anti-RhoU, goat anti nucleolin, anti-rabbit Alexa fluor 488, anti-goat Alexa fluor 594 and DAPI. STATTIC was used for STAT3 inhibition and the IL-6 cytokine was used to stimulate the IL-6R. Migration assays were set-up using 5mm transwells with standard controls, cell count was done by 3 minutes reads in FACS Canto.
Results: Here we provide data showing that RhoU is overexpressed in BMSC-dependent MM cells, but not in malignant plasma cells from the peripheral blood of Plasma Cell Leukemia (PCL) patients. We also demonstrate that its expression was positively modulated to the same extent by BMSC conditioned media and by IL-6, a major growth factor for MM cells. In MM cell lines, a blockade in STAT3 activation inhibited RhoU mRNA transcription in a dose dependent manner, and caused a clear impairment in migration/motility capacity of these cells. Interestingly, we observed an unexpected subcellular localization for this protein in the nucleolus of BMSC-dependent MM cells, which could be independent from STAT3 regulation.



RhoU staining in INA-6 MM cell line after 6h treatment with different doses of STATTIC. Blue: Nucleus; Green: RhoU; Bars: 10μm

Figure 1.

Summary/Conclusions: RhoU GTPase is widely overexpressed in malignant plasma cells from BM extracts of MM patients but not in cells purified from the peripheral blood of PCL patients. Its expression is positively modulated by IL-6 stimulus through the activation of STAT3 transcription factor. We describe for the first time a localization of this G protein in the nucleolus of MM cells. These results put to light the pleiotropic features that RhoU could display in this malignancy. We firmly believe that RhoU might be important in MM pathogenesis and could become a suitable target to disrupt the MM plasma cell interaction with protective BM niches.

E1238

DEREGULATION OF MAJOR DNA REPAIR MECHANISMS CORRELATES WITH CLINICAL OUTCOME OF ANTI-MYELOMA THERAPY. THE BENEFICIAL EFFECT OF DNA REPAIR INHIBITION

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Background: Deregulation of DNA repair pathways in malignant plasma cells mediates onset of disease and survival. The precise nature of dysregulated DNA repair and how it improves multiple myeloma (MM) cells survival or is co-opted during antitumor therapy is as yet unexplored and requires extensive study. If fully understood, DNA repair dysregulations could be exploited to selectively kill MM cells by targeting the remaining repair pathways that are critical for their survival.
Aims: We investigated the mechanistic basis for the link between DNA repair efficiency and response to anti-myeloma therapy.
Methods: We evaluated major DNA repair mechanisms [nucleotide excision repair (NER), interstrand cross-links repair (ICL/R), double-strand breaks repair (DSB/R)] in MM cell lines (melphalan-sensitive, RPMI8226; melphalan-resistant, LR5), peripheral blood mononuclear cells (PBMCs) from 25 healthy controls (HC; 14M/11F, median age 58 years), as well as bone marrow plasma cells (BMPCs) and PBMCs from 15 newly diagnosed MM patients (8M/7F; median age 61 years), responders (≥PR, n=9) and non-responders (
Results: Following ex vivo melphalan treatment, responders' primary cells (both BMPCs and PBMCs) showed significantly slower rates of NER and DSB/R compared to non-responders (P=0.0015). Similar rates of ICL/R were found in primary cells from all individuals. Moreover, apoptotic rates of the primary cells were inversely correlated with individual DNA repair efficiencies, being significantly higher in responders' cells compared to non-responders ones (P=0.0015). In line with these data, RPMI8226 cells showed slower rates of NER and DSB/R, but not ICL/R, and higher apoptotic rates than LR5 cells. Interestingly, co-treatment of all types of cells with DSB/R inhibitors significantly reduced the rates of DSB/R and increased melphalan sensitivity of the cells. Moreover, in untreated PBMCs from MM patients, we found an inverse correlation between the local chromatin condensation and the repair capacity of the cells. Particularly, we observed a progressive, significant increase in the looseness of the local chromatin structure, from HC to MM patients, with responders showing more condensed chromatin structure compared to non-responders (all P<0.05). Finally, microarray analyses of untreated PBMCs from MM patients consistently point to an altered expression of several DNA damage response-related genes in MM patients compared to HC. Particularly, responders' PBMCs showed upregulation of ATR, CHEK2, XPA, XRCC1 and CHEK2 genes and downregulation of ATM, MPG, UNG, CDKN1A and CDC25C compared to non-responders (in all cases, fold changes between groups were >2, P<0.001), suggesting that perturbation in the molecular components of DDR/R pathways also plays an important role in the therapeutic action of genotoxic drugs.
Summary/Conclusions: Responders to melphalan therapy are characterized by lower efficiencies of NER and DSB/R mechanisms resulting in higher accumulation of the extremely cytotoxic ICLs and DSBs lesions which in turn, triggers the induction of the apoptotic pathway. Moreover, the potentiation of melphalan cytotoxicity by DSB/R inhibition offers a promising strategy toward improvement of existing regimens.

E1239

BAX ACTIVATION IS A POTENTIAL BIOMARKER OF RESPONSE TO FILANESIB (ARRY-520) ALONE AND IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE

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Background: Pomalidomide in combination with dexamethasone (PD) has demonstrated activity in refractory MM patients, but novel combinations are needed to improve its efficacy. In this context, the kinesin spindle protein (KSP) inhibitor, filanesib (Arry-520), has demonstrated clinical anti-myeloma effect and previous data from our group and others have demonstrated the preclinical synergy of pomalidomide with dexamethasone and filanesib (PDF) what set the stage for the recently activated clinical trial POMDEFIL being run by the Spanish MM group investigating this combination in relapsed MM patients.
Aims: In this abstract, we gained insights into the mechanism of the combination and aimed at identifying potential biomarkers that could predict response.
Methods: MM cells were transiently transfected with non-targeting control short interfering RNA (NT-SiRNA), Bax siRNA ON TARGET plus SMART pool siRNA using the cell line Nucleofector Kit V. *In vitro* action was evaluated in MM cells by Annexin V staining using flow cytometry. The mechanism of action was analyzed by Western Blot, immunohistochemistry and immunofluorescence techniques.
Results: All 11 MM cell lines tested were sensitive to filanesib and sensitivity to this agent correlated with basal levels of Bax and Bax/Mcl-1 protein ratio. Subcellular fractionation of MM1S cells indicated that filanesib triggered translocation of Bax from the cytoplasm to the mitochondria after 24 and 48 hours, and its subsequent cleavage into the very potent proapoptotic 18 kDa fragment. A simultaneous decrease in the levels of Mcl-1 was observed. Knock-down of Bax in MM1S

by using small interfering RNA decreased the sensitivity of these cells to filanesib, as treatment with 10 nM for 24 hours induced apoptosis in only 26% of the siRNA-Bax cells as compared with 50% in the non-targeted cells (as compared with 58% *vs* 61% for bortezomib, used as a control for this experiment). Next, we evaluated changes of Bax protein levels in comparison with others in MM1S cells after treatment with vehicle, F, PD and PDF treatments. The levels of the proapoptotic protein, Bax, were significantly increased in both cytosolic and mitochondrial fractions with PDF treatment, as assessed by Western blotting after 12 or 48 hours of treatment. Of note, this event preceded the initiation of the apoptosis process as evaluated by cleavage of PARP and annexin V staining. Moreover, total cellular antiapoptotic protein Mcl-1 levels declined with the combination, although less than with F treatment and no significant changes occurred in the mitochondrial and cytosolic compartments by western blot. These results were confirmed *in vivo* in a model of subcutaneous plasmacytoma in tumors with big plasmacytomas, that after two days of treatment with vehicle, PD, F or PDF were sacrificed and tumors were fixed, paraffin-embedded and stained with anti-Bax and counterstained with haematoxilin for analyses. *In vivo* treatment with PDF also induced a clear increase in Bax that was mainly present in cells with aberrant monopolar spindles (a hallmark of the KSP inhibition induced by filanesib) and with an increase of apoptosis evaluated by TUNEL ($p<0.001$).

Summary/Conclusions: Bax protein is specific involved in the response to filanesib in monotherapy and plays a significant role in the synergy of its combination with pomalidomide and dexamethasone, being one of the earlier events observed. Therefore, Bax protein could be a potential biomarker of response to filanesib and also this synergistic combination.

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E1240

COMBINATION DRUG THERAPIES WITH THE NOVEL RNA POLYMERASE I INHIBITOR CX-5461 IMPROVE EFFICACY IN THE TREATMENT OF MULTIPLE MYELOMA

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Background: Ribosome biogenesis is dysregulated in malignancy, which is thought to contribute to cellular transformation and proliferation. Selective targeting of RNA polymerase I (Pol I), the enzyme responsible for transcribing the ribosomal RNA genes, with the novel small molecule inhibitor CX-5461 (Drygin *et al*., Cancer Res 2011) induces cell death using both p53-dependent and -independent pathways in both haematological and solid tumours. In a murine model of B-cell lymphoma, treatment with CX-5461 significantly increased survival (Bywater *et al*., Cancer Cell) and the drug is now in early stage clinical trials.

Aims: To determine the efficacy of CX-5461 in the treatment of multiple myeloma (MM), both alone and in combination with standard and emerging MM therapies.

Methods: A panel of human myeloma cell lines (HMCLs) were treated with CX-5461 prior to measuring proliferation, cell death and cell cycle distribution. Western blots were performed at serial time points for markers of DNA damage signalling and cell cycle control. CX-5461 was tested in combination with a variety of therapeutic agents that have demonstrated clinical or preclinical efficacy against MM.

Results: Testing CX-5461 against our panel of HMCLs reveals a wide range of sensitivity to Pol I inhibition. Our data show that sensitivity to CX-5461 is not solely dependent on proliferation rate or p53 status. Treatment of HMCLs with CX-5461 leads to a rapid increase in total and serine-15-phosphorylated p53 protein levels, in line with published data in other malignancies. Interestingly, CX-5461 induced phosphorylation of the checkpoint proteins Chk1 and Chk2, even in mutant p53 expressing cell lines, suggesting activation of p53-independent cell cycle checkpoints. The combination of CX-5461 with drugs having differing mechanisms of action shows increased inhibition of proliferation and induction of cell death compared to single agents alone. Increased efficacy is seen in combination with dexamethasone, everolimus, JQ1, ABT-199 and dinaciclib, with the most dramatic results being seen when CX-5461 is combined with bortezomib, carfilzomib or panobinostat.

Summary/Conclusions: Combination drug therapy is necessary to delay acquired drug resistance to single therapeutic agents and increase overall survival. Our data suggest that the novel Pol I inhibitor CX-5461 can be combined with a broad spectrum of agents, with bortezomib, carfilzomib and panobinostat showing the most promise.

E1241

CHARACTERIZATION OF A PERK KINASE INHIBITOR WITH ANTI-MYELOMA ACTIVITY

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Background: Due to the immunoglobulin production, multiple myeloma (MM) plasma cells are dependent on the unfolded protein response process (UPR), which controls protein production and ensures its proper translation and folding. A study by Michallet et al (2011) showed that knockdown of one of the three well-known arms of the UPR, PERK (protein kinase R (PKR)-like ER kinase) in MM cells resulted in autophagic cell death. This outcome indicated the important role of PERK activation for the metabolic shift of plasma cell to myeloma cell but also its ability to impede the apoptotic effect.

Aims: In this study we used a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding region, while displaying ≥385 fold selectivity over c-kit, Aurora B, BRK and many other kinases.

Methods: mRNA expression of PERK, CHOP and ATF4 in MM patients and cell lines was determined by real-time PCR. To deterine the effects of GSK2606414 on MM cell proliferation and apoptosis WST1 proliferation assay Annexin-PI staining was used respectively. To determine the effects of GSK2606414 in key genes of the UPR, UPR RT2 profiler PCR array was used.

Results: We initially screened 25 CD138+ MM patients and 6 human myeloma cell lines (HMCLs) for PERK mRNA expression. Our results showed that PERK mRNA is highly expressed in almost all patients (5-10 fold higher than the mean PERK expression of HMCLs). To test the effect of GSK2606414 on the proliferation of MM cells, 4 HMCLs were treated with different doses of GSK2606414 at two time points (24 and 48 hours). Treatment of cells with 3-30μM GSK2606414 resulted in a dose-dependent inhibition of cell proliferation in all HMCLs ranging for 20-95% reduction of proliferative activity, thus, indicating the dependency of these cells on PERK activity. Treatment with GSK2606414 at 20μM in H929 and L363 HMCLs for 24 hours resulted 25% and 15% increase in apoptotic cells by Annexin-PI staining respectively compared to the untreated cells. However, the most important finding was a significant synergistic effect of GSK2606414 with bortezomib in these cells. Specifically when H929 and L363 cells were treated with 5nM bortezomib in combination with 20μM GSK2606414, synergistic effect was seen where apoptotic cells reached 99% and 77% respectively, compared to bortezomib-treated cells (87% and 42% respectively). To determine the gene target effects of GSK2606414, ATF4 and CHOP mRNA expression levels were determined in H929 cell line after 24 hour of treatment. Treatment with GSK2606414 alone did not alter the expression levels of CHOP but reduced more than 50% the expression levels of ATF4. When combined with bortezomib CHOP and ATF4 levels were reduced 20% and 60% respectively while treatment with bortezomib alone increased the levels of CHOP and ATF4 by 50-100%. H929 and L363, were pre-treated with GSK2606414 and then subjected to ER stress conditions by treatment with tunicamycin (TM). In the presence of exogeneous UPR inducers, such as TM, GSK2656157 had a significant effect on the expression of the above genes at much lower IC_{50} range (≤100 nM) suggesting the in a more active UPR (sustained ER stress) the effects are more pronounced. Thus, the combination of GSK2606414 with bortezomib may inhibit one of the mechanisms of MM cells to escape ER stress induced by bortezomib. To determine the effects of the GSK2606414 on myeloma cells, a UPR RT^2 Profiler PCR array containing 84 key genes for the UPR pathway, was used in order to screen for differentially expressed genes between treated and non-treated cells. Results of this array will be presented at the meeting.

Summary/Conclusions: In conclusion, given the on-target pharmacological effects of PERK inhibitor on MM, development of PERK inhibitors may offer a therapeutic advantage that would affect MM pathogenesis and treatment.

E1242

EXPLORING THE BONE MARROW NICHE IN MULTIPLE MYELOMA USING AN ADHESION-INDEPENDENT THREE-DIMENSIONAL CO-CULTURE MODEL

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Background: The interaction between malignant plasma cells and their bone marrow (BM) niche is crucial for MM pathogenesis. The BM niche provides a specific tumor microenvironment which leads to quiescence, drug resistance and ultimately progression of myeloma. We and others have previously observed that reduced sensitivity to bortezomib, pomalidomide or vorinostat in the presence of BM stromal cells (BMSCs) can be restored by adding the adhesion inhibitors AMD3100 and NOX-A12. However, little is known about the mechanisms of how the MM niche mediates protection.

Aims: Our focus was to develop a novel bone-derived *in vitro* 3D co-culture platform and assess the cellular composition and function of the BM niche by studying the effects of niche cell subfractions on MM growth.

Methods: An adhesion-independent three-dimensional co-culture model was used to mimic the BM niche. This model consisted of an agarose matrix interlayer containing 100 microwells/cm² (Fig.1A). Each microwell was 1.5mm in depth and permeable for oxygen and cytokines, but not for BMSCs (Fig.1B). U266, RPMI-8226, OPM-2 and primary BM patient (pt) cells were utilized with

and without (w/o) HS-5 *vs* M210B4 stroma support (Fig.1C.a+b: stroma-support effect and pt characteristics). Analyses included trypan blue, Annexin/PI, MTT, FACS, cell cycle analyses and H2B-mCherry/cytochrome c-GFP assays (Udi, BJH 2013). To examine the effect of distinct BM niche cell populations on MM growth, niche cell subsets from C57BL6J mice were acquired, digested and FACS-sorted to collect cell subfractions of mesenchymal stem and progenitor cells (MSPC), endothelial cells, osteoblasts, premature CD146+ MSPCs (PαS) and CXCL12-abundant reticular cells (CaRs; Fig.1C.d).

Results: Human MM cell lines (MMCLs) and pt samples were cultured with 1, 10 or 100 cells/microwell, showing a growth advantage with *vs* w/o stroma support. Pt samples after 7 days (d) of culture benefitted from both murine M210-B4 and human HS-5 co-culture (Fig.1C.a). Expression of the chemokine CXCL12 and its corresponding receptor CXCR4 in U266 cells decreased after 7d with stroma support, thereby reflecting the dynamic regulation of the CXCL12-CXCR4 axis *in vitro* (Fig.1C.c). FACS-sorting of murine bone and BM cells led to valid subset separation, illustrated by a multipolar morphology of CD31+ endothelial cells and CD31-, CD45-, Sca1+ MSPCs with fibroblast-like bipolar appearance (Fig.1C.e), whereas PαS cells remained undifferentiated and positive for CD146. We observed that C57BL6J-derived murine cells differed in terms of their growth support for OPM-2 cells after 6d of co-culture: MSPCs from murine BM were more beneficial than those derived from murine bone (Fig.1C.f). BM-MSPCs also induced stronger support than premature MSPCs and CXCL12-abundant reticular cells, commonly considered as crucial mediators of adhesion in the murine niche. Current analyses focus on the generation of BM cell subsets from both MM pts and non-MM pts, thus overcoming the limitation of using C57BL6J mice.

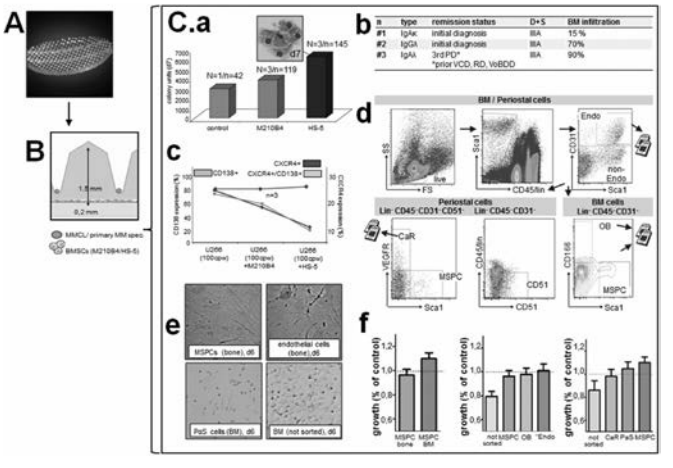

Figure 1.

Summary/Conclusions: We present a 3D culture model which reflects stromal protection for MM cells and may prove more reliable for *ex-vivo* drug screening and longitudinal studies. Assessment of the cellular composition of the MM niche in comparison to the normal BM microenvironment will help to better understand and selectively target stroma-mediated protection in MM.

E1243

ACTIVATION OF TOLL-LIKE RECEPTOR 4 PROMOTES MULTIPLE MYELOMA CELL GROWTH AND SURVIVAL THROUGH UPR PATHWAY AND IN A CASPASE-DEPENDENT MANNER

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Background: Toll-like receptor family (TLR) of receptors is an essential part of innate immunity. Expression and function of TLRs in Multiple Myeloma (MM) has recently become the focus of several studies and although the regulatory role of TLRs in MM plasma cells has been reported, the molecular mechanism remains unclear. During infection, human immune cells sense the presence of invading pathogens through TLRs which then activate transcriptional programs that orchestrate adaptive responses and induce the endoplasmic reticulum (ER) unfolded protein response (UPR) to accommodate essential protein translation. Studies have shown that prolonged ER stress occurs in response to microbes and specifically when cells are exposed to lipopolysaccharide (LPS), a TLR4 activator. The prolonged stress, possibly arising from a massive increase in protein synthesis, has shown to suppress CHOP, an apoptosis biomarker, in ER-stressed macrophages, while low levels of CHOP expression promote B cell survival.

Aims: The aim of our study was to investigate TLR4 signaling in myeloma cells and to explore possible implications with endoplasmic reticulum unfolded protein response as a potential mechanism of drug resistance.

Methods: mRNA expression of TLR4 and CHOP a in MM patients and cell lines was determined by real-time PCR. To deterine the effects of LPS on MM cell proliferation and apoptosis WST1 proliferation assay and Annexin-PI staining was used respectively. Protein expression of CHOP, ATF4, peIF2a, NFkB and caspases was assesed by immunoblotting analysis.Bottom of Form

Results: We found that TLR4 mRNA is expressed at increased levels (2-10 fold) both in human myeloma cell lines (HMCL) and primary cells. To investigate whether TLR4 signaling may suppress CHOP expression during sustained UPR response JJN3 and U266 cells, were pre-treated with LPS (5 ug/mL) and then subjected to ER stress conditions with a typical ER stressor, tunicamycin (TM). We found that ER stress-induced CHOP expression was suppressed by prior engagement of TLR4, by LPS pre-treatment. To test the relevance of these results, pre-treated LPS and TM samples were also subjected to Annexin-PI staining to determine the amount of apoptosis. As expected, pre-treated LPS HMCLs exposed to TM had almost 30% lower Annexin-FITC stained cells compared to the TM-stressed cells only. Thus, downregulation of CHOP by TLR4 ligands may confer resistance to apoptotic stimuli and promote the growth and survival of MM cells. To determine the potential therapeutic impact of TLR4 signaling in myeloma, we evaluated the activity of bortezomib in LPS pre-treated above cell lines.LPS pre-treatment partially abrogated the efficacy of bortezomib in these cell lines by decreasing its anti-proliferative effects compared to the non-LPS-pretreated cell lines as tested by the WST1 assay. In addition extract of cells were immunoblotted for CHOP protein expression. Pre-treatment of MM cells with low dose LPS selectively suppressed CHOP in bortezomib treated cells compared to the non-LPS treated cells. CHOP suppression was also accompanied with suppression of PERK, ATF4 and peiF2a, protein expression. In order to determine whether the escape from the apoptotic pathway which was achieved by LPS was preceded in caspase-dependent manner, the protein expression of caspase-8 and caspase-9 was explored upon treatment with LPS. Indeed, LPS stimulation was able to partially inhibit caspase-8 and caspase-9 expression in bortezomib-induced JJN3 cells. Furthemore pretreatment of LPS was able to completely abort NFκB inhibition induced by bortezomib. Then, we examined the impact bortezomib therapy on TLR4 and CHOP mRNA expression in primary tumors cells, collected before and at day 7 after bortezomib-based therapy from 6 myeloma patients. In 5 out of 6 cases TLR4 expression was significantly up-regulated and was accompanied with a coupled down-regulation of CHOP mRNA expression.

Summary/Conclusions: In conclusion, our data suggest that the TLR4 signaling pathway might provide a translational control pathway which disables cells to undergo the programmed cell death by apoptosis via CHOP branch. Further exploration of this pathway is needed to establish its role as a potential mechanism of drug resistance of MM cells which may direct future development of novel therapeutic targets.

E1244

LONG NON-CODING RNA MALAT1 WAS ASSOCIATED WITH PROGRESSION OF MULTIPLE MYELOMA AND INDUCED BY CELLULAR STRESS

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Background: Recent transcriptome-wide analyses have revealed an overwhelming amount of transcribed but not translated non-coding RNAs capable of influencing diverse cellular processes such as proliferation, apoptosis, and motility. Long non-coding RNA (lnc RNAs), which are commonly defined as transcripts >200 nt in length, have emerged as a class of key regulatory RNA. LncRNAs are deregulated in diverse human cancers and associated with disease progression; however little is available in multiple myeloma (MM).

Aims: To elucidate the role of lnc RNA in MM, we studied expression pattern of several well-known lnc RNAs in plasma cells of MM, MGUS and plasmacytoma, and function in MM cell lines in vitro. Moreover, to reveal the distinct lnc RNA signature more comprehensively, we performed next generation sequencing based RNA sequencing, which is potentially able to discover novel lnc RNAs.

Methods: Purified CD138+ plasma cells from bone marrow (BM) mononuclear cells obtained from 110 of MM patients, 48 of MGUS patients, 19 of control BM and 5 of extramedullary disease (EMD) are subjected to the study after obtaining informed consent. The expression levels of lnc RNAs were determined by RQ-PCR. RNase H-activating LNA™ GapmeR antisense oligonucleotides were used to knockdown lnc RNA in vitro in MM cell lines. The cell lines were treated with bortezomib, MG132, doxorubicin to evaluate the effects of cytotoxic stress for the lnc RNA expression. RNA sequencing was performed with Illumina NextSeq 500. This study was approved by IRB of Gunma University Hospital under Declaration of Helsinki.

Results: We found significant higher level of MALAT1 expression in BM plasma cells of MM compared to MGUS and control (control *vs* MM, p<0.001; control *vs* MGUS, p=0.01; MGUS *vs* MM, p<0.001). MALAT1 expression in EMD was higher by 140 fold than BM MM cells. MALAT1 expression was higher in MM with t(4;14) and del 17p (p=0.049, p=0.03), but was not different among ISS (p=0.87). Higher MALAT1 expression is associated shorter progression free survival (p<0.05), and tendency of shorter overall survival (p=0.09). MM cell

lines KMS12PE, OPM2, KMS11 treated with bortezomib showed elevated MALAT1 expression by 4.3 -21.8 fold and ANRIL by 2.2-4.7 fold. Cytotoxic drug doxorubicin also elevated both lnc RNAs in the cell lines. MALAT1 knockdown by GapmeR did not affect cell proliferation. The reported cell motility genes HMMR, CTHRC1 and ROD1 did not alter in MALAT1 knockdown MM cell lines, but RNA sequencing revealed the changes of many gene expressions. Finally, RNA sequencing of MM, MGUS and EMD samples revealed distinct lnc RNA expression signature as well as protein coding genes.
Summary/Conclusions: Significant upregulations of lnc RNAs MALAT1 might be associated with MM progression. Given that MALAT1 is associated with lung cancer metastasis, MALAT1 might be strongly associated with EMD formation. Genotoxic and ER stress induced by therapeutic drugs might upregulate MALAT1 leading extramedullary extension. Revealing distinct lnc RNA signature of MM is current important issue to clarify molecular mechanisms underlying MM progression and to development novel therapies.

E1245

COMBINATION OF T(4;14), DEL(17P13), DEL(1P32) AND 1Q21 GAIN FISH PROBES IDENTIFIES CLONAL HETEROGENEITY AND ENHANCES DETECTION OF ADVERSE CYTOGENETIC PROFILES IN 233 NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Cytogenetic analyses play a leading part in the risk stratification of MM due to the prognostic and therapeutic impacts of cytogenetic abnormalities. However, with a metaphase cytogenetic approach only 35% of patients present abnormal karyotypes, often associated with an advanced stage of the disease. Practice guidelines now recommend interphase fluorescence in-situ hybridization (FISH) on isolated CD138-expressing plasma cells (PC) as the initial cytogenetic analysis for MM. The most pertinent markers target the deletion of 17p13 (*TP53* deletion) and the t(4;14)(p16;q32) *FGFR3-IGH* translocation, and partial aneuploidies of chromosome 1 (1q21 gain and 1p32 deletion) are retained as more relevant additional markers.
Aims: Our aim was to fix the FISH combination of del(17p13), t(4;14), 1q21 gain and del(1p32) in a prospective study of 233 newly diagnosed MM with an analysis of the association between abnormalities and the number of clones.
Methods: Between January 2013 and August 2015, 233 BM samples were collected from 233 patients during diagnostic at the Cytogenetic Laboratories in Valenciennes General Hospital, and Versailles General Hospital. The institutional ethics committee approved the study. PC were enriched from BM mononuclear cells, using a magnetic cell sorting CD138 MicroBeads kit (Miltenyi Biotec; BergischGlabach, Germany). The FISH panel included *TP53/CEP17* probe (Amplitech, Compiègne, France), *1p32/CDKN2C-FAF1-1q21/CKS1B* probe (Amplitech), t(4;14)(p16;q32) probe (MetaSystems, Altlussheim, Germany), and *IGH* break-apart probe (MetaSystems). Technical thresholds were determined for each probe using isolated CD138-expressing PC from patients without MM, on the basis of the same method as patients with MM. Thresholds were assessed after counting 200 cells for each negative sample, and established by "mean+3 DS" calculation.
Results: Cytogenetic abnormalities were identified in 79.0% of cases, with one or more adverse abnormalities in 51.9%. We observed a del(17p13) in 15.0%, a t(4;14) translocation in 11.5%, a 1q21 gain in 37.8%, and a del(1p32) in 8.7% of patients with statistically significant associations between 1q21 gain and t(4;14) ($p=0.001$), and del(1p32) and del(17p13) ($p=0.01$). Adding 1p32/1q21 FISH probe has enabled us to identify one or more adverse abnormalities in 39.0% of patients with absence of *TP53* deletion or t(4;14). Clonal heterogeneity was observed in 51.1% of cases. Adverse abnormalities were significantly more frequent when the number of clones was greater than or equal to 2, with a frequency of 85.1% against 45.6% when 1 single clone was identified ($p<0.0001$). We observed a greater number of MM with 1q21 gain when clonal heterogeneity was present (≥2 clones): 81.6% *versus* 18.4% when 1 single clone was identified ($p<0.0001$). In the case of marked clonal heterogeneity (≥3 clones), a higher involvement of del(1p32) was found with a frequency of 28.0% against 5.8% when only 2 related clones were present ($p=0.002$). In the subgroup $t(4;14)^+/1q21^+$ with 2 or more identified clones (n=14), 1q21 gain was found to be significantly more often present in the minor clone compared to the clone with t(4;14) (10/14) ($p=0.01$).
Summary/Conclusions: We were able to identify adverse abnormalities or derivative anomalies, and related clones or clonal evolution by FISH analysis. We confirm the presence of clonal heterogeneity and accumulation of adverse abnormalities in the first diagnostic analysis. The prognostic impact of these parameters should be evaluated, and could be included in cytogenetic classifications.

E1246

EXPRESSION OF HUMAN CRBN SENSITIZES MOUSE MULTIPLE MYELOMA CELLS TO LENALIDOMIDE

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Background: The immunomodulatory drugs (IMiDs) lenalidomide, thalidomide, and pomalidomide are highly active in the treatment of multiple myeloma. We and others have shown that IMiDs inhibit growth of multiple myeloma cells by inducing ubiquitination and degradation of the lymphoid transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) by the cereblon (CRBN) E3 ubiquitin ligase. Recently, we identified another lenalidomide-induced substrate of the CRBN E3 ligase, Casein Kinase 1A1 (CK1α), the degradation of which is responsible for lenalidomide's activity in del(5q) myelodysplastic syndrome. However, in contrast to human cells, mouse cells are resistant to lenalidomide-induced degradation of CK1α due to a single amino acid change in the mouse CRBN (mCRBN) protein at position 391 (valine in human CRBN (hCRBN)), isoleucine in mCRBN) that can be overcome by expression of the human variant.
Aims: To determine whether expression of *hCRBN* and $mCrbn^{I391V}$ sensitizes mouse multiple myeloma cells to lenalidomide.
Methods: We studied the mouse multiple myeloma cell line MOPC-315.BM that has been previously shown to induce multiple myeloma in BALB/c mice after transplantation. The different *CRBN* forms were expressed by retroviral vectors.
Results: Western blot analyses showed that MOPC-315.BM multiple myeloma cells had high protein levels of IKZF1 and IKZF3 as compared to non-myeloma mouse cells such as Ba/F3 pro-B cells and myeloblast-like 32D cells. However, lenalidomide treatment of MOPC-315.BM neither induced degradation of IKZF1 and IKZF3 nor had an impact on proliferation. Using retroviral transduction we expressed *mCrbn, hCRBN,* and $mCrbn^{I391V}$ in MOPC-315.BM cells. After lenalidomide treatment, we observed effective degradation of IKZF1 and IKZF3 proteins by western blot analysis in *hCRBN* and $mCrbn^{I391V}$ expressing MOPC-315.BM cells but not in those expressing *mCrbn* or empty vector. Immunoprecipitation analysis revealed that in the presence of lenalidomide IKZF3 bound to hCRBN and $mCrbn^{I391V}$ but not mCRBN, indicating that the isoleucine present in mCRBN at position 391 prevents direct interaction with IKZF3. Next, we examined the effect of expressing different CRBN forms on drug sensitivity of MOPC315.BM cells. As in the parental cells, *mCrbn*-expressing cells were insensitive to lenalidomide. In contrast, lenalidomide inhibited proliferation of *hCRBN* and $mCrbn^{I391V}$ expressing cells in a dose-dependent fashion. Similar results were obtained for pomalidomide and thalidomide. Expression of a dominant negative *IKZF3* isoform that inactivates IKZF3 and IKZF1 inhibited proliferation of MOPC315.BM cells, demonstrating that these cells depend on both transcription factors as their human counterparts. Expression of *hCRBN* and $mCrbn^{I391V}$ did not sensitize Ba/F3 or 32D cells to lenalidomide, demonstrating the multiple myeloma-specific effect of IMiDs. In MOPC315.BM cells, expression of *hCRBN* or $mCrbn^{I391V}$ had no effect on the sensitivity of other potent anti-multiple myeloma drugs including bortezomib and dexamethasone, confirming the specific role of CRBN as a drug target for IMiDs.
Summary/Conclusions: These results demonstrate that mouse multiple myeloma cells can be specifically sensitized to IMiDs by expression of *hCRBN* or $mCrbn^{I391V}$ and provide a basis for further development of *in vivo* models for IMiDs.

E1247

INFLUENCE OF HISTONE DEACETYLASE INHIBITORS (HDACIS) ON THE EXPRESSION OF ADHESION MOLECULES AND MODERN TARGET STRUCTURES IN MULTIPLE MYELOMA (MM)

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Background: During the last decades the vital role of the bone marrow (BM) niche was increasingly explored to elucidate the progression and drug resistance in multiple myeloma (MM), this leading to advances of treatment strategies to modulate the interaction between malignant plasma cells (PCs) and the microenvironment. Small molecules (IMiDs, plerixafor, NOX-A12) and biologicals (elotuzumab, daratumumab, indatuximab) have been developed and are in pre- and clinical use.
Aims: We assessed the influence of histone deacetylase inhibitors (HDACis) on the BM niche. For this purpose various HDACis were assessed alone and in combination with antimyeloma agents in a novel 3-dimensional (3D) co-culture, to predict most rational combination use targeting both PCs and BM niche.
Methods: To best mimic the microenvironment, whilst ensuring easy handling, we have established an agarose matrix-based 3D coculture model, suitable for high-throughput screening. The cells are seeded in conical microwells and their proliferation, viability and apoptosis are assessed. These properties are compared both in presence or absence of stroma and to 2D cultures. Cells are incubated with HDACis, their synergisms with established antimyeloma drugs and

stroma effects upon the treatment can be visualized via flow-cytometry and viability assays. The expression profile of relevant surface molecules (CXCR4, CD38, SLAM-F7, CD138) in primary MM cells and MM cell lines (MMCLs) are determined using fluorescent labeled antibodies and flow-cytometry.
Results: Inside the microcavities of the agarose matrices, PCs indeed formed 3D cell clusters and showed a similar cell proliferation rate compared to 2D cultures. Cell growth and expression pattern of surface proteins were influenced by the presence of BM stroma cells (BMSCs), leading to a decrease in CXCR4 but persistence of CD138 expression. Our current results suggest evident differences concerning growth and treatment response in our 3D *versus* 2D culture systems, which we currently further validate using HDACi as a novel substance class in MM within this easy to use culture system. Therein, the novel selective HDAC-6 inhibitor JS28 was selected from more than 20 hydroxamic acid derivates due to trypsin-dependent *in vitro* HDAC-inhibition assay results, with an IC_{50} of 59nM and a both 200-fold selectivity for HDAC 6 and HDAC 8 as compared to HDAC 1, respectively. The effect on target could be demonstrated in 2 different cell lines (HeLa and HL30) via western blot, quantifying a higher amount of acetylated tubulin, which is mainly degraded via HDAC 6 and consequently an evident marker for HDAC 6 activity. The phenotypic HDACi effect was determined with assessment in 2 MMCLs U266 and RPMI 8226: treatment of U266 and RPMI cells were performed with use of panobinostat or JS28 for 48 hours, either alone or combined with bortezomib, to test synergism. 2, 4 and 6nM bortezomib were combined with same ratios of both HDACi panobinostat with 2, 4 and 6nM or JS28 with 20, 40 and 60µM. The calculated combination indices (CI) in both MMCLs were <1 (=synergistic) within a range of approximately EC_{40} to EC_{55} for both combined panobinostat/bortezomib and JS28/bortezomib treatment schedules.

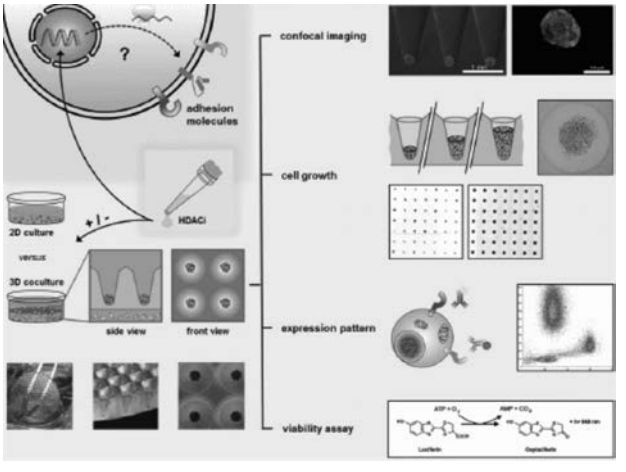

Figure 1.

Summary/Conclusions: Our current data underline the importance of accurate 3D co-culture models to mimic *in vivo* proliferation and drug resistance and to predict later clinical treatment success more effectively. So far, there is insufficient data that conclusively unravel the complex interactions between HDACis, their different subtype selectivity and the rapidly increasing number of treatment options in MM. In this context, our ongoing investigations will focus on cytokine secretion patterns, expression of adhesion molecules, their targeting and their alteration by HDACis.

E1248

HEME OXYGENASE 1 (HO-1) PROTECTS MYELOMA CELLS AGAINST BORTEZOMIB THROUGH NUCLEAR TRANSLOCATION AND REGULATION OF ER STRESS AND AUTOPHAGY PROTEINS

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Background: Despite recent advances in proteasome inhibitor and immunomodulatory drug-based therapies, MM remains largely incurable, primarily owing to acquired resistance. HO-1 is a cytoprotective microsomal enzyme that catalyzes the degradation of heme. We have recently shown that the protective effect of HO-1 on drug-induced cytotoxicity in leukemic cells does not involve its enzymatic byproducts, but rather its nuclear translocation following proteolytic cleavage. It has been recently described that Bortezomib (BTZ) is able to increase HO-1 expression.
Aims: We investigated about the role of BTZ-induced HO-1 in MM cell lines (U266, SKM-M1).
Methods: Cell Proliferation was performed by ATPlite one step assay (Perkinelmer) and using flow cytometry Annexin V/ iodure propidio. ROS formation was evaluated by flow cytometry. Endoplasmic reticulum (ER) associated proteins, autophagy-related proteins and HO-1 protein were evaluated by western blot assay. The fluorescent images were obtained using a Confocal Laser Scanning Microscopy (CLSM, Zeiss LSM700, Milan, Italy).
Results: As expected, we observed that Btz (15 nM) induced apoptosis after 24h ($p<0.001$). Flow cytometric analysis revealed increased levels of ROS after 1h ($p<0.0001$) of treatment with a peak after 3h ($p<0.001$). Btz was able to induce a significant increase in HO-1 mRNA levels after 3h ($p<0.0001$) of treatment with a maximum peak after 6h ($p<0.0001$). Since HO-1 is one of the major endoplasmic reticulum (ER) associated heme protein, we analyzed the ability of BTZ to induce ER stress. BTZ was able to induce the expression of ER stress proteins (Bip, IRE1α, Ero1, PERK and CHOP) in MM cells after 6h ($p<0.001$) with a peak after 24h ($p<0.0001$). Moreover, we observed that BTZ was able to induce autophagy-related genes such as ATFG5 and BENC1 in U266 cell lines. Silencing HO-1 using siRNA, we observed reduction of proteins described above indicating that induction of ER stress and then autophagy pathways by BTZ is HO-1 mediated. Furthermore, by confocal microscope we observed that HO-1 localized both in the cytoplasm and in the nucleus of MM cells. Interestingly, blockage of nuclear translocation by E64, a selective inhibitor of the protein cleavage, induced MM cells to become more sensitive to BTZ ($p<0.001$). No change in cell viability was observed inhibiting HO-1 enzymatic activity by using TIN (zync protoporphirin, 10 uM). Since nuclear HO-1 it has been reported to be a regulator of DNA repair activities, we also explored its role in genomic instability of MM cells. Using the cytochinesis-block micronucleus (CBMN) assay, we observed that pre-treatment of U266 with E64 for 24h led to a significant reduction of the percentage of micronuclei ($p<0.01$) and nucleolasmic bridges ($p<0.05$) observed in binucleated cells. Next, we evaluated U266 ability to activate G2/M checkpoint after UV damage using CBPI (cytochinesis block proliferation index) assay. The percentage of monucleated cells (G2/M checkpoint activated) was higher in cells pre-treated with E64 than control ($p<0.05$).
Summary/Conclusions: Our data suggest that BTZ-induction of HO-1 is probably linked to the activation ER stress and autophagy pathway by BTZ through HO-1 activation. HO-1 nuclear translocation may be involved in MM BTZ resistance. In addition, nuclear HO-1 may be involved in genomic instability of MM cells.

E1249

ANTI-MYELOMA EFFECT OF CANNABINOIDS

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Background: It is well studied the antiproliferative and proapoptotic effects of cannabinoids in neoplasias of the central nervous system, where the cannabinoid receptor type 1 (CB1) exhibits the highest levels of expression. In contrast, the cannabinoid receptor type 2 (CB) is found also exclusively in the hematopoietic and immune system. Despite this, the potential effect of cannabinoids on hematologic malignancies has been poorly determined.
Aims: Our purpose is to investigate the anti-tumor effect, both in vitro and *in vivo*, of different cannabinoids in multiple myeloma (MM) and know the main signaling pathways implicated in the effect of cannabinoids.
Methods: We evaluated the effect of five cannabinoid compounds in cell viability of six different human multiple myeloma cell lines, primary myeloma plasma cells and healthy primary cells from patients and donors. We studied the involvement of the main signaling pathways in the effect of cannabinoid as well as the expression of capases and some proteins of family Bcl-2 and also of the SPT enzyme by Western-blot. We used selective antagonists of cannabinoid receptors to know if the effect antiproliferative of tested cannabinoids is mediated by CB2. The role of ceramide was examined by immunohistochemistry and by pharmacological inhibition of the synthesis route. In addition we evaluated the synergism of the combination of cannabinoids with antimyeloma agents, such as dexamethasone and melphalan. Finally, we evaluated the effect of cannabinoids in MM xenograft models.
Results: We demonstrate that cannabinoids induce a selective apoptosis in myeloma cell lines and in primary malignant plasma cells from MM patients, without affecting the viability of normal cells from healthy donors, including hematopoietic stem cells. This antiproliferative effect is mediated by activation of caspases, mainly caspase 2, and is partially prevented by a pan-caspase inhibitor. Cannabinoid-induced apoptosis was correlated with an increased expression of Bax and Bak and a decrease of Bcl-xL and Mcl-1. In addition, cannabinoid treatment induced a biphasic response of Akt/PKB and significantly increased the levels of ceramide in MM cells. Remarkably, the blockade of ceramide synthesis prevented cannabinoid-induced apoptosis, indicating that ceramides play a key role in the pro-apoptotic effect of cannabinoids in MM cells. Furthermore, blockage of the cannabinoid receptor CB2 also inhibited cannabinoid induced apoptosis. Cannabinoid WIN-55 enhanced the antimyelo-

ma activity of dexamethasone and melphalan overcoming resistance to melphalan in vitro. Finally, administration of cannabinoid WIN-55 to plasmacytoma-bearing mice significantly suppressed tumor growth *in vivo*.
Summary/Conclusions: Our findings suggest that cannabinoids induce a selective apoptosis in tumor cells without adverse effects and thus they may be considered as potential therapeutic agents in the treatment of multiple myeloma.

E1250

BONE MARROW ADIPOCYTES PROMOTE MYELOMA CELL PROLIFERATION THROUGH INTERLEUKIN-6 SIGNALLING

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Background: Multiple myeloma (MM) is a malignancy characterised by the proliferation of monoclonal plasma cells within the bone marrow (BM) and its progression crucially involves support from the BM microenvironment. The BM microenvironment consists of many cell types not directly involved in haematopoiesis including the adipocyte, which accounts for over 50% of the older adults BM by volume. Adipocytes are known to promote the growth and survival of breast, ovarian and prostate cancers. Subsequently, we hypothesised that BM adipocytes could play a role in mediating MM cell survival and proliferation.
Aims: To investigate the role of BM adipocytes in regulating the survival and proliferation of MM cells.
Methods: Primary myeloma cells and MM cell lines were cultured on patient derived adipocytes which were obtained following informed consent under approval from the UK National Research Ethics Service (LRCEref07/H0310/146). MM cell proliferation was determined using flow cytometry cell count and BrdU uptake while cell survival was determined by annexin V/PI staining. Immunocytochemistry using a lipid specific dye and nuclear staining was performed to determine free fatty acid (FFA) transfer between adipocytes and MM cells with and without the inhibitor acipimox. ß-oxidation rates in MM cells were determined by Seahorse XF analysis using palmitate and etomoxir. PCR and cytokine analysis was used to determine the levels of interleukin-6 (IL-6) and other factors.
Results: We report that BM adipocytes promote MM cell survival and proliferation. BrdU analysis and annexin V/PI staining revealed that adipocyte/MM cell co-culture led to increased MM cell proliferation and decreased MM cell apoptosis compared to MM cells cultured alone. Furthermore, MM cells cultured on adipocytes induced adipocyte lipolysis and initiated lipid transfer from adipocytes to MM cells. This process was inhibited by acipimox. In addition, MM cell/adipocyte co-culture also led to increased MM cell β-oxidation rates indicating that MM cells use BM adipocyte derived FFAs to produce ATP. Furthermore, pharmacological inhibition of ß-oxidation using etomoxir led to a significant decrease in survival and proliferation of MM cells. PCR and cytokine analysis identified an increased expression of IL-6 in MM cells co-cultured with adipocytes. Further analysis revealed that IL-6 derived from myeloma cells induced the breakdown of triglycerides into FFAs (lipolysis) in BM adipocytes.
Summary/Conclusions: Results indicate that adipocytes provide energy to MM cells in the form of FFAs for survival and growth in vitro. Moreover, we found that the signal which induces adipocyte lipolysis is myeloma derived IL-6.

E1251

RETROSPECTIVE STUDY OF PARAPROTEINEMIA PREVALENCE AND DEVELOPMENT OF HEMATOLOGICAL MALIGNANCIES IN HBSAG POSITIVE *VERSUS* HBSAG NEGATIVE PATIENTS

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Background: Hepatitis B virus is lymphotropic and persists in peripheral blood mononuclear cells for years after active infection. In chronic infection, persistent antigenic stimulation of lymphocytes occurs. Whether these effects of the virus on B cells increase the risk of paraproteinemia and its progression is unknown.
Aims: We proposed to compare HBsAg+ *versus* HBsAg negative patients identified in the same time frame for prevalence of paraproteins and risk of development of hematological malignancy (HM).
Methods: Adults in Montefiore Medical Center with a HBsAg test between Jan 1st 2001 and Dec 31st 2011 were identified. Those with a positive serum protein electrophoresis (SPEP) test were included. SPEP results were classified into faint (F-SPEP) and discrete (D-SPEP). Demographics, results of Hepatitis C and HIV serology were obtained. Cancer registry data were reviewed for a diagnosis of biopsy confirmed HM.
Results: 216,522 patients were tested for HBsAg. 3,177 were positive. 346 of 3,177 HBsAg+ (10.9%) had an SPEP test. 66 of 346 (19%) had a +SPEP. 15 (4.5%) had a D-SPEP and 51 (14.5%) had a F-SPEP. Of the 213,345 HBsAg-, 21,291 (9.9%) had a SPEP. 2,226 of 21,291 (10.5%) were SPEP+. 739 (3.3%) had a D-SPEP and 1487 (7.2%) had a F-SPEP. HBsAg+ patients were more likely to be SPEP+ than HBsAg-. (19% *vs* 10.5%, p<0.0001). Patients with +SPEP among HBsAg+ and HBsAg- were compared. (Table 1) HBsAg+ SPEP+ patients were more likely to be male (69.7% *vs* 47.9%, p<0.001), more likely tested for HIV (OR:3.7, 95% CI: 2.1-6.4) and to be HIV positive (OR:2.3, 95% CI: 1.3-4.1). HBsAg+ SPEP+ patients were 6.7 times more likely to have a HM compared to HBsAg- SPEP+ patients. (OR: 6.7, 95% CI 3.5-12.7) The types of HM identified in the groups is described in Table 2.

Table 1. HBsAg- SPEP+ *versus* HBsAg+ SPEP+ patients.

Characteristic	HBsAg- SPEP+ (n=2226)	HBsAg+ SPEP+ (n=66)	p value
Male (n, %)	1067 (47.9%)	46 (69.7%)	<0.0001
HCV serology tested	2047 (92%)	65 (98.5%)	0.052
HCV positive/ tested for HCV	370 /2047 (18.1%)	12/65 (18.5%)	0.937
HIV serology tested	974 (43.8%)	49 (74.2%)	<0.0001
HIV positive / tested for HIV	319/974 (32.8%)	26/49 (53.1%)	0.003
D-SPEP positive	739 (33.2%)	15 (22.7%)	0.074
F-SPEP positive	1487 (66.8%)	51 (77.3%)	0.074
HM positive	79 (3.5%)	13 (19.7%)	<0.001

Table 2. Malignancies in HBsAg+ and HBsAg- patients with +SPEP.

Type of HM	HBsAg- SPEP+ HM+ (n=79)	HBsAg+ SPEP+ HM+ (n=13)
Plasma cell neoplasm	18 (21.4%)	8 (44.4%)
High grade B cell lymphoma/ Acute lymphoblastic leukemia	14 (16.7%)	1 (5.6%)
Low grade B cell lymphoma	26 (31%)	2 (11.1%)
T cell or NK cell lymphoma	8 (9.5%)	0
Acute myeloid leukemia or myelodysplasia	9 (10.7%)	1 (5.6%)
Myeloproiliferative syndromes	4 (4.8%)	1 (5.6%)

Summary/Conclusions: Our study shows that paraproteinemia is more prevalent in HBsAg+ patients, and HBsAg+SPEP+ patients are at a greater risk of developing HM compared to HBsAg- SPEP- Virus dependent B cell proliferation may be involved in Hepatitis B lymphomagenesis and paraproteins may be a marker. Modest frequency of HIV testing in our retrospective cohort is a significant limitation.

E1252

PROTEOMIC SIGNATURE PREDICTING COMPLETE RESPONSE TO PAD IN REFRACTORY MULTIPLE MYELOMA PATIENTS QUALIFIED FOR AUTOPBSCT AND VD CONSOLIDATION-MULTICENTER, POLISH MYELOMA CONSORTIUM 001 STUDY

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Background: Despite the new drugs introduced to multiple myeloma (MM) treatment the disease remains incurable due to increasing resistance to therapy. Understanding biology of disease and identification of biomarkers of resistance is a key research challenge and may enable the individualization of treatment.
Aims: We aimed to identify biomarkers and signaling pathways differing plasma cells (PCs) of patients (pts) with refractory/relapsed MM (RRMM) who achieved complete response (CR) after PAD (bortezomib, adriamycin, dexamethasone) from those with lower response. All the patients were qualified to undergo a high dose melphalan and a VD (bortezomib, dexamethasone) consolidation afterwards.
Methods: Comparative proteome analysis was performed on purified by positive selection (EasySep), pretreatment PCs from pts, who were refractory to the first line (CTD) and qualified for PAD chemotherapy followed by high dose melphalan and 2 cycles of VD consolidation according to standard recommendations of Polish Myeloma Group. Treatment response was assessed using IMWG criteria. PCs acquired from pre-treatment bone marrow after obtaining informed consent from pts (IRB# 434/14 UMP) were lysed and protein was isolated. Proteins from each patient were analyzed using two different, independent proteomic methods: Isobaric Tag for Relative and Absolute Quantitation iTRAQ (4-plex) and Label Free-based (LF) proteomic approach. Peptides were analyzed using Q-Exactive hybrid quadrupole-Orbitrap mass spectrom-

eter coupled to the chromatograph Dionex 3000 Ultimate nanoLC (Thermo Scientific). Proteins which accumulation in the analyzed subgroups differed by at least 50% in both methods (iTRAQ and LF) were considered to differentiate. The dysregulated proteins were subjected to REACTOME and PANTHER bioinformatic tools for identifying enriched signaling pathways and networks.
Results: As of January 31th 2016 30/45 (66%) pts (median age: 60 (38-74)) received 6 planned PAD. Treatment was interrupted due to toxicity for 8 pts (G3 neuropathy, G2 infection) and disease progression (PD) (7 pts - 15%). After PAD 8 pts achieved CR (17%), 14 VGPR (30%), 14 PR (30%) and 9 - PD (20%). 4 pts died due to PD during PAD. Transplantation was so far performed in 12 pts and consolidation planned 3 months after autoPBSCT in - 7. Proteomic analysis was performed on PCs from 28 pts. Out of 499 proteins identified (FDR<1%), 56 showed significant differences in accumulation between patients, who, after treatment with PAD, achieved CR and patients with worse response. Accumulation of 13 proteins was up-regulated in resistant patients, among them thioredoxin domain-containing protein 5 (TXNDC5), proteasome activator complex subunit 2 (PSMA2) and peroxiredoxin-5 whereas relative abundance of 42 proteins were down-regulated in resistant patients. Among the latter were vimentin, annexin A1. Based on the log of p-value from Fisher's test and number of identified differential proteins, the most affected pathways constitute: proteasome pathway, apoptosis and programmed cell death, signalling by Rho GTPases, detoxification of ROS and activation of DNA fragmentation factor and apoptosis induced DNA fragmentation.
Summary/Conclusions: We indicated pathways involved in resistance to investigated regimen in RRMM and confirmed our previous finding in newly diagnosed MM and RRMM (Dytfeld BJH 2015, ASH 2015). We pointed significant biomarkers of resistance and potential therapeutic targets as basis for individualized MM therapy.

E1253

NOTCH PATHWAY AND INTELEUKIN-6 COOPERATE TO SUPPORT MULTIPLE MYELOMA CELL PROLIFERATION

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Background: Multiple myeloma (MM) is a malignant plasma cells (PC) disorder characterized by PCs accumulation in the bone marrow (BM) and a close interaction between PCs and the surrounding microenvironment. Infact, BM stromal cells (BMSCs) sustain the survival and proliferation of tumor cells through adhesion molecules and the production of several cytokines, especially interleukin-6 (IL6). At the initial stage, MM cells strictly depend on IL6 produced by BMSCs and during disease progression may acquire independence and/or the ability to produce autonomously this cytokine. The Notch pathway is highly conserved and plays a crucial role in cell-fate decision, tissue patterning and morphogenesis. Recent evidences suggest a key role of Notch pathway in MM progression, by its ability to positive regulate cell proliferation, drug resistance and BM infiltration through the overexpression of both receptors (Notch1 and 2) and ligands (Jag1 and 2).
Aims: We investigated the cooperation between the Notch pathway and IL6 signaling in the promotion of MM cells proliferation. Particularly, we analyzed how Notch upregulation during MM progression may support tumor cells growth. Moreover, we evaluate if it could promote the activation of an IL6 autocrine loop in MM and favor IL6 paracrine production by the surrounding BMSCs.
Methods: Notch signaling modulation was induced in MM cell lines and primary MM cells as follows: upregulation by 5µg/mL soluble Jag1; down-regulation by 50µM DAPT or Jag1-2 RNA interference. qPCR was performed using SYBR Green. Absolute cells count and evaluation of IL6 protein expression was achieved by flow cytometry. Immunohistochemistry (IHC) for Hes6, IL6 and Ig light chain was performed on BM biopsies at different stages of MM.
Results: Gene expression profiling (GEP) analysis evidenced the upregulation of Jag1 and Hes5 (Notch transcriptional target) in MM and pPCL (primary plasma cell leukemia) cases compared to normal controls, and the overexpression of Notch2, Hes5 and Hes6 in the worst prognosis, MAF-traslocated MM patients. These data support the hypothesis that over-expression of Notch pathway members correlate with progression or high-risk MM. Notch activity was modulated in MM cell lines, allowing us to demonstrate that the dysregulation of Notch signal can substitute IL6 stimulation. Infact, Notch activation in IL6-dependent cells may stimulate their proliferation in the absence of the cytokine. On the opposite, upon Notch blockade, IL6-indipendent cells became dependent on IL6 for their growth. Notch pathway is also able to support MM cells proliferation through the promotion of an IL6 autonomous production. Indeed, IL6 expression in U266 cells depends upon Notch signaling and IHC study confirmed an association between Notch activation and IL6 immunoreactivity in MM cells. Since the most important source of IL6 are BMSCs, we focused our attention on the interaction between MM cells and the surrounding niche. We showed, by IHC staining, that MM cells induced IL6 expression in the nearby BMSCs. By co-culture systems, we demonstrated that MM cell-derived Jag ligands are able to activate Notch pathway in BMSCs, promoting IL6 secretion and MM cell proliferation, whereas Notch inhibition reverts this effect. Our results were further confirmed in *ex-vivo* co-cultures of MM cells and BMSCs, collected from patients.
Summary/Conclusions: Notch signaling in MM cells and in surrounding BMSCs promotes MM cell growth by boosting IL6 production. These results support the rationale for a Notch-directed approach in MM and suggest that Jag ligands may be promising molecular targets in MM therapy.

E1254

INTEGRATIVE ANALYSIS OF DNA COPY NUMBER, DNA METHYLATION AND GENE EXPRESSION IN MULTIPLE MYELOMA REVEALS ALTERATIONS RELATED TO RELAPSE

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Background: Multiple myeloma (MM) remains incurable despite the introduction of novel agents and a relapsing course is observed in the majority of patients. Although the development of genomic technologies has greatly improved our understanding of MM pathogenesis, the mechanisms underlying relapse have been less investigated.
Aims: To investigate the genomic changes generated in the transition of myeloma cells from diagnosis to relapse, analyzing DNA imbalances, methylation, and transcriptomic modifications.
Methods: Twenty patients with symptomatic newly diagnosed MM were included in the study. Paired bone marrow (BM) samples were obtained at diagnosis and relapse in all the patients. A CD138 positive PC isolation using the AutoMACs automated separation system was carried out in all the BM samples (purity was above 90%). Genome-wide detection of CNA was carried out using the standard Affymetrix CytoScan 750k assay protocol (Affymetrix); DNA methylation was assessed using the "Human DNA Methylation 3x720K CpG Island Plus RefSeq Promoter Array" according to standard procedures by NimbleGen Systems, and gene expression profiling was performed by means of the Human Gene 1.0 ST Array (Affymetrix).
Results: Overall, the acquisition of abnormalities at relapse was much more frequent than the lost of lesions present at diagnosis, and DNA losses were significantly more frequent at relapse than in diagnosis samples. Interestingly, copy number abnormalities involving more than 100 Mb of DNA at relapse significantly impact the gene expression of these samples, provoking a particular deregulation of IL-8 pathway. On the contrary, no relevant modifications of gene expression were observed in those samples with less than 100 Mb affected by chromosomal changes. Although different statistical approaches were used to uncover genes whose abnormal expression at relapse was regulated by DNA methylation, only two genes significantly deregulated in relapse samples (*SORL1* and *GLT1D1*) showed a negative methylation-expression correlation. A deeper analysis demonstrated that DNA methylation was involved in regulation of *SORL1* expression in MM. Finally, relevant changes in gene expression observed in relapse samples, such us downregulation of *CD27* and *P2RY8*, were not apparently preceded by alterations in corresponding DNA.
Summary/Conclusions: Cross-platform integration of three different microarrays data revealed that genomic heterogeneity of MM already described at diagnosis is subject to change at relapse, both at the DNA and RNA level. This study, also, showed a limited effect of DNA methylation changes observed at relapse on the transcriptome of myeloma cells.

E1255

DETAILED FISH PROFILING OF MULTIPLE MYELOMA PATIENTS IDENTIFIES A GROUP OF PATIENTS WITH ONLY SECONDARY CHROMOSOMAL ABERRATIONS

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Background: Using targeted fluorescence in situ hybridization (FISH) analyses of plasma cells, chromosomal aberrations are identified in more than 90% of patients (pts) with multiple myeloma (MM). It is postulated that early oncogenesis in MM is driven by two different (almost) exclusive genetic events: hyperdiploidy (HYP) and IGH translocations (IGH-R), and that clonal evolution occurs with acquisition of secondary aberrations (SA). Recent studies showed that some pts have both types of aberrations (IGH-R+HYP) and that myeloma relapses are sometimes related to clones quite different to those at diagnosis. However, the characteristics of MM pts with secondary aberrations only (SA) have not being extensively studied.
Aims: Our aim was to detect MM pts with SA and to analyze if these pts show dif-

ferent biological and clinical characteristics than MM pts with primary aberrations.
Methods: We performed FISH on CD138+ plasma cells of 163 MM pts consecutively diagnosed in our department since 2012. We used an extensive FISH panel to analyze five IGH-translocations, five different trisomies and five probes labeling recurrent secondary aberrations. Ten patients were analyzed by targeted next-generation sequencing (NGS) technology using the Agilent HaloPlex library preparation approach. Genes bearing hotspots for mutations in hematological malignancies were included in the sequencing panel.
Results: Chromosomal (chr) aberrations were detected in 98% (160/163) and hyperdiploidy was the most frequent primary aberration in 59% (94/160), IGH-R occurred in 29% (47/160) and SA in 12% (19/160) of MM pts. Overlapping IGH-R and HYP was found in three pts with HYP proceeding the developing of t(4;14), in line with recent publications (1,2). In comparison to all MM pts those with SA showed higher frequencies of IgA MM isotypes (6/19) and light chain-only MM (6/19). Moreover, they showed more aggressive MM with ISS3 in 74% (14/19) of pts at the time of analysis. 13q14 was the most frequent chromosomal locus involved in almost all pts (18/19) with equally distribution of deletion 13q14 (9/18) and monosomy 13 (9/18). Gain 1q21 was the second most frequent aberration present in even 68% (13/19) of SA pts. Deletion 1p32, cMYC and 14q32 (IGH)-abnormalities were discovered in 21% (4/19), 21% (4/19) and 26% (5/19), respectively. A total of 53 chr aberrations were detected in 19 MM pts with SA showing a high level of chr complexity compared to all MM pts. Interestingly, two of the analyzed pts, one with three SA and one with six, did not show additional mutations via NGS technology.
Summary/Conclusions: Our results reveal a progressive MM cell type with as yet unknown initiating oncogenetic events, but highly activated mechanisms of clonal proliferation. Moreover they raise two questions, 1. whether pts without detectable primary genetic events, but with occurrence of unfavorable and high-risk secondary cytogenetic lesions represent a distinct MM subgroup and 2. how these pts should be best assessed and treated. Using NGS technology in a large MM pt cohort should allow to gain more insight in the pathogenesis of this subgroup of MM pts.

References
1. Pawlyn C. et al. Blood. 2015;125:831-40;
2. Heuck C. et al Blood 2015;126(23).

E1256

LONG NON-CODING RNAS IN MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is the second most common hematological malignancy in the world. It is characterized by increasing rate of various genetic mutations and dysregulated pathways. This work aims to find out if this observed dysregulation may be partly caused by a subgroup of non-coding RNAs, the so-called long non-coding RNA molecules (lncRNA). These molecules are over 200 nt long, primarily localized in the nucleus, and it seems increasingly obvious that lncRNAs play a crucial role in human diseases including cancer.
Aims: The goal of this study was to identify a disease-specific cellular lncRNA signature using a cohort of MM patients in comparison to healthy donors.
Methods: Fifty CD138+ samples obtained from newly diagnosed MM patients and healthy donors (HD) were evaluated for this study. Screening analysis of 83 lncRNA was performed on 6 MM patients and 6 HD using RT2 lncRNA PCR Array-Human lncRNA Finder (Qiagen). Significantly deregulated lncRNAs between MM *vs* HD were validated by qPCR using relative quantification approach $2^{-\Delta Ct}$ on a larger cohort of patients and HD. Receiver Operating Characteristic (ROC) analysis was used to calculate specificity and sensitivity of each lncRNA. P values<0.05 were considered significant.
Results: RT2 lncRNA PCR Array profiling revealed 27 deregulated lncRNAs (all p<0.01) between MM patients and HD. ZFAS1, UCA1, BDNF-AS, NEAT1 and FAS-AS1 expression levels were further verified on a bigger cohort of MM and HD samples. UCA1 was significantly down-regulated (p<0.0001), NEAT1 and BDNF-AS were up-regulated in MM samples when compared with HD (both p<0.00000001). To discriminate MM from HD, receiver operating characteristic (ROC) curve was calculated. It revealed sensitivity of 100% and specificity of 100%, and area under curve (AUC)=1.000 for NEAT1 and BDNF-AS lncRNA expression and sensitivity of 95.24% and specificity of 75%, and area under curve (AUC)=0.905 for UCA1 expression levels. We suppose that these dysregulated lncRNAs could have a biological relevance in MM since BDNF-AS is an antisense RNA for BDNF, a significant stimulating factor of osteoclasts in MM, NEAT1 and UCA1 were described as dysregulated in several types of cancer, including hematological malignancies.
Summary/Conclusions: Altogether, our first observations demonstrate that cellular lncRNA UCA1, NEAT1 and BDNF-AS may be involved in pathophysiological processes occurring in MM cells and prompt further studies in this field.
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E1257

NEXT GENERATION FLOW CYTOMETRY IS A RELIABLE TECHNIQUE FOR DETECTING MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA PATIENTS IN COMPLETE REMISSION: A STUDY COMPARING SHORT *VS* LONG TERM REMISSION
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Background: 4-6 colours flow cytometry has been validated in the past years as a powerful and feasible technique able to detect MRD in multiple myeloma patients thus furnishing a clinical and prognostic tool to the physician. Although its applicability and specificity can be high, it requires good quality bone marrow aspirate and expert and trained personnel for the analysis. However, recent results have confirmed that 4-6 colours flow-MRD has a lower sensitivity than molecular techniques such as allele-specific oligonucleotyde (ASO)-PCR and next generation sequencing (NGS). Recently, two 8 colours tubes panel developed by the EuroFlow Consortium showed to be able to detect MRD with an increased sensitivity and to be applicable as standardized method.
Aims: Complete remission (CR) is a prerequisite for long term responses, progression free survivals, and ultimately overall survivals and cure, but while many studies have looked at MRD status soon after autologous or allogeneic stem cell transplantation with flow or molecular techniques, little is known about long term remission patients (>5-10 years) and in particular if more sensitive techniques such as NGF or NGS can still detect minimal disease in those patients.
Methods: We tested the feasibility of NGF-MRD to CR MM patients with a lyse-wash-and-stain sample preparation protocol, staining an high numbers of bone marrow (BM) cells ($\geq 2x10^6$ cells/tube) with 2-tubes optimized 8-colours antibody panel, (OneFlow PCST e PCD BD Biosciences) for accurate identification of BM plasma cells (PCs) and discrimination between phenotypically aberrant (aPC) and normal PC (nPC). Patients with a CR/stringent CR were tested for MRD in BM and peripheral blood samples. Patients with 5-10 years of remission duration were analysed as well as patients with 2 to 4 years of remission. Clinical characteristics will be reported as well as treatment received.
Results: Applicability was 100% of patients studied. A preliminary result on the first 20 patients analysed showed an MRD+status in 12/20 patients predefined as CR. In particular, 2/20 patients did show residual disease also in the PB. Interestingly when studying patients with >5 years CR status, 1/5 did show an MRD+. A plan to analyse patients in CR is ongoing within the Tuscan Group of Multiple Myeloma (GTMM). The target is to test NGF-MRD in >100 CR patients comparing different treatments (auto *vs* chemo, *vs* new agents), age (<65 years *vs* >65 years), CR duration (<5 ys *vs* >5 ys).
Summary/Conclusions: Next generation flow is able to detect minimal residual disease in approximately 60% of predefined CR patients. The sensitivity can be quite comparable to the molecular techniques, but with an applicability largely superior. Patients with long term CR seem to have more chances to be MRD negative, although these findings nee to be demonstrated in a large cohort of patients. To confirm these data a study is currently ongoing of the GTMM and results will be presented.

E1258

BONE DISEASE IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: RESULTS FROM A SCREENED POPULATION-BASED STUDY
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Background: Monoclonal gammopathy of unknown significance (MGUS) is a precursor condition that precedes multiple myeloma (MM). Bone disease is a major manifestation of MM, and includes osteolytic lesions, fractures, and osteoporosis. Previous population-based studies have shown that individuals with MGUS have an increased risk of fractures, although the mechanism behind this increased risk remains unknown. Furthermore, these studies have been performed on clinically established cohorts with a risk of bias due to underlying comorbidity.
Aims: Our aim was to analyze bone mineral density (BMD), bone volume, and risk of fractures among individuals with MGUS in a screened population.
Methods: We performed a screening for MGUS in the Age Gene/Environment Susceptibility Reykjavik study (AGES-Reykjavik) cohort, consisting of 5,764 elderly Icelandic men and women. Through serum protein electrophoresis and free light chain analyses, 300 individuals with MGUS (159 men and 141 women) and 52 individuals with light chain MGUS (LC-MGUS; 35 men and 18

women) were identified. Quantitative computerized tomography (QCT) was performed in the lumbar spine (L1/L2), hip and mid-femoral shaft to evaluate cortical, trabecular and integral BMD as well as bone geometry in all individuals. Analysis of variance and Tukey's honest significance test were used to compare individuals with MGUS and LC-MGUS with others. Hospital records with International Classification of Diseases were used to record fractures from the individuals' enrollment into the study with a mean follow up time of 6.9 years. Cox proportional hazard models were used to compare risk of fractures in MGUS and others. Results were adjusted for age and sex.

Results: No difference was found in BMD between subjects with MGUS and others at the spine (Table 1; mean for MGUS (M_{MGUS})=0.197 mg/cm^3, mean for others (M_{others})=0.194 mg/cm^3, p=0.21) or total hip (M_{MGUS}=0.239 mg/cm^3, M_{others}=0.237 mg/cm^3, p=0.22). Volumetric measurements showed that individuals with MGUS had a statistically significant increase in bone volume compared to others in the lumbar spine (M_{MGUS}=41.5 cm^3, M_{others}=39.7 cm^3, p<0.001) and total hip (M_{MGUS}=106.3 cm^3, M_{others}=100.1 cm^3, p<0.001). A significant difference was found in bone volume in total hip in MGUS men, compared to other men (M_{MGUS}=123.6 cm^3, M_{others}=119.3 cm^3, p=0.04). Overall, the risk of fractures was not increased in individuals with MGUS as compared to others (hazard ratio (HR): 1.19; 95% confidence interval (CI): 0.94-1.50). Men with MGUS had a significantly increased risk of fractures, compared to other men (HR: 1.49; 95% CI: 1.05-2.12).

Table 1.

TABLE 1

	Mean measurements				ANOVA	Tukey's HSD
SPINE		**No MGUS**	**MGUS**	**LC-MGUS**		
Average L1 and L2 integral BMD (mg/cm^3)	Men	0.206	0.206	0.191	P=0.179	NS
	Women	0.185	0.186	0.181	P=0.850	NS
	All	0.194	0.197	0.188	P=0.207	NS
Mean volume of L1 and L2 (cm^3)	Men	45.96	46.37	46.13	P=0.741	NS
	Women	35.16	35.43	34.91	P=0.842	NS
	All	**39.70**	**41.45**	**42.13**	**P<0.001**	**P_{MGUS}<0.001 $P_{LC-MGUS}$=0.017**
TOTAL HIP						
Integral BMD (mg/cm^3)	Men	0.249	0.250	0.230	P=0.143	NS
	Women	0.228	0.226	0.216	P=0.536	NS
	All	0.237	0.239	0.225	P=0.221	NS
Integral volume of total hip (cm^3)	**Men**	**119.31**	**123.59**	**125.29**	**P=0.020**	**P_{MGUS}=0.043 $P_{LC-MGUS}$=0.287**
	Women	85.63	84.65	84.98	P=0.760	NS
	All	**100.12**	**106.28**	**111.50**	**P<0.001**	**P_{MGUS}<0.001 $P_{LC-MGUS}$<0.001**

Summary/Conclusions: Our results from a screened population show that individuals with MGUS do not have a decreased BMD at the lumbar spine or hip. Surprisingly, however, we find that bone volume is increased in individuals with MGUS, especially in men, who also have an increased risk of fractures. This suggests an effect of MGUS on bone metabolism in men that is not noted in women, probably as a result of other stronger risk factors in women. Further reaserch is needed to explain the increased bone volume in men with MGUS.

E1259

COMPENSATORY STRESS PATHWAYS IN MODULATING ER STRESS IN BORTEZOMIB RESISTANT MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) remains a predominantly incurable malignancy despite high-dose chemotherapy, autologous stem cell transplants and novel agents. Nonetheless, proteasome inhibitors (PI) such Bortezomib (Bz) have been effective in treating the disease to date. The 26S proteasome inhibitor indirectly targets the unfolded protein response (UPR) by inhibiting proteasomal degradation of ubiquitinated paraprotein, subsequently leading to the lethal accumulation of paraprotein within the cell. Initial treatment is effective, however most patients eventually develop resistance to the drug. The mechanism of resistance is still unknown, though one possible mechanism contributing to Bz resistance could be chaperone-mediated autophagy (CMA). Upregulated in a number of tumours, CMA specifically targets and degrades soluble cytosolic proteins. It is therefore likely that this pathway could be an alternative stress management pathway in Bz resistance.

Aims: We aimed to assess CMA in sensitive and resistant MM, and its role in alleviating ER stress in bortezomib resistance.

Methods: QPCR, western blotting, cell viability assays, ER imaging and shRNA assays were performed to assess the role and importance of UPR and autophagy in Bz resistant MM, and was correlated to Bz sensitivity.

Results: Molecularly and morphologically, we identified reduced UPR activity as a result of increasing Bz resistance in both resistant MM patients and in resistant KMS11 cells. We further identified that the chaperone-mediated autophagy (CMA) marker, LAMP2A, was highly expressed in resistant cells compared to sensitive cells in cell lines and patients. CMA activity was also seen to increase in 4 different MM cell lines under various levels of ER induced stress, strongly suggesting the pathways importance in ER stress management. Upon further investigation, preliminary data has also shown that macroautophagy may provide as a compensatory mechanism in cells with impaired CMA.

Summary/Conclusions: CMA is upregulated in Bz resistant MM and functions as a compensatory stress mechanism in alleviating ER stress in cells with reduced UPR activity. Partial inhibition of the pathway subsequently results in the activation of macroautophagy. To effectively treat MM and potentially overcome Bz resistance, new therapies targeting autophagy are required.

Myeloma and other monoclonal gammopathies - Clinical

E1260

CARFILZOMIB, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (KCYD) IN ELDERLY NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS: INITIAL RESULTS OF A PHASE 1 STUDY

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Background: The combination of carfilzomib at the dose 20/36 mg/m^2, cyclophosphamide and dexamethasone has been demonstrated to be well tolerated and highly effective (Bringhen S. et al, Blood 2014; 124:63-9). A further dose escalation could improve clinical efficacy. Herein we present initial results of the dose-escalation phase 1 portion of a multicenter, phase 1/2 study evaluating the safety and the efficacy of the combination KCyd in elderly NDMM patients (pts).
Aims: The primary objective was to determine the maximum tolerated dose. The secondary objectives were to determine the safety and the efficacy of KCyd, including response rate, time to progression, progression free survival, time to next therapy and overall survival. ated results will be presented.
Methods: Newly diagnosed pts not eligible for autologous stem cell transplantation due to age or co-morbidities were eligible. Carfilzomib was administered intravenously on days 1,2,8,9,15,16, cyclophosphamide was administered orally on days 1,8,15 and dexamethasone was administered orally once weekly. Dose-escalation used a standard 3+3 schema with dose-limiting toxicities (DLTs) assessed during cycle 1. All pts received carfilzomib at 20 mg/m^2 on days 1 and 2 of cycle 1. Beginning on day 8 of cycle 1, carfilzomib was escalated in 3 successive dose levels from 45 to 70 mg/m^2 in combination with the standard doses of cyclophosphamide 300 mg/m^2 and dexamethasone 40 mg. KCyd induction was administered for 9 28-day cycles. Adverse events (AEs) were graded by NCI-CTCAE v4. Response was assessed according to the modified International Uniform Response Criteria.
Results: Between June 2014 and March 2015, 9 pts were enrolled in the completed phase 1 dose-escalation portion of the study. Median age was 74 years (range 67-82), 44% had ISS stage III, 67% had unfavorable FISH profile [t(4;14) or t (14;16) or del17p or amp1]. No DLTs were observed in the 3 pts of cohort 1, with carfilzomib at 45 mg/m^2. In cohort 2 carfilzomib was escalated to 56 mg/m^2 and 1/6 pt had a DLT with acute kidney injury. Therefore, the MTD of carfilzomib was established at 56 mg/m^2. Drug-related AEs occurring in >20% of pts included anaemia (56%), fever (44%), thrombocytopenia (33%), diarrhoea (33%), vomiting (33%), infections (33%), hypertension (33%), heart failure (22%), acute kidney injury (22%). Grade 3 AEs occurring in >10% of pts included anaemia (22%), infections (22%), acute kidney injury (22%) and heart failure (11%). Only 1 grade 4 AE was reported (thrombocytopenia). Six pts completed induction treatment, 2 pts discontinued treatment due to AEs and 1 pt due to pt decision. After a median follow-up of 14.2 months, 2 pts achieved a complete response (CR), 4 pts a very good partial response (VGPR) and 3 pts a partial response (PR), for an overall response rate of 100%, at least VGPR of 66% and at least CR of 22%. No progressive disease or death occurred during induction.
Summary/Conclusions: The MTD of carfilzomib was established at 56 mg/m^2. KCyd combination given to untreated, symptomatic, elderly pts with myeloma is well tolerated and highly effective with an overall response rate of 100%, including a CR rate of 22%. Enrollment in the phase 2 portion of the study is ongoing and updated results will be presented.

E1261

AN ONGOING MULTINATIONAL OBSERVATIONAL STUDY IN MULTIPLE MYELOMA (PREAMBLE): PRELIMINARY REPORT ON PROGRESSION-FREE SURVIVAL

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Background: Multiple myeloma (MM) is associated with significant disease burden, and long-term prognosis is poor, with relapse almost inevitable. Immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) have improved patient outcomes; however, data on real-world clinical effectiveness of these available treatments are limited.
Aims: PREAMBLE (Prospective REsearch Assessment in multiple Myeloma: an oBservationaL Evaluation; NCT01838512) is an ongoing, international, prospective, observational cohort study to improve understanding of the real-world outcomes of IMiDs, PIs, and combination therapy in relapsed/refractory MM (RRMM). Here we report preliminary progression-free survival (PFS) data.
Methods: Patients with RRMM (≥1 prior therapy) who initiated treatment with an IMiD, PI, or IMiD+PI within 90 days before or 30 days after study enrollment were eligible. Data were analyzed using a Cox proportional hazard regression model that included patient demographics, baseline disease characteristics, and MM treatment-related variables as covariates.
Results: At the time of data cut-off (December 7, 2015), data were available for 764 patients and median duration of follow-up was 15 months. The median age of patients was 68 years and 55% were male. Of the 764 patients, 368 (48%) were receiving an IMiD (81% of whom were receiving lenalidomide, n=297), 347 (45%) were receiving a PI (80% of whom were receiving bortezomib, n=278), and 49 (6%) were receiving an IMiD+PI (59% of whom were receiving lenalidomide/bortezomib, n=29). Most patients (n=325; 43%) had 1 prior line of therapy, 203 (27%) had 2 prior lines, 111 (15%) had 3 prior lines, and 123 (16%) had >3. The majority of patients had relapsed MM (n=599, 78%). 161 (21%) patients were refractory to their last line of treatment; 260 (34%) patients discontinued 1 prior line of therapy due to progression, 61 (8%) discontinued 2 prior lines due to progression, and 25 (3%) discontinued >2 prior lines. 40% of patients had International Staging System (ISS) stage III disease. Median PFS was 9.26 months (95% CI 8.21–10.12); 1-year PFS rate was 40.9% and 2-year PFS rate was 19.7%. Approximately half of the patients (50.7%) were estimated to respond to treatment before then progressing. At 2 years, all patients in this analysis had either responded to treatment or progressed without response. Median (95% CI) duration of response was 14.2 (10.87–16.39) months and was longer for patients receiving an IMiD *versus* a PI (16.4 [11.73–not estimable] months *vs* 10.9 [7.13–14.69] months). Number of lines of therapy discontinued due to progression was most strongly associated with higher rates of progression/death (Figure; HR p<0.001 for all comparisons). Other factors associated with higher rates of progression or death were number of prior lines of therapy (2 *vs* 1, HR 1.38, p=0.016; 3 *vs* 1, HR 1.48, p=0.012; >3 *vs* 1, HR 1.63, p<0.001) and ISS stage at study entry (III *vs* I, HR 1.45, p=0.032).

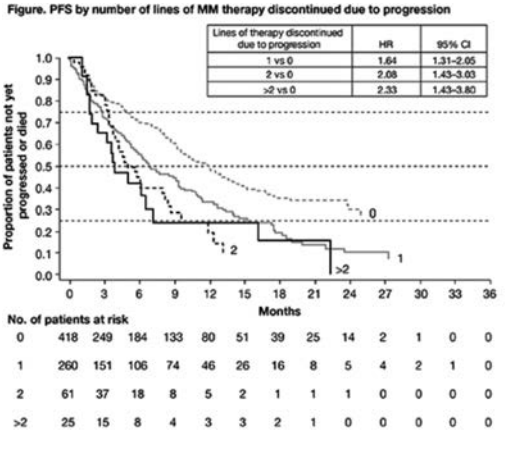

Figure 1.

Summary/Conclusions: Preliminary analysis of real-world data from PREAMBLE suggests that the number of lines of therapy discontinued due to progression, lines of therapy overall, and disease stage are key risk factors associated with the rate of progression/death in patients with MM, demonstrating an unmet need.
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E1262

ROBUSTNESS OF THE PROGNOSTIC VALUE OF THE SKY92 MARKER *VERSUS* FISH MARKERS ACROSS NINE MULTIPLE MYELOMA COHORTS

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Background: Multiple Myeloma (MM) is recognized as a heterogeneous group of patients with varying response and outcome of their disease, associated with various risk factors including genetic aberrations. With increasing availability of genetic data, ever more reliable conclusions can be drawn about the relevance of individual and combined biomarkers. Here we compare multiple stratifications of MM to evaluate their value in relation to overall survival (OS). All available biomarkers were used and included interphase FISH (iFISH), virtual FISH (vFISH), SKY92, ISS and some combinations thereof. The other combination is SKY92+ISS recently identified to be superior to all other combinations of one or two biomarkers for identification of both high risk and low risk patients.
Aims: To demonstrate and compare the robustness of the prognostic value of the SKY92, ISS, SKY92+ISS and various FISH marker risk classification methods across a wide variety of clinical datasets including NDMM and RRMM, young/elderly patients and a wide diversity of treatments.
Methods: Nine GEP datasets publicly available or obtained by analysis of the CE IVD MMprofiler assay were used (Table 1, rows) and evaluated for 17 biomarkers (Table 1, columns). In each analysis the Cox proportional hazard ratio (HR) and its *p*-value are calculated. Interphase FISH (iFISH) data was not available for all datasets, therefore, the so-called Virtual FISH markers (as classified by GEP signatures with high accuracy) were determined using the MMprofiler assay algorithms. Note that there is no virtual FISH signature for del(17p). We combined the subtypes [t(14;16)+t(14;20)] since they are rare but both predictors of poor outcome.
Results: All available data was used to compare prognostic HR and *p*-values for overall survival in nine MM cohorts, see Table 1. Note that HOVON-65/GMMG-HD4 was the training set for SKY92. iFISH data was only available for a limited set of studies. Therefore, additionally, vFISH results are shown (HR in white). For some of the datasets, the microarray platform was not HG-U133Plus2 - for which the MMprofiler vFISH software was developed - such that no vFISH call was available. There was also missing ISS data for 4 study cohorts. SKY92 could be determined in all datasets and was always prognostic (Table 1, white across the column). ISS and the combination of ISS+SKY92 could be determined in 5 datasets and were significant in all 5 of the studies. The FISH markers did not show robust prognostic values across the evaluated datasets. Only gain(1q) showed prognostic value in 4 out of 6 datasets. Hazard ratios are given in Table 1 and cell-shading is white where *p*-values were significant (<0.05).

Table 1. Prognostic value (hazard ratios for OS) of ISS and genetic biomarkers in nine clinical datasets including NDMM and RRMM. White=significant (p<0.05); grey=n.s.; dark grey=NA / not available. 1) HOVON-65/GMMG-HD4 |n=329|, 2) HOVON-87/NMSG-18 |n=143|, 3) MRC-IX |n=246|, 4) MMGI |n=91|, 5) TT3 |n=139|, 6) TT6 |n=55|, 7) Czech E-MTAB-1038 |n=66|, 8) TT2 |n=351|, 9) APEX |n=264|. FISH column HR with underscores are based on vFISH, others on iFISH. Inf=infinitely large.

	SKY92	SKY92 + ISS	ISS	iV FISH t(4;14)	iV FISH t(11;14)	iV FISH t(14;16)/t(14;20)	iV FISH del(13q)	iV FISH gain(1q)	iV FISH gain(9q)	i FISH del(17p)
HOVON-65/GMMG-HD4	4,7	12,2	4,6	1,5	0,8	2,8	1,7	1,3	0,7	3,4
HOVON-87/NMSG-18	2,9	3,8	2,2	1,3	0,8	2,5	1,6	2,0	0,6	2,5
MRC-IX	2,2	5,7	2,9	1,4	0,7	1,1	1,3	1,6	1,0	1,7
MMGI	6,2	10,1	3,4	0,0	2,5	13,4	1,2	3,9	0,9	NA
TT3	5,2	NA	NA	1,6	0,3	1,2	1,5	1,6	0,7	NA
TT6	10,3	NA	NA	4,3	0,2	62,7	4,2	9,6	0,8	NA
Czech E-MTAB-1038	2,6	inf	inf	0,3	NA	NA	NA	1,4	NA	1,7
TT2	3,4	NA	NA	NA	NA	NA	NA	NA	NA	NA
APEX	3,0	NA	NA	NA	NA	NA	NA	NA	NA	NA

Summary/Conclusions: SKY92, and its combination with ISS, have demonstrated to be robust prognostic markers in MM while the evaluated FISH markers do not show robustness for prognostic purposes.

E1263

DARATUMUMAB MONOTHERAPY COMPARED WITH REAL-WORLD HISTORICAL CONTROL DATA IN HEAVILY PRETREATED PATIENTS WITH HIGHLY REFRACTORY MULTIPLE MYELOMA: AN ADJUSTED TREATMENT COMPARISON

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Background: Daratumumab (DARA) received FDA approval in November 2015 based on data from single-arm phase 2 studies, following fast track/breakthrough therapy designation based on preliminary clinical evidence showing substantial improvements over available therapy for the treatment of heavily pretreated/refractory patients with multiple myeloma (MM). Recent analyses of pooled DARA studies and real-world US data revealed median overall survival (OS) of 19.9 (95% confidence interval [CI], 15.1-not evaluable) months and 7.9 (95% CI, 6.9-8.9) months in heavily pretreated and refractory patients with MM, respectively (Usmani S, et al. *Blood*. 2015;126(23): Abstract 4498 and Usmani S, et al. *Blood*. 2015;126(23): Abstract 29). In the absence of randomized head-to-head trials, evidence on the relative treatment efficacy of DARA *versus* physician's choice (PC) based on an adjusted comparison of data from a single arm trial *versus* a historical control group of comparable patients can inform the decisions of both clinicians and payers.
Aims: To estimate the comparative efficacy of DARA, as measured by overall survival, *versus* real-world historical control (PC) based on a comparison of data from DARA monotherapy clinical studies *versus* real-world US data, adjusted for confounding factors between patient cohorts.
Methods: Patient-level data were pooled from DARA monotherapy clinical studies (patients treated with DARA 16 mg/kg in the SIRIUS and GEN501 studies) and from two independent US databases (IMS-LifeLink and OPTUM), which reflect treatments utilized in a real-world cohort of patients with MM who received at least 3 prior therapy lines or were double refractory to a proteasome inhibitor and immunomodulatory drug. Using a multivariate proportional hazards regression model, the relative treatment effect of DARA compared with PC was estimated, adjusting for imbalances in patient characteristics between patient cohorts; co-variates included gender, age, albumin, hemoglobin, number of prior therapies, prior pomalidomide/carfilzomib-exposure, and refractory status.
Results: Baseline characteristics that differed between patients treated with DARA (N=148) and the real-world US historical control (N=658) included median age (64 *vs* 69 y), median lines of prior therapy (5 *vs* 4), prior treatment with pomalidomide (55% *vs* 15%) and carfilzomib (41% *vs* 28%), and triple/quadruple refractory status (64% *vs* 14%). The adjusted OS-hazard ratio (HR) for DARA *versus* PC was 0.32 (95% CI, 0.22-0.46) compared with 0.44 (95% CI, 0.33-0.58) for unadjusted HR. The impact of adjustment was mainly driven by differences in refractory status and prior exposure to pomalidomide/carfilzomib.
Summary/Conclusions: This adjusted treatment comparison suggests that DARA demonstrates improved OS compared with real-world historical control data in heavily pretreated and refractory MM patients. Such comparisons allow evaluation of novel agents in the absence of head-to-head comparison studies.

E1264

IMPACT OF FAMILY HISTORY ON RISK OF PROGRESSION OF MGUS TO MULTIPLE MYELOMA: A POPULATION-BASED STUDY

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Background: Multiple myeloma (MM) is characterized by a neoplastic proliferation of plasma cells in the bone marrow and overproduction of monoclonal immunoglobulins in serum or urine. MM is always preceded by a premalignant condition called monoclonal gammopathy of undetermined significance (MGUS). Individuals with MGUS have a 1-1.5% annual risk of developing MM or a related disease. Furthermore, familial aggregation of MM and MGUS has been described, and first-degree relatives of MGUS and MM patients have a 2-3 fold increased risk of a lymphoproliferative disorder (LP). We have previously shown that survival among MM patients with a family history of a LP have a significantly superior survival compared to sporadic MM. However, the effect of family history of LP on risk of progression among MGUS is unknown.
Aims: The aim of our study was to compare the risk of progression of MGUS to MM or other LPs in MGUS individuals with a family history of LPs compared to sporadic MGUS.
Methods: Individuals with MGUS and their first-degree relatives were identified using nationwide Swedish Registries. Information on malignancies in relatives of MGUS patients was obtained by record-linkages to the Swedish Cancer Registry. A positive family history of LP was defined as MGUS patients having at least one first-degree relative diagnosed with MM, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, Waldenström's macroglobulinemia, or Hodgkin's lymphoma. Cox proportional hazard model was used to obtain hazard ratios (HR) and 95% confidence intervals (95% CIs). In statistical analysis all data were adjusted for age, sex, and year at diagnosis.
Results: A total of 24,126 individuals diagnosed with MGUS in 1958-2013 were included in the study of whom 12,504 were male. Median age at diagnosis was 69.7 and 64.6 years in the sporadic and familial groups, respectively. In a total of 1666 MGUS individuals, the disorder progressed to MM. When compared to patients with sporadic MGUS, patients with MGUS and family history of any LP had similar risk of progression to MM (HR 1.17, 95% CI 0.90-1.53, p=0.23). The same was true when compared only to individuals with family history of

MM (HR 0.79, 95% CI 0.52-1.20, p=0.26). Subgroup analyses, stratified by gender and different age groups gave similar results (data not shown).
Summary/Conclusions: In this large population-based study we have shown that family history of LPs is not a risk factor for progression among MGUS individuals. Our findings are of importance for counseling MGUS individuals with family history and provide information regarding the biology of hereditary LPs.

E1265

THE SWEDISH MULTIPLE MYELOMA REGISTER: MEDIAN 5-YEAR SURVIVAL OF 7.3 YEARS IN PATIENTS 65 YEARS AND YOUNGER, POPULATION-BASED DATA ON 3,876 MULTIPLE MYELOMA PATIENTS DIAGNOSED 2008-2013

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Background: The Swedish Multiple Myeloma Register (SMMR) is a prospective observational register designed to document real-world management and outcomes in newly diagnosed multiple myeloma (MM), and was designed to improve the quality of the management of MM patients in Sweden
Aims: With high representation and good data quality we can present survival data on a whole MM population.
Methods: SMMR, initiated in 2008, comprises data on all patients diagnosed with MM, plasmocytoma, and plasma cell leukemia treated in Sweden. Report sheet are sent to all clinicians diagnosing MM. Another request is sent to the clinician one year after diagnosis, requesting information on initial treatment and complications. Through linkage with the Register of Total Population, we collected information on vital status until August 6, 2015. This report contains data on patients reported to SMMR between 2008 and 2013 and follow-up data from the first year on patients with symptomatic MM 2008-2012, with a follow-up till August 6, 2015, with focus on survival at 1, 3 and 5 years. Analyses of incidence, characteristics at baseline, proportion of patients given intensive treatment, obtaining very good partial remission rate (VGPR) and overall survival (OS) were estimated. Survival data was analysed with respect to age and ISS stage at diagnosis.

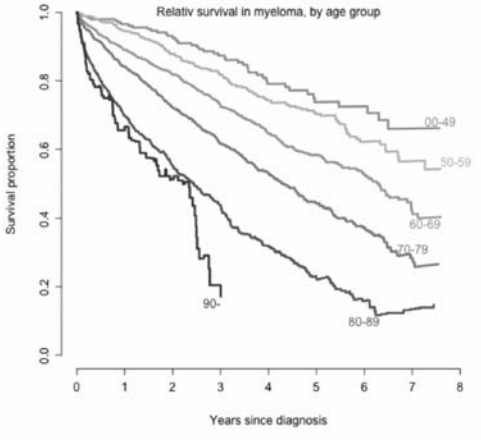

Figure 1.

Results: The SMMR has a current coverage of 98% compared to the Swedish Cancer Register on patients 2008-2013, to which reporting is obliged by law. Clinical data at baseline was available for 3876 patients and one year follow-up data was available on 2284 symptomatic MM patients (92% of all symptomatic MM cases initially reported to the register), from 70 different centers in Sweden. The median time of follow-up was 4.6 years. The age adjusted incidence was 6.2 MM cases per 100 000 inhabitants and year. The median age was 71 years, 70 years for men, and 73 years for women. Two third of patients were 65 years or older. Of the reported patients, 29% were in ISS stage I, 42% in stage II, and 29% in stage III. Overall, 80% of patients 65 years or younger received ASCT and 4% of patients above 65 years. In patients 66-70 years, ASCT was performed in 19%. In the year 2012, 67% of patients received one of the new drugs (thalidomide, bortezomib, or lenalidomide) as induction, (87% of patients<66 and 60% >65), an improvement compared to earlier years. The response grade >VGPR increased from 36% to 46% in the study period. The 1-, 3- and 5-year relative survival was 92%, 79%, and 66% for patients 65 years and younger and 79%, 56%, and 39% for the patients >65 years, respectively. The median relative survival was 7.3 and 3.7 years for patients<66 years and >65 years, respectively. Early death (<1year after diagnosis) was observed in 19% of patients. The relative median survival according to ISS stage was 3.2 years and 6.0 years for stages III and II, while not reached for stage I. Patients with no reported stage had the same survival as stage III patients.
Summary/Conclusions: The SMMR is an instrument for increased quality in the management of MM in Sweden. This report shows encouraging survival data on the MM population of Sweden. There was a significant difference in survival for younger and older patients that cannot be explained by age alone. The 19% early death rate (<1 year survival) can be compared to 48% in the period 1964 - 1968 (Swedish Cancer register data) and illustrates the enormous development in the care of MM patients in Sweden during the last 50 years.

E1266

CARFILZOMIB AND DEXAMETHASONE *VERSUS* BORTEZOMIB AND DEXAMETHASONE: SUBGROUP ANALYSIS OF THE PHASE 3 ENDEAVOR STUDY TO EVALUATE THE IMPACT OF PRIOR TREATMENT ON PATIENTS WITH RELAPSED MULTIPLE MYELOMA

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Background: In a randomized phase 3 study (ENDEAVOR; NCT01568866), carfilzomib and dexamethasone (Cd) significantly improved median progression-free survival (PFS) *versus* bortezomib (BTZ) and dexamethasone (Bd) (18.7 *vs* 9.4 months; hazard ratio [HR] 0.53; 95% confidence interval [CI]: 0.44, 0.65; p<0.0001) in 929 patients with relapsed multiple myeloma (RMM).
Aims: This subgroup analysis evaluated treatment with Cd *versus* Bd in patients after first relapse *versus* ≥2 prior lines of therapy as well as the effect of previous exposure to BTZ or lenalidomide (LEN).
Methods: Adult patients with RMM who had received 1-3 prior lines of therapy were randomized 1:1 to Cd or Bd. Patients in the Cd arm received carfilzomib (30-min intravenous [IV] infusion) on days 1, 2, 8, 9, 15, and 16 (20mg/m^2 on days 1 and 2 of cycle 1; 56mg/m^2 thereafter) and dexamethasone 20mg on days 1, 2, 8, 9, 15, 16, 22, and 23 of a 28-day cycle. Patients in the Bd arm received BTZ 1.3mg/m^2 (IV or subcutaneous) on days 1, 4, 8, and 11 and dexamethasone 20mg on days 1, 2, 4, 5, 8, 9, 11, and 12 of a 21-day cycle. Treatment was continued until disease progression or unacceptable toxicity. Primary end point was PFS, secondary end points included overall survival, overall response rate (ORR; ≥partial response), duration of response (DOR), rate of grade ≥2 peripheral neuropathy (PN) and safety.
Results: Efficacy outcomes by prior lines of therapy are shown in the Table. Median PFS for Cd *vs* Bd was 15.6 months *vs* 8.1 months, respectively (HR: 0.56; 95% CI: 0.44, 0.73) for patients with prior BTZ exposure and NE *vs* 11.2 months, respectively (HR: 0.48; 95% CI: 0.36, 0.66) for patients without prior BTZ exposure. Median PFS for Cd *vs* Bd was 12.9 months *vs* 7.3 months, respectively (HR: 0.69; 95% CI: 0.52, 0.92) for patients with prior LEN exposure and 22.2 months *vs* 10.2 months, respectively (HR: 0.43; 95% CI: 0.32, 0.56) for patients without prior LEN exposure. ORRs for Cd *vs* Bd were 71.2% *vs* 60.3% (OR: 1.63; 95% CI: 1.12, 2.36) in patients with prior BTZ exposure, 83.6% *vs* 65.3% (OR: 2.72; 95% CI: 1.72, 4.31) in patients without prior BTZ exposure, 70.1% *vs* 59.3% (OR: 1.60; 95% CI: 1.03, 2.49) in patients with prior LEN exposure and 81.2% *vs* 64.6% (OR: 2.37; 95% CI: 1.62, 3.47) in patients without prior LEN exposure. Grade ≥3 adverse events (AEs) were reported in 69.8% (Cd) and 63.9% (Bd) of patients with 1 prior line and 76.6% (Cd) and 69.9%

(Bd) of patients with ≥2 prior lines. Grade ≥3 hypertension, dyspnea, and cardiac failure were more common with Cd *vs* Bd. The rate of grade ≥2 PN was lower with Cd *vs* Bd in patients with 1 prior line (6.5% *vs* 30.0%; OR: 0.16; 95% CI: 0.09, 0.29) and ≥2 prior lines (5.6% *vs* 34.1%; OR: 0.12; 95% CI: 0.06, 0.22).

Table 1. Efficacy outcomes and AEs of interest by prior lines of therapy.

Outcome	1 prior line		≥2 prior lines	
	Kd (n=232)	Vd (n=233)	Kd (n=232)	Vd (n=232)
Median PFS, months	22.2	10.1	14.9	8.4
HR for Kd vs Vd (95% CI)	0.447 (0.330–0.606)		0.604 (0.466–0.783)	
Best overall response, n (%)				
Stringent complete response	6 (2.6)	6 (2.6)	2 (0.9)	3 (1.3)
Complete response	21 (9.1)	12 (5.2)	29 (12.5)	8 (3.4)
Very good partial response	117 (50.4)	53 (22.8)	77 (33.2)	51 (21.9)
Partial response	46 (19.8)	80 (34.5)	58 (25.0)	77 (33.0)
Minimal response	11 (4.7)	21 (9.1)	13 (5.6)	32 (13.7)
Stable disease	16 (6.9)	24 (10.3)	24 (10.3)	29 (12.4)
Progressive disease	6 (2.6)	15 (6.5)	19 (8.2)	16 (6.9)
Not evaluable	9 (3.9)	21 (9.1)	10 (4.3)	17 (7.3)
ORR, % (95% CI)	81.9 (76.3–86.6)	65.5 (59.0–71.6)	72.0 (65.7–77.7)	59.7 (53.1–66.0)
Median DOR, months	21.3	14.1	NE	10.3
Grade ≥3 AEs of interest, n (%)[a]				
Hypertension[b]	24 (10.3)	8 (3.5)	17 (7.4)	4 (1.7)
Dyspnea[b]	12 (5.2)	5 (2.2)	13 (5.6)	5 (2.2)
Cardiac failure[b]	5 (2.2)	2 (0.9)	5 (2.2)	1 (0.4)
Acute renal failure[b]	2 (0.9)	3 (1.3)	7 (3.0)	2 (0.9)

[a]In the 1 prior line group, 232 (Kd) and 227 (Vd) patients were evaluable for safety; in the ≥2 prior lines group, 231 (Kd) and 229 (Vd) patients were evaluable for safety.

[b]Preferred term.

AE, adverse event; CI, confidence interval; DOR, duration of response; HR, hazard ratio; Kd, carfilzomib and dexamethasone; NE, not estimable; ORR, overall response rate; PFS, progression-free survival; Vd, bortezomib and dexamethasone.

Summary/Conclusions: A clinically meaningful improvement in PFS was seen for patients with RMM who were treated with Cd compared with Bd, regardless of the number of prior lines of treatment. The improvement was greatest for those who had received 1 prior line, where median PFS was over 1 year longer in patients treated with Cd *vs* Bd. PFS benefit with Cd *vs* Bd was also maintained regardless of prior exposure to BTZ and LEN. A higher ORR was also observed with Cd *vs* Bd across all subgroups. Cd had an acceptable benefit-risk profile in this study.

E1267

CARFILZOMIB AND DEXAMETHASONE *VS* BORTEZOMIB AND DEXAMETHASONE: SUBGROUP ANALYSIS OF PATIENTS WITH RELAPSED MULTIPLE MYELOMA BY BASELINE CYTOGENETIC RISK STATUS (PHASE 3 ENDEAVOR STUDY)

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Background: In a previous study, single-agent carfilzomib demonstrated activity in patients with relapsed and refractory multiple myeloma with high-risk cytogenetic abnormalities. In the phase 3 study (NCT01568866; N=929 patients) carfilzomib plus dexamethasone (Cd) significantly improved progression-free survival (PFS) by 2-fold compared with bortezomib/dexamethasone (Bd) in patients with relapsed multiple myeloma (RMM) (18.7 months *versus* 9.4 months; hazard ratio [HR]: 0.53; 95% confidence interval [CI]: 0.44–0.65; P<.0001).
Aims: A pre-planned subgroup analysis of efficacy and safety outcomes in patients treated with Cd *vs* Bd according to patients' baseline cytogenetic risk status.
Methods: Adults with RMM who had received 1–3 prior lines of therapy received either carfilzomib (30-minute intravenous [IV] infusion on days 1, 2, 8, 9, 15, and 16 [20mg/m^2 on days 1 and 2 of cycle 1; 56 mg/m^2 thereafter]) and dexamethasone (20mg on days 1, 2, 8, 9, 15, 16, 22, and 23 of a 28-day cycle) (Cd) or bortezomib (1.3mg/m^2 IV bolus or subcutaneous injection on days 1, 4, 8, and 11) and dexamethasone (20mg on days 1, 2, 4, 5, 8, 9, 11, and 12 of a 21-day cycle) (Bd). Treatment continued until disease progression, withdrawal of consent or unacceptable toxicity. Primary end point was PFS, secondary end points included overall survival, overall response rate (ORR), duration of response (DOR), rate of grade ≥2 peripheral neuropathy (PN) and safety. The high-risk group was defined (using fluorescence in situ hybridization analysis of baseline bone marrow samples) as those patients with the genetic subtypes t(4;14) or t(14;16) in ≥10% of screened plasma cells or deletion 17p in ≥20% of screened plasma cells; the standard-risk group consisted of patients without these genetic subtypes.
Results: Of the 929 patients, 464 were randomized to receive Cd and 465 to receive Bd. Baseline cytogenetic risk status was balanced between the treatment arms (high-risk: Cd, 20.9%; Bd, 24.3%; standard-risk: Cd, 61.2%; Bd, 62.6%; unknown: Cd, 17.9%; Bd, 13.1%). Efficacy and safety end points by baseline cytogenetic risk status are presented in the Table. In the high-risk group, median PFS was 8.8 months (95% CI: 6.9, 11.3) for Cd *vs* 6.0 months (95% CI: 4.9, 8.1) for Bd (HR: 0.646; 95% CI: 0.453, 0.921). In the standard-risk group, median PFS was not estimable (NE) for Cd (95% CI: 18.7, NE) *vs* 10.2 months (95% CI: 9.3, 12.2) for Bd (HR: 0.439; 95% CI: 0.333, 0.578). ORRs (≥partial response) in the high-risk group were 72.2% (Cd) *vs* 58.4% (Bd) and 79.2% (Cd) *vs* 66.0% (Bd) in the standard-risk group. Median DOR in the high-risk group was 10.2 months for Cd *vs* 8.3 months for Bd. Median DOR in the standard-risk group was NE for Cd *vs* 11.7 months for Bd. Grade ≥3 adverse events (AEs) were reported at higher rates with Cd *vs* Bd in both the high- and standard-risk groups (70.1% *vs* 63.1% and 73.9% *vs* 68.3%). Grade ≥2 PN occurred less frequently with Cd *vs* Bd regardless of cytogenetic risk status (high-risk group: 3.1% *vs* 35.1%; odds ratio: 0.059; 95% CI: 0.018, 0.198 and standard-risk group: 6.4% *vs* 33.4%; odds ratio: 0.135; 95% CI: 0.079, 0.231).

Table 1. Efficacy outcomes and AEs of interest by baseline cytogenetic risk status.

Outcome	High-Risk		Standard-Risk	
	Kd (n=97)	Vd (n=113)	Kd (n=284)	Vd (n=291)
Median PFS, months	8.8	6.0	NE	10.2
HR for Kd vs Vd (95% CI)	0.646 (0.453–0.921)		0.439 (0.333–0.578)	
Best overall response, n (%)				
Stringent complete response	2 (2.1)	3 (2.7)	6 (2.1)	6 (2.1)
Complete response	13 (13.4)	2 (1.8)	31 (10.9)	17 (5.8)
Very good partial response	30 (30.9)	29 (25.7)	130 (45.8)	63 (21.6)
Partial response	25 (25.8)	32 (28.3)	57 (20.1)	105 (36.1)
Minimal response	8 (8.2)	11 (9.7)	12 (4.2)	36 (12.4)
Stable disease	9 (9.3)	17 (15.0)	21 (7.4)	28 (9.6)
Progressive disease	6 (6.2)	10 (8.8)	15 (5.3)	16 (5.5)
Not evaluable	4 (4.1)	9 (8.0)	12 (4.2)	20 (6.9)
ORR, % (95% CI)	72.2 (62.1–80.8)	58.4 (48.8–67.6)	79.2 (74.0–83.8)	66.0 (60.2–71.4)
Median DOR, months	10.2	8.3	NE	11.7
Select grade ≥3 AEs of interest, n (%)[a]				
Hypertension[b]	6 (6.2)	4 (3.6)	30 (10.6)	8 (2.8)
Dyspnea[b]	5 (5.2)	1 (0.9)	16 (5.7)	6 (2.1)
Cardiac failure[b]	3 (3.1)	2 (1.8)	7 (2.5)	1 (0.3)
Acute renal failure[b]	3 (3.1)	0	6 (2.1)	3 (1.0)
Peripheral neuropathy[c]	1 (1.0)	8 (7.2)	7 (2.5)	29 (10.1)
Treatment discontinuations due to an AE, n (%)[a]	18 (18.6)	22 (19.8)	56 (19.8)	62 (21.6)

[a]In the high-risk group, 97 (Kd) and 111 (Vd) patients were evaluable for safety; in the standard-risk group, 283 (Kd) and 287 (Vd) patients were evaluable for safety.

[b]Preferred term.

[c]Grouped term.

AE, adverse event; CI, confidence interval; DOR, duration of response; HR, hazard ratio; Kd, carfilzomib and dexamethasone; NE, not estimable; ORR, overall response rate; PFS, progression-free survival; Vd, bortezomib and dexamethasone.

Summary/Conclusions: Patients treated with Cd had a clinically meaningful improvement in PFS compared with Bd regardless of baseline cytogenetic risk status. Higher response rates, greater depth of response and longer DOR were also reported with Cd *vs* Bd. Cd had a favorable benefit–risk profile in patients with high-risk relapsed MM.

E1268

INCREASED LEVELS OF SOLUBLE SYNDECAN-1 AND LDH ARE MAJOR PROGNOSTIC FACTORS FOR SUBSEQUENT OVERALL SURVIVAL AND PROGRESSION FREE SURVIVAL AFTER A RELAPSE IN MULTIPLE MYELOMA

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Background: Despite the recent advances in the therapy of Multiple Myeloma (MM), a relapse is almost a universal phenomenon in the clinical course of the disease. Nevertheless only few are known about the prognostic factors that characterize the relapses in MM, since robust and easily accessible prognostic factors as the levels of soluble Syndecan-1 (sS-1) or the ratio of involved to uninvolved free light chains in the serum (FLCR) have been verified only in newly diagnosed cases of MM.

Aims: A series of 245 MM patients with at least one relapse in their disease course, was studied to identify the prognostic factors that govern the prognosis of relapse in MM.
Methods: sS-1 levels in the serum were measured through the commercially available Diaclone™ ELISA kits. Survival statistical analysis was performed using the SPSS v22 statistical software. The running log rank method was used for the establishment of the optimal threshold of numerical variables. Clinical and laboratory values used were the ones closest to an established relapse and prior to the salvage treatment.
Results: From 01/01/2000 till 20/06/2014, 245 patients with symptomatic MM and at least one relapse were recorded for a total of 803 relapses.56 patients had 1 relapse, 51 patients 2 relapses, 48 patients 3 relapses and 90 patients more than 3. The clinical and laboratory characteristics of the relapses are presented in Table 1. The median Overall Survival (OS) and median Progression Free Survival (PFS) after a relapse were 1.7 and 0.9 years (yr) respectively. For the first relapse OS and PFS were 3.3 and 1.3 years, for the second relapse OS and PFS were 2.1 and 1 yr, for the third relapse OS and PFS were 1.2 and 0.8 yr, while for the fourth and above OS and PFS were 0.8 and 0.4 yr respectively. Statistically significant variables for the prediction of subsequent OS after a relapse in univariate analysis were: ISS stage III (HR:1.26, P<0.001), male sex (HR:1.39, P<0.001), sS-1>245 ng/mL (HR:2.56, P<0.001), LDH>480 IU/L (HR:1.99, P<0.001), number of prior relapses (HR:1.02, P=0.002) and response to treatment (HR:1.16, P<0.001). For the prognostication of subsequent PFS, the following factors were significant in univariate analysis: age≥65 years (HR:1.07, P=0.02), sS-1>245 ng/mL (HR:2.29, P=0.001), LDH>480 IU/L (HR:1.53, P=0.003), number of prior relapses (HR:1.14, P<0.001) and response to treatment (HR:1.12, P<0.001). Multivariate analysis revealed sS-1>245 ng/mL and LDH>480 IU/L as the only statistically significant factors for both OS and PFS. sS-1>245 ng/mL and LDH>480 IU/L retained their statistical significance both for univariate and multivariate analysis even when the subgroup of relapses treated with novel agents was independently studied.

Table 1. Characteristics of MM relapses.

Variable	n/N (%)
Age ≥ 65 yr	340/803 (42%)
Male Sex	452/803 (57%)
Albumin < 3.5g/dL	72/318 (23%)
B2M ≥ 3.5mg/L	461/732 (63%)
FLCR>100	345/524 (66%)
Monoclonal Immunoglobulin > 6000 mg/dl	80/614 (13%)
Creatinine ≥ 2.0 mg/dL	51/433 (11%)
Abnormal LDH	107/437 (24%)
sS-1> 245 ng/ml	22/74 (30%)
ISS stage I	184/732 (25%)
ISS stage II	216/732 (30%)
ISS stage III	332/732 (45%)
Salvage treatment novel agents	425/802 (53%)
Salvage treatment autologous transplant	55/802 (7%)

Summary/Conclusions: In conclusion both OS and PFS are gradually declining after each relapse. While both the number of prior relapses and the response to salvage treatment are important prognostic factors, subsequent OSand PFS are mainly dictated by the levels of sS-1 and LDH that characterize the MM relapse.

E1269

11C- METHIONINE PET SCAN FOR THE DETECTION OF MULTIPLE MYELOMA LESIONS

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Background: Multiple Myeloma (MM) is a plasma cell neoplasia that represents the 10-15% of hematological malignancies. The myeloma diagnostic criteria have recently changed based on the availability of more sensitive tools for early detection of the disease. This particularly apply to imaging techniques such as CT, MRI and PET. ^{11}C-methionine is a radiolabeled amino acid that has proven to have higher avidity for protein synthesis. Some recently published works show that ^{11}C-MET PET/TAC is useful for detecting bone and extramedullary lesions in MM and might be a promising tool in the diagnosis and follow up of this disease.
Aims: The purpose of this study is first to evaluate the performance of ^{11}C-MET PET/CT in MM as a diagnostic method compared to ^{18}FDG-PET/CT, and second to analyze the correlation between the metabolic activity and biological parameters of the disease.
Methods: We have reviewed retrospectively the results of 14 patients (50% women, ranged 31-79 years) with MM (12 cases), Smoldering Multiple Myeloma (one case) and solitary plasmacytoma (one case), who simultaneously underwent ^{11}C-MET and ^{18}FDG- PET/CT at our institution. We have analyzed the number and the uptake value (SUV_{max}) of the lesions observed with both techniques and we have correlated these findings with the biological parameters of the disease (M component, plasma cell percentage in bone marrow, free light chains, immunoglobulins, calcium, renal function). The concordance and correlation between both techniques were analyzed using Cohen's kappa coefficient and Pearson coefficient (SPPS version 19).
Results: A total of 54 lesions were studied (mean 3.8 lesions/patient). The number of lesions observed with ^{11}C-MET was higher than with ^{18}FDG in 57% of the patients. Only in two cases ^{18}FDG -PET/CT revealed lesions that were not seen with ^{11}C-MET, but interestingly in one of them a biopsy confirmed the non-malignant nature of the lesion. In most cases the SUV_{max} was higher in ^{11}C-MET for the same lesion compared to ^{18}FDG. Cohen's kappa coefficient between both tests was calculated with a result of 0.29 that means a fair agreement between ^{18}FDG and ^{11}C-MET. This coefficient also reflects a 35% of false negative lesions seen in ^{18}FDG-PET. The following biological parameters were correlated with a significant increase in the uptake value in ^{11}C-MET PET/CT: M-component (r=0.28; p=0.02), serum IgG (r=0.58; p=0.002), involved free light chain (r=0.28; p=0.04) and percentage of plasma cells in the bone marrow (r=0.32; p=0.03). These correlations were not significant with the ^{18}FDG PET/CT.

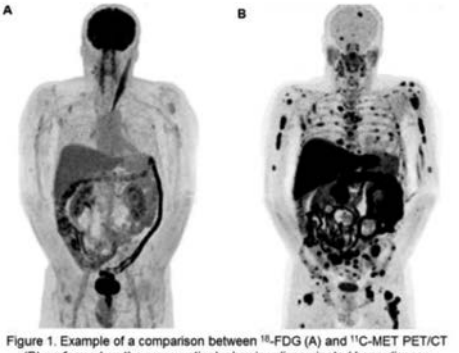

Figure 1. Example of a comparison between ^{18}-FDG (A) and ^{11}C-MET PET/CT (B) performed on the same patient, showing disseminated bone disease evident only with ^{11}C-MET.

Figure 1.

Summary/Conclusions: In our experience, PET with ^{11}C- MET could be a highly sensitive tool to evaluate MM lesions. Our data, despite the small number of patients, suggest that it may be superior to conventional ^{18}FDG-PET. Furthermore, there seems to be a significant correlation between some biological parameters, such as FLC, MC or immunoglobulins levels and the avidity of uptake. Larger series are needed to confirm the present findings.

E1270

ACY-241, A NOVEL, ORAL TABLET HDAC6 SELECTIVE INHIBITOR COMBINES SAFELY WITH POMALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA (ACE-MM-200 STUDY)

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Background: Histone deacetylase (HDAC) enzymes are attractive therapeutic targets, but non-selective HDAC inhibitors have toxicities limiting exposure in patients (pts), particularly in combination with other agents. Ricolinostat (ACY-1215), is a first-in-class liquid orally available HDAC inhibitor 11-fold more selective for HDAC6, that synergizes *in vitro* and *in vivo* in models of multiple myeloma (MM) with bortezomib (Santo, *Blood*, 2012) and with carfilzomib (Mishima, *Br J Haematol*, 2015) and with lenalidomide (Len) and pomalidomide (Pom) (Quayle *Blood* 2013;122:1952). Ricolinostat, an oral liquid, has an excellent safety profile. (Raje, *Haematologica*, 2014, Suppl 1). We now identify ACY-241 as a structurally related, selective inhibitor of HDAC6 in tablet form.
Aims: Determine the safety, tolerability and preliminary efficacy of ACY-241 monotherapy and combination with pom and dexamethasone (dex).
Methods: Based on clinical experience with ricolinostat and non-clinical pharmacokinetics of ACY-241, we designed a first-in-human phase 1a/1b clinical

trial of a single-cycle of ACY-241 monotherapy followed by ACY-241 in combination with Pom and dexamethasone (Dex) in MM pts. A monotherapy/combination trial (mono/combo) design was chosen to grant pts access to combo therapy with an active regimen while exploring the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of ACY-241 monotherapy. Pts with relapsed or relapsed-and-refractory MM (RRMM) previously treated with ≥2 cycles of Len and a proteasome inhibitor were eligible. Cohorts of 3 pts had ACY-241 PO QD as monotherapy (180, 360 and 480 mg) on days 1-21 of a 28 day cycle. If no mono DLT was noted, pts continued to cycle 2 of combo therapy with ACY-241/Pom/Dex. Safety review committee meetings for mono and combo therapy were separate. PK of ACY-241, Pom and Dex were analyzed. PD assessment were acetylated tubulin (HDAC6) and histones (HDAC 1, 2 and 3) in peripheral blood mononuclear cells.
Results: Since June 2015, 13 safety-evaluable pts have enrolled. Median age was 62 (46-82) years and median number of prior regimens 2 (1-7). All pts had RRMM. 62% were refractory to len and 46% to both Btz and Len. Fifty percent of pts had high risk cytogenetics. No dose relationship of the number or severity of AEs in either monotherapy or combination therapy were noted. Common toxicities in the safety population included grade 1/2 fatigue (38%), cough, back pain and dizziness (23% each), diarrhea, constipation and nausea (20% each). Grade 3/4 toxicities included neutropenia (4 pts, 31%), thrombocytopenia (23%) and anemia (8%). No MTD was identified up to the highest dose of ACY-241 at 480 mg QD monotherapy and 180 and 360 mg po QD in combo with Pom/Dex. 360 mg QD was recommended for further clinical exploration of ACY-241 based on safety and PD data. PK results show dose-linear increase in exposure with no accumulation, drug-drug interaction with Pom and Dex, or exposure plateau (previously observed with ricolinostat.) Selective increase in acetylated tubulin was seen at 180 mg with increasing levels of acetylated tubulin and histones at higher doses. Confirmed efficacy data (minimum follow-up 3 months) for combo treatment in this refractory pt population (8 efficacy evaluable pts) shows 4 PR, 2 MR and 2 PD. Three pts had only one efficacy cycle reported and 2 are early.
Summary/Conclusions: ACY-241 is well-tolerated in combination with Pom/Dex with at least dose proportional PK. Early response data to combo treatment parallel those observed with ricolinostat/Pom/Dex and compare favorably to historic controls of Pom/Dex. Cohort expansion at biologically relevant combination doses is ongoing. Updated data will be presented.

E1271

TREATMENT OF PRE-MYELOMA STATES: A CLINICAL DECISION ANALYSIS
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Background: Recent studies suggest that treatment of pts with pre-myeloma disease may result in reduced progression to myeloma and improvement in overall survival. However, there is a lack of data to determine which patients would benefit and what type of treatments are optimal. A decision analysis to synthesize available data and quantify the benefits and risks of different treatments in different at-risk populations can help clarify both individual and policy decision-making.
Aims: To quantify the benefit and risks of different treatments in different at-risk populations. To develop a decision framework that informs both individual and policy decision-making for treatment of pre-myeloma states.
Methods: Monte Carlo simulations using a Markov Model were used to simulate the life course of patients diagnosed with pre-myeloma states in the modern era, either treated pre-emptively or monitored per standard of care. The primary outcome was number needed to treat (NNT) to save one life from myeloma, along with life expectancy (LE) and number needed to harm (NNH) given treatment risks. Pre-myeloma risk states modeled included standard risk MGUS (21% 20-yr progression), high risk MGUS (58% 20-yr progression), and smoldering myeloma (78% 20-yr risk of progression). Three interventions were modeled: (1) "high-intensity" treatment (e.g. lenalidomide+dexamethasone) with a conservatively modeled 50% reduction in risk of progression but also an increased risk of developing a secondary hematologic malignancy; (2) "low-intensity" treatment with a 10% reduction in risk of progression; and (3) observation until progression. Rates of progression, increased risk of hematologic malignancy, and death rates from myeloma and secondary malignancies were estimated by literature review. The life course of 500,000 patients for every combination of pre-myeloma state, treatment, and ages ranging from 35 to 75 was simulated. The model was validated against existing cohort data. Probabilistic sensitivity analyses were conducted to generate 95% "confidence intervals" [95% CI] given uncertainty in model parameters.
Results: NNT and NNH varied substantially by age, pre-myeloma state, and type of treatment (Figure). For 65 year old patients with high risk MGUS, the high and low-intensity treatments yielded NNTs of 8.3 [6.8-9.8] and 47.6 [33.6-90.9] respectively, an increase in LE of +0.9 [0.3-1.5] and +0.2 [+0.1-0.4] yrs respectively, and an NNH of 29.4 [95% CI 18.2-47.1] for the high-intensity treatment. For 75 yr old patients with MGUS, the high-intensity regimen yielded a lower NNH (27.8 [17.6-58.7]) than NNT (38.5 [27.2-58.1]), suggesting that more patients would be harmed by secondary malignancies than be benefited with treatment, with a non-statistically significant decrease in LE of -0.1 [-0.3-0.1]. In the same cohort, the low-intensity treatment had an NNT of 200 [105.6-887.5] and no benefit in LE (mean=0 [0-0.1]). In general, younger patients with higher-risk disease benefitted more from treatment. In 35 yr old patients with smoldering myeloma, the high and low intensity treatments had NNT of 5.3 [4.8-6.5] and 41.7 [30.3-121.9] respectively, with estimated LE increase of +6.5 [5.5-7.7] and +1.0 [0.4-1.3] yrs, respectively, and a NNH for the high-intensity regimen of 71.4 [37.7-154.2].

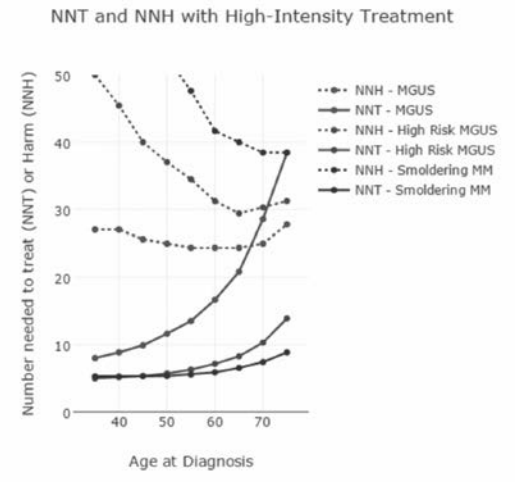

Figure 1.

Summary/Conclusions: The benefit/risk ratio of treatment for pre-myeloma patients varies considerably depending on age, risk of myeloma progression, and intensity/risk of treatment. For younger higher-risk pre-myeloma patients, the benefit/risk ratio of high-intensity pre-myeloma intervention is increased, whereas for older patients with lower-risk disease a lower-intensity or no treatment is preferred. Though cost and quality of life under these treatments also need to be considered, our results suggest there are substantial subgroups of patients with pre-myeloma disease who would benefit from treatment. As we develop improved therapies and risk-stratification, using such a quantitative framework will help inform decision-making.

E1272

PHASE I/IIA CLINICAL STUDY OF AUTOLOGOUS DENDRITIC CELL THERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA
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Background: Cellular immunotherapy using dendritic cells is emerging as a useful immunotherapeutic modality to treat multiple myeloma (MM). We have developed potent immunotherapeutic agent (VAX-DC/MM) generated by dendritic cells loaded with the ultraviolet B-irradiated autologous myeloma cells.
Aims: In this study, we evaluated the safety and efficacy of VAX-DC/MM in patients with relapsed or refractory MM.
Methods: This trial enrolled relapsed or refractory MM patients who had received thalidomide- and bortezomib-containing regimen. Patients received the intradermal VAX-DC/MM injection every week for four weeks. Before the first injection of VAX-DC/MM, low-dose cyclophosphamide (375 mg/m^2, i. v) was administered to stimulate immune response at D-3. In a phase I trial, each three patients were treated with 5 x 10^6, and 10 x 10^6 cell, respectively. After higher dose was established as the tolerable dose, an additional 6 patients were enrolled at 10 x 10^6 cell doses.
Results: Median time to VAX-DC/MM therapy from diagnosis was 56.6 months (28.5-130.5). Patients had received a median of five prior treatments, and 75% had received autologous stem cell transplantation. VAX-DC/MM therapy was well tolerated, and most frequent adverse event was grade 1-2 myalgia (33.3%). In 8 of 9 patients who received 10 □ 10^6 cell, immunologic response (88.9%) was observed by interferon-gamma ELISPOT assay or mixed lymphocyte reaction assay for T-cell proliferation. Clinical benefit rate was 66.7% including 1 minor response (11.1%) and 5 stable disease (55.6%), and 3 patients (33.3%) showed a progression disease. The median progression free survival was 3.1 months (95% CI, 2.8-3.5 months), and all patients are alive.
Summary/Conclusions: In conclusion, VAX-DC/MM therapy was well-tolerated, and has activity in heavily pretreated MM. Further studies are needed to increase the efficacy of VAX-DC/MM in patients with MM.

E1273

ECONOMIC EVALUATION OF CARFILZOMIB+LENALIDOMIDE+DEXAMETHASONE (KRD) *VS* LENALIDOMIDE+DEXAMETHASONE (RD) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (R/RMM)

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Background: In ASPIRE, KRd achieved superior progression free survival (PFS) (HR=0.69; p=0.0001) and improved quality of life (QoL) (p<0.001) over Rd in patients (pts) with R/RMM.
Aims: We assessed the cost-effectiveness (CE) of KRd *vs* Rd from a US payer perspective with data from ASPIRE and long-term overall survival (OS) data from the Surveillance, Epidemiology, and End Results Program (SEER).
Methods: A partitioned-survival model with progression-free (PF), post-progression (PP) and death states was built with a 30 year horizon. Treatment (Tx) effect for PFS and OS was estimated with parametric regression models and applied over the time horizon. Since 60% of pre-specified OS events were observed, post-trial OS transition probabilities were estimated by matching SEER data to ASPIRE pts. Grade ≥3 adverse events (AEs) with >2% incidence in any ASPIRE arm were included. Utilities were derived from literature and adjusted for the relative improvement in QoL increase observed when pts were PF in ASPIRE. Costs related to drug (WAC prices) as well as wastage (30% for carfilzomib), administration, monitoring, AE management, and subsequent Tx were considered. Tx duration was derived fitting ASPIRE data and carfilzomib was dosed up to 18 cycles. The base case assessed the CE of adding carfilzomib to Rd, and considered only additional costs of carfilzomib, *i.e.* neglecting Rd drug costs in both arms. A scenario including Rd costs in both arms was conducted. A 3% discount rate was applied to costs and outcomes.
Results: KRd was more effective compared to Rd, providing 1.134 PF life year, 1.927 life year, and 1.616 quality-adjusted life year (QALY) gains over the modelled horizon. KRd incurred $123,524 in total additional costs. Incremental CE ratio (ICER) was $76,416/QALY. With the inclusion of Rd costs, KRd incurred $179,863 additional costs with an ICER of $111,270/QALY.
Summary/Conclusions: The model predicts that KRd delivers nearly 2.86 additional undiscounted life year's over the standard of care (Rd), and is cost-effective *vs* Rd at a willingness-to-pay threshold of $120,000/QALY. Therefore, carfilzomib when added to Rd delivers considerable incremental value to patients and payers.

E1274

CARFILZOMIB AND DEXAMETHASONE *VS* BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA: ANALYSIS OF THE PHASE 3 ENDEAVOR STUDY BY AGE SUBGROUP

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Background: The selective proteasome inhibitor carfilzomib is approved in the United States (US) as a single agent for the treatment of relapsed and refractory multiple myeloma (MM) and in combination with dexamethasone for relapsed MM. It is also approved in the US and Europe in combination with lenalidomide and dexamethasone for the treatment of patients with relapsed MM (RMM). In the phase 3 study (ENDEAVOR; NCT01568866) carfilzomib and dexamethasone (Cd) doubled progression-free survival (PFS) compared with bortezomib and dexamethasone (Bd) in patients with RMM (median 18.7 *vs* 9.4 months; hazard ratio [HR], 0.53; 95% confidence interval [CI], 0.44, 0.65; 1-sided p<0.0001).
Aims: A pre-planned subgroup analysis of the ENDEAVOR study according to patients' age (<65, 65–74, and ≥75 years of age).
Methods: Adults with RMM (1–3 prior regimens) were randomized to either the Cd arm (carfilzomib 30-min intravenous [IV] infusion on days [D] 1, 2, 8, 9, 15, and 16 [20mg/m^2 on D1, 2 in cycle 1; 56mg/m^2 thereafter]) and dexamethasone (20mg on D1, 2, 8, 9, 15, 16, 22 and 23 of 28-day cycles) or the Bd arm (bortezomib 1.3mg/m^2 IV or subcutaneous on D1, 4, 8, and 11) and dexamethasone (20mg on D1, 2, 4, 5, 8, 9, 11, and 12 of 21-day cycles). Treatment continued until disease progression, withdrawal of consent or unacceptable toxicity. Primary end point was PFS and secondary end points included overall survival, overall response rate (ORR), duration of response (DOR), safety, and rate of peripheral neuropathy (PN).
Results: Of the 929 patients enrolled, in the<65 years subgroup: 223 patients received Cd and 210 received Bd; in the 65–74 years subgroup: Cd, n=164; Bd, n=189; and the ≥75 years subgroup: Cd, n=77; Bd, n=66. Patient and disease characteristics were balanced across treatment arms within each age subgroup. For all age subgroups, median PFS was improved with Cd *vs* Bd (<65 years: not estimable *vs* 9.5 months [HR, 0.58; 95% CI, 0.44, 0.77]; 65–74 years: 15.6 months *vs* 9.5 months [HR, 0.53; 95% CI, 0.38, 0.73]; ≥75 years: 18.7 months *vs* 8.9 months [HR, 0.38; 95% CI, 0.23, 0.65]) (Table). ORRs in each age group were also consistently higher in the Cd arm *versus* the Bd arm (<65 years: 74% *vs* 61% [odds ratio, 1.82; 95% CI, 1.21, 2.74]; 65–74 years: 77% *vs* 66% [odds ratio, 1.80; 95% CI, 1.12, 2.89]; ≥75 years: 84% *vs* 59% [odds ratio, 3.75; 95% CI, 1.71, 8.24]). Grade ≥3 hypertension, dyspnea, cardiac failure, renal failure were more common with Cd *vs* Bd. However, rates of grade ≥2 PN were lower in the Cd arm compared with the Bd arm (<65 years: 6% *vs* 27% [odds ratio, 0.17; 95% CI, 0.09, 0.32]; 65–74 years: 8% *vs* 34% [odds ratio, 0.17; 95% CI, 0.09, 0.32]; ≥75 years: 3% *vs* 43% [odds ratio, 0.04; 95% CI, 0.01, 0.16]). Deaths within 30 days post-study drug due to adverse events occurred at a similar frequency in the Cd and Bd arms (<65 years: 3% *vs* 3%; 65–74 years: 5% *vs* 3%; ≥75 years: 4% *vs* 5%).

Table 1. Efficacy outcomes and grade ≥3 adverse events of interest.

Outcome	<65 years		65–74 years		≥75 years	
	Kd (n=223)	Vd (n=210)	Kd (n=164)	Vd (n=189)	Kd (n=77)	Vd (n=66)
Median PFS, months	NE	9.5	15.8	9.5	18.7	8.9
HR for Kd vs Vd (95% CI)	0.58 (0.44–0.77)		0.53 (0.38–0.73)		0.38 (0.23–0.65)	
Best overall response, n (%)						
Complete response or better	35 (16)	16 (8)	19 (12)	11(6)	4 (5)	2 (3)
Very good partial response or better	118 (53)	64 (30)	88 (54)	54 (29)	46 (60)	15 (23)
ORR, % (95% CI)	74 (68–80)	61 (54–68)	77 (70–84)	66 (58–72)	84 (74–92)	59 (46–71)
Median DOR, months	NE	11.1	NE	10.3	21.3	10.2
Select grade ≥3 AEs of interest, n (%)[a]						
Hypertension[b]	20 (9)	6 (3)	12 (7)	4 (2)	9 (12)	2 (3)
Dyspnea[b]	8 (4)	3 (1)	11 (7)	6 (3)	6 (8)	1 (2)
Cardiac failure[b]	2 (1)	1 (1)	5 (3)	1 (1)	3 (4)	1 (2)
Renal failure[b]	3 (1)	0	3 (2)	2 (1)	1 (1)	0
Treatment discontinuations due to an AE, n (%)[a]	37 (17)	31 (15)	35 (21)	41 (22)	20 (26)	23 (35)

[a]In the <65 year subgroup, 223 (Kd) and 208 (Vd) patients were evaluable for safety; in the 65–74 year subgroup, 163 (Kd) and 183 (Vd) patients were evaluable for safety; in the ≥75 year subgroup, 77 (Kd) and 65 (Vd) patients were evaluable for safety.

[b]Preferred term

AE, adverse event; CI, confidence interval; DOR, duration of response; HR, hazard ratio; Kd, carfilzomib and dexamethasone; NE, not estimable; ORR, overall response rate; PFS, progression-free survival; Vd, bortezomib and dexamethasone.

Summary/Conclusions: Cd significantly improved PFS and ORR compared with Bd across all age subgroups. A trend toward a greater improvement was observed in the eldest-age subgroup (≥75 years) *versus* the two younger-age subgroups (<65 and 65-74 years). In the eldest-age subgroup treatment with Cd was associated with an increased incidence of select grade ≥3 adverse events of interest, including cardiac failure and hypertension, compared with treatment with Cd in the younger-age subgroups. Hypertension is a common and manageable complication in elderly patients and should be monitored. Cd has a favorable benefit-risk profile in patients with RMM, irrespective of age.

E1275

TREATMENT AND OUTCOME PATTERNS IN PATIENTS WITH RELAPSED WALDENSTRÖM'S MACROGLOBULINEMIA: DEVELOPMENT OF A LARGE OBSERVATIONAL PAN-EUROPEAN DATA PLATFORM

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Background: There are few randomized trials and no well-established treatment standards for Waldenström's Macroglobulinemia (WM). Data on treatment choices and their outcome in patients (pts) outside clinical trials are lacking.
Aims: The goal of this observational chart review was to generate real-world data on diagnosis, treatment patterns, and outcomes for WM over a decade in a large effort involving several European countries.
Methods: Physicians completed retrospective electronic records for pts who fit the following criteria: confirmed WM, symptomatic disease at treatment initiation, frontline treatment initiated between Jan 2000-Dec 2010, treatment with at least one salvage regimen (excluding maintenance therapy), and availability of clinical/biologic evaluation at diagnosis or initial therapy. Study endpoints included initial/subsequent lines of treatment, progression-free survival (PFS), and overall survival (OS). The number of pt records per country was prespecified to balance distribution between countries.
Results: Electronic records were reviewed for 368 pts from France (n=85), United Kingdom (n=62), Germany (n=51), Spain (n=45), Italy (n=40), Greece (n=25), Netherlands (n=21), Poland (n=14), Czech Republic (n=13), and Austria (n=12). Data were summarized across 2nd/3rd-lines for 368/155 pts. Median age at initiation of frontline treatment was 63 yr (range, 29-89); 61% were male. Reasons for initiating treatment were cytopenias (77%; anemia [75%]), constitutional symptoms (56%), IgM-related symptoms (55%), and organomegaly (26%). Choice of therapy varied with line of treatment and age; monotherapy fell from 35% in frontline to 21%/22% in 2nd/3rd-lines (Table 1) and age ≥70 was associated with greater use of monotherapy (40%) *vs* combination therapy (29%) in the frontline. Combination therapy with rituximab increased from 36% in frontline to 63%/56% in 2nd/3rd-lines. Across all lines, rituximab and cyclophosphamide were the most common agents, excluding steroids, used as monotherapy or in any combination. Median PFS decreased with successive lines of treatment from 31 mo for frontline to 24/16 mo for 2nd/3rd-lines. Median PFS varied by country and choice of agents (Table 1), but was similar for pts<50 and ≥50 y. Improved median PFS was observed for pts who received rituximab in 2nd-line *vs* pts who did not (26 *vs* 19 mo, *P*=0.014). Treatment outcomes were similar in 3rd-line with regards to rituximab use (15 *vs* 16 mo, *P*=0.69). The use of rituximab in the frontline did not affect median PFS of subsequent lines of therapy (2nd-line: 24 *vs* 23 mo, *P*=0.87; 3rd-line: 13 *vs* 16 mo, *P*=0.12). Median OS for the overall population was not reached, but was significantly lower in pts ≥75 yr (70 mo; *P*<0.0001) or in pts with high-risk IPSSWM risk score (91 mo; *P*=0.0014). The current data does not indicate a difference in OS with frontline rituximab use. Other malignancies were reported in 14% after receipt of at least one line of treatment for WM.

Table 1. Use of monotherapy or combination regimens and PFS in 2nd- and 3rd-Line settings overall and by country.

	Cases, n		Monotherapy, %		Combination Therapy With Antibody†, %		Combination Therapy Without Antibody†, %		Median PFS, Months (95% CI)	
Country	2nd line	3rd line	2nd line	3rd line	2nd line	3rd line	2nd line	3rd line	2nd line	3rd line
Overall	368	155	21	22	63	56	14	20	24.0 (20-27)	16.0 (10-18)
France	85	43	26	16	66	70	8	14	30.0 (20-37)	16.0 (9-32)
United Kingdom	62	19	23	21	55	42	23	37	25.0 (11-36)	13.0 (9-33)
Germany	51	17	8	24	80	53	8	12	24.0 (16-35)	8.0 (3-16)
Spain	45	19	36	42	49	53	13	0	17.0 (12-28)	11.0 (9-24)
Italy	40	19	18	16	75	68	3	16	30.0 (18-50)	17.0 (4-21)
Eastern European*	27	11	11	9	41	0	48	91	20.0 (16-26)	20.5 (4-38)
Smaller European**	58	27	21	26	67	63	12	11	16.0 (13-25)	16.0 (7-26)

*Includes Czech Republic and Poland, **Includes Austria, Greece, and Netherlands
† Antibodies other than rituximab, <1%

Summary/Conclusions: For WM pts treated in Europe, the most common reasons for initiating therapy are anemia and constitutional symptoms. Rituximab was the most commonly used agent across all lines of treatment and use of rituximab was associated with improvement in median PFS in the 2nd-line. Outside clinical trials, monotherapy is widely used even at first relapse with notable differences between countries. This large observational dataset will be an important tool to promote understanding of treatment practices and survival of WM pts outside of a clinical trial setting.

E1276

EFFICACY AND SAFETY OF ORAL IXAZOMIB-LENALIDOMIDE-DEXAMETHASONE (IRD) *VS* PLACEBO-RD IN RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS: IMPACT OF PRIOR THERAPY IN THE PHASE 3 TOURMALINE-MM1 STUDY

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Background: The TOURMALINE-MM1 study (NCT01564537) demonstrated improved progression-free survival (PFS) with the all-oral combination of IRd *vs* placebo-Rd (median 20.6 *vs* 14.7 months; HR 0.74) in patients (pts) with relapsed/refractory multiple myeloma (RRMM)(Moreau et al, ASH 2015). Based on this study ixazomib was approved by the US FDA in combination with Rd for the treatment of pts with MM who have received at least one prior therapy.
Aims: To analyze the efficacy and safety of IRd *vs* placebo-Rd according to prior proteasome inhibitor (PI) and prior thalidomide (thal)/lenalidomide (R) exposure.
Methods: Pts with RRMM were randomized 1:1 to receive IRd or placebo-Rd (ixazomib 4 mg or matching placebo on days 1, 8, and 15, plus lenalidomide 25 mg on days 1–21 and dexamethasone 40 mg on days 1, 8, 15, and 22, in 28-day cycles) until disease progression or unacceptable toxicity. Pts were stratified by number of prior therapies (1 *vs* 2 or 3), PI-exposed *vs* PI-naïve status, and International Staging System (ISS) stage I or II *vs* III. Pts who had received prior therapy with PI- and thal/R-based regimens, and pts who were refractory to thal, were eligible for inclusion. However, pts who were refractory to PI- or R-based prior therapy were not included.
Results: Of 722 pts, 69% had prior PI therapy (<1% prior carfilzomib) and 55% had prior thal/R therapy, including 45% prior thal (12% thal-refractory) and 12% prior R. Prior therapies were balanced between arms. At the primary analysis (median follow-up ~15 months), consistent PFS benefit was seen with IRd regardless of prior PI or thal/R exposure (Table). Overall response rate (ORR) with IRd *vs* placebo-Rd appeared generally similar across subgroups (PI-naïve: 81% *vs* 74%; PI-exposed: 77% *vs* 70%; thal/R-naïve: 80% *vs* 77%; R-naïve: 78% *vs* 73%) but slightly lower in thal-refractory pts (70% *vs* 57%), while ORR in the placebo-Rd arm appeared somewhat lower in thal/R-exposed (77% *vs* 67%) and R-exposed pts (77% *vs* 59%). Complete response plus very good partial response (CR+VGPR) rates with IRd *vs* placebo-Rd appeared generally similar in PI-naïve (54% *vs* 37%), PI-exposed (46% *vs* 40%), thal/R-naïve (51% *vs* 44%), thal/R-exposed (45% *vs* 35%), R-naïve (48% *vs* 39%), and R-exposed (45% *vs* 36%) pts, but lower in thal-refractory (30% *vs* 27%) pts. At a 23-month analysis, IRd safety profile was generally consistent regardless of prior PI or thal/R exposure. Rates of grade ≥3 AEs with IRd *vs* placebo-Rd were 76% *vs* 66% in PI-naïve, 73% *vs* 70% in PI-exposed, 75% *vs* 71% in thal/R-naïve, and 73% *vs* 67% in thal/R-exposed pts. Rates of individual AEs (all-grade and grade ≥3) with IRd *vs* placebo-Rd were similar and consistent with those reported for the overall patient population regardless of prior PI or thal/R exposure, including common AEs such as GI and hematologic toxicities, rash, and neuropathy, with the exception of neutropenia in the PI-naïve population; overall rates were 29% *vs* 20%, including 21% *vs* 15% grade ≥3, whereas IRd and placebo-Rd rates were similar in the overall population and other subgroups. Rates of serious AEs with IRd *vs* placebo-Rd were 47% *vs* 40% in PI-naïve, 46% *vs* 53% in PI-exposed, 48% *vs* 51% in thal/R-naïve, and 45% *vs* 48% in thal/R-exposed pts.

Table 1.

Median PFS, months	IRd, N=360	Placebo-Rd, N=362	HR
PI naïve (n=110 vs 109)	NE	15.7	0.75
Bortezomib (Btz) naïve (n=112 vs 112)	NE	15.9	0.75
PI exposed (n=250 vs 253)	18.4	13.6	0.74
Btz exposed (n=248 vs 250)	18.5	13.6	0.75
Thal/R naive (n=167 vs 158)	20.6	13.6	0.70
R (n=316 vs 318) / Thal naive (n=203 vs 192)	20.6 / 20.6	13.6 / 13.6	0.77 / 0.69
Thal/R exposed (n=193 vs 204)	NE	17.5	0.74
R exposed (n=44 vs 44)	NE	17.5	0.58
Thal exposed (n=157 vs 170) / refractory (n=40 vs 49)	NE / 16.6	15.7 / 13.0	0.75 / 0.73

NE, not estimable

Summary/Conclusions: The benefit of IRd *vs* placebo-Rd appeared consistent across subgroups defined by prior PI and thal/R exposure; IRd safety profile was also broadly consistent across subgroups.

E1277

PRACTICE PATTERNS AND OUTCOMES IN U.S. PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) AND COMORBID RENAL DYSFUNCTION (RD) AND/OR CARDIOVASCULAR DISEASE (CVD)

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Background: Multiple myeloma (MM) is a disease of the elderly, many of whom often present with comorbidities including RD and CVD (Gupta 2015; Dimopoulus 2015). While the presence of RD and/or CVD may influence RRMM treatment choices, there is limited real-world data on prescribing patterns and outcomes in these patient populations (Castelli 2014; Larocca 2015).
Aims: To describe the prevalence of RD and CVD over time, and the variation in prescribing patterns and clinical outcomes (progression-free survival (PFS) and overall survival (OS)) by comorbidity profile in RRMM pts initiating second line therapy (2LT).
Methods: This was a retrospective cohort study using a large national EMR database in the U.S. Newly diagnosed adult MM patients initiating first line therapy (1LT) between 1/2008 and 12/2014 were followed until death/loss to follow up or the end of study period (6/30/2015). 2LT was identified by: 1) retreatment after a treatment gap of >3 months after 1LT discontinuation, or 2) a switch to another drug combination after starting 1LT. Comorbid RD was based on presence of a diagnosis (dx) code or a lab value (creatinine clearance<40ml/min or serum creatinine >2 mg/dL) in the 12 months prior to start of 1LT through the start of 2LT; and comorbid CVD was based on dx codes in the same timeframe. Time to new treatment (TTNT) or death was used as a surrogate for PFS. Kaplan-Meier analyses were performed for TTNT/OS from start of 2LT.

Table 1.

Presence of Comorbid Condition prior to 1LT	No RD or CVD N=329	RD only N=172	CVD only N=48	RD and CVD N=79
Type of Therapy in the First Line, %				
PI-based	35.9	55.2	18.8	49.4
Bortezomib ± non-IMID	35.6	55.2	18.8	49.4
Carfilzomib ± non-IMID	0.3	0.0	0.0	0.0
IMID-based	37.4	23.3	50.0	31.7
Lenalidomide ± non-PI	31.3	21.5	43.8	29.1
IMID (other than lenalidomide) ± non-PI	6.1	1.7	6.3	2.5
PI + IMID-based	19.8	17.4	14.6	11.4
Lenalidomide + bortezomib ± other	16.7	14.0	10.4	11.4
Bortezomib + IMID (other than lenalidomide) ± other	2.7	2.9	4.2	0.0
Carfilzomib + IMID ± other	0.3	0.6	0.0	0.0
Other, not categorized elsewhere	7.01	7.0	4.1	16.7
Presence of Comorbid Condition prior to 2LT	**No RD or CVD N=202**	**RD only N=197**	**CVD only N=55**	**RD and CVD N=174**
Type of Therapy in the Second Line, %				
PI-based	32.2	34.0	34.6	33.3
Bortezomib ± non-IMID	29.2	27.4	30.9	26.4
Carfilzomib ± non-IMID	3.0	6.6	3.6	6.9
IMID-based	48.0	43.2	32.7	44.3
Lenalidomide ± non-PI	39.6	37.1	20.0	36.2
IMID (other than lenalidomide) ± non-PI	8.4	6.1	12.7	8.1
PI + IMID-based	8.4	9.6	10.9	8.1
Lenalidomide + bortezomib ± other	4.5	6.1	5.5	5.2
Bortezomib + IMID (other than lenalidomide) ± other	0.5	0.5	0.0	1.2
Carfilzomib + IMID ± other	3.5	3.1	5.5	1.7
Other, not categorized elsewhere	11.4	13.2	21.8	14.4

Key: IMID – immunomodulator; PI – proteasome inhibitor; Other – includes: cyclophosphomide, melphalan, vincristine, (liposomal) doxorubicin, interferon, pomalidomide, thalidomide, panobinostat bendamustine, vorinostat, single agent steroid

Results: Among 628 RRMM pts (mean age 69 years), 27.4% had comorbid RD, 7.6% had comorbid CVD, and 12.6% had both RD and CVD prior to 1LT; this increased to 31.4%, 8.8%, and 27.7%, respectively, prior to 2LT. The distribution of treatments across 1LT and 2LT is shown in Table 1. In 1LT, pts with RD±CVD were most likely to receive proteasome inhibitor (PI)-based therapies; in contrast, pts with CVD only were most likely to receive immunomodulatory drug (IMID)-based therapies. In 2LT, the use of IMID-based therapies was most common, except among pts with CVD only where a similar proportion used IMID-based or PI-based therapies (approx. one-third). Additionally, intensive regimens (PI+IMID) were more common for all pts regardless of comorbidity in 1LT compared to 2LT. Median TTNT from 2LT initiation was 14.3 months (95% CI: 9.2, 19.1) for pts with CVD only, 13.7 months (95% CI: 11.4, 17.8) for pts with RD only, 12.3 months (95% CI: 8.4, 16.6) for pts with RD and CVD, and 20.1 months (95% CI: 16.6, 24.4) for pts without CVD or RD. OS at 1 yr and 2 yrs from 2LT initiation were 77.8% and 64.1% for those with CVD only, 77.4% and 58.5% for those with RD only, 72.7% and 51.6% for those with CVD and RD, and 88.2% and 78.4% for pts without CVD or RD (log-rank test, $P<0.05$).
Summary/Conclusions: Among pts receiving 2LT for RRMM, prevalence of RD and/or CVD increased from 48% to 68% during the MM clinical course. The type of therapy varied by presence of comorbidity for 1LT but to a lesser extent for 2LT. Pts with RD and/or CVD had a shorter TTNT and a lower 1- and 2-yr OS probability relative to those without these comorbidities.

References

1. Castelli, et al. *Expert Rev. Anticancer Ther.* 2014;14:2:199–215;
2. Dimopoulus, et al. *Cancer Treatment Reviews*. 2015; 41:827–835;
3. Gupta, et al. *Journal of Geriatric Oncology*. 2015;6:178-184;
4. Larocca, et al. *Blood*. 2015;126:19:2179-2185.

E1278

BORTEZOMIB-BASED TREATMENT MAY OVERCOME THE ADVERSE EFFECT OF FOPNL GENOMIC VARIANT ON THE PROGNOSIS OF MULTIPLE MYELOMA

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Background: In a recent genome-wide association study (GWAS), the *FOPNL* (fibroblast growth factor receptor 1 oncogen partner N terminal like gene) single nucleotide polymorphism rs72773978 was identified as a novel adverse prognostic factor in multiple myeloma (MM).
Aims: The aim of our study was to investigate the associations of *FOPNL* polymorphism with clinical characteristics (sex, age, ISS, cytogenetic abnormalities) as well as treatment outcome (progression free survival, [PFS] and overall survival, [OS]) in a cohort of Hungarian MM patients.
Methods: Data of 373 MM patients (188 male/185 female; mean age: 60.5±11.2 year) diagnosed from 2005 to 2013 were retrospectively analysed. As a first line treatment, older regimens (containing no proteasome inhibitor) were administered in 132 cases, while PI based therapy was applied in 241 cases. The minimal follow up was 24 month. LightCycler melting analysis was applied to identify rs72773978 polymorphism.
Results: In the whole cohort applying a dominant model, the distribution of rs72773978 polymorphism was as follows: AA:328/373 (87.9%) *vs* AT&TT: 45/373, (12.1%). We found no significant difference in sex, age, ISS stage or citogenetics stratified subgroups. In our cohort *FOPNL* polymorphism showed difference in the prognostic effect depending on the treatment applied. In line with the previous GWAS study, carriership of the minor allele was significantly associated with adverse PFS among patients treated with non-PI based therapy (p=0.042). PFS at 24 months was 43.5±4.5% for AA patients (n=124) compared to 12.5±11.7% for AT&TT patients (n=8). Suprisingly in the PI treatment group, PFS at 24 months was 51.7±3.5% for AA (n=204) compared to 62.2±8% for AT&TT (n=37) patients (p=0.082). As significant interaction between *FOPNL* and PI treatment was observed (p=0.003), we performed multivariate analyses (Cox regression considering considering sex, age and ISS as covariates) in both treatment groups separately. The difference did not remain significant in either groups (non PI group: p=0.22; HR: 1.78, 95% CI: 0.7-4.5, PI group: p=0.094; HR: 0.7, 95% CI: 0.47-1.06). Similar comparisons for OS revealed a similar effect of *FOPNL* variant in both treatment groups. In the non-PI treated group adverse prognosis was observed in minor allele carriers: OS at 72 months for AA patients was 49±4.6% compared to AT&TT with 18.8±15.8% (p=0.022). In the PI-based group, OS at 72 months for AA patients was 40.9±4.5% compared to AT&TT 67.3±8.5% (p=0.048). The test for interaction between FOPNL and treatment was significant (p<0.001). Separate multivariate analyses revealed the independent adverse effect of rs72773978 on survival in the non PI group (p=0.029; HR: 2.9, 95% CI: 1.1-7.6); but not in PI treatment group: p=0.155; HR: 0.63, 95% CI: 0.34-1.19).
Summary/Conclusions: In the present study, we confirmed the adverse prognostic effect of *FOPNL* rs72773978 polymorphism in case of non-PI based treatment regimens. On contrary this adverse effect was overcome by the application of newer, proteasome inhibitor containing treatment protocols.

E1279

HIGH RESPONSE RATES BUT INEFFECTIVE PRE-SELECTION STRATEGIES FOR TREATMENT WITH HDM AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA BETWEEN 65-70 YEARS IN THE NETHERLANDS

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Background: The Dutch national clinical guideline recommends bortezomib-based induction chemotherapy (inductionCT) followed by high dose melfalan (HDM) and autologous stem cell transplantation (ASCT) for patients with symptomatic multiple myeloma (symMM) aged ≤65 years. In addition, ASCT should also be considered for patients with symMM aged 66-70 years.
Aims: To evaluate how these recommendations were applied in daily practice in the Netherlands for patients with symMM aged ≤70 years and, secondly, what percentage of patients aged 66-70 years was offered ASCT as their first line treatment.
Methods: From the nationwide population-based Netherlands Cancer Registry, we identified 476 patients aged ≤70 years diagnosed with symMM in 2014 (60% male). Of these patients, 318 (67%) were ≤65 years and 158 (33%) were 66-70 years. InductionCT was defined as bortezomib-based chemotherapy regimens (i.e. BD, PAD, VCD or VTD) with or without (+/-) subsequent HDM and ASCT. Overall response rate (ORR) was defined as achieving complete or partial response *i.e.* CR, VGPR and PR as best achieved response outcome. Data were analyzed for the total cohort, as well as stratified by age at diagnosis.
Results: Overall, of patients ≤65 years, 283 (89%) were treated with inductionCT, of which VCD was most frequently used (N=231/283;82%), followed by PAD (N=30/283;11%) and BD (N=22/283;8%). The remaining patients received other types of chemotherapeutic regimens (2%) or no chemotherapy at all (9%). Of patients who received inductionCT, 217 of 283 (77%) received the planned HDM+ASCT. Of patients aged 66-70 years, 61 (39%) received inductionCT consisting of either VCD (N=38/61;62%), BD (N=19/61;31%) or PAD (N=4/61;7%). The remaining patients were mostly treated with VMP (N=77/158;49%), while 8% (N=13/158) did not receive any treatment. In contrast to patients aged ≤65 years, only 29 of 61 (48%) patients underwent ASCT after inductionCT. The ORRs were 86% (242/283) and 72% (44/61) for patients ≤65 and 66-70 years who received inductionCT +/- ASCT, respectively. The ORR for patients aged 66-70 years who received VMP was 34% (26/77), which is markedly lower than previously reported in the VISTA trial (ORR, 71%). The ORR for patients who received inductionCT, but did not proceed to ASCT was lower as compared to patients who did proceed to ASCT (51% *vs* 96%) irrespective of age group. Moreover, the ORR of patients aged 66-70 years who received inductionCT (+/- ASCT) was higher than the ORR of patients in the same age group who received VMP (72% *vs* 34%).
Summary/Conclusions: Our results show that most patients aged ≤65 years start with inductionCT and 77% of these are able to proceed to HDM+ASCT. In addition, among patients aged 66-70 years, 39% start with inductionCT of whom only 48% received subsequent ASCT. Although based on modest patient numbers, the ORR of inductionCT +/- ASCT in patients between 66-70 years was lower as compared to the ORR in the younger patient group, while higher than the ORR in the VMP group. This population-based analysis demonstrates that inductionCT is frequently offered to older patients with similar efficacy as in younger patients. However, pre-selection strategies for intensive treatment are insufficient since only half of the older patients subsequently receive their planned ASCT.

E1280

SURVIVAL AND TREATMENT PATTERNS IN PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA (MM) IN A REAL-WORLD SETTING

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Background: Survival rates for patients (pts) with MM have improved with the advent of new therapies, however data on real-world survival outcomes are limited.
Aims: Retrospective analysis using the Registry of Monoclonal Gammopathies database of the Czech Myeloma Group.
Methods: Pts diagnosed with symptomatic MM (May 2007–June 2014), were followed from diagnosis until death, loss to follow-up or June 2015. The primary analysis was to estimate overall survival (OS) from diagnosis; secondary analyses included treatment-line-specific OS and progression-free survival (PFS), treatment patterns and response. Exploratory analyses included OS and PFS from treatment initiation by International Staging System (ISS) stage, International Myeloma Working Group (IMWG) risk and therapy type (lenalidomide plus dexamethasone [Ld] or bortezomib [bor]-based regimens).
Results: 2513 pts were included. Median age at diagnosis was 67.0 years. Similar proportions of pts had ISS stage I, II or III disease. Median OS (months, 95% confidence interval [CI]) from diagnosis was 50.3 (46.1–54.5) and decreased from 47.5 (43.1–52.0) at 1st line to 13.2 (11.3–15.2) at 3rd (Figure). Median PFS (months, 95% CI) decreased from 16.3 (15.4–17.2) at 1st line to 6.0 (5.4–6.6) at 3rd. Main treatments at 1st line (n=2446) were bor- (46%) or thalidomide-based (40%). At subsequent lines, pts frequently received bor - or lenalidomide-based regimens (2nd line [n=1118], 49% and 31%; 3rd line [n=547], 26% and 47%, respectively). Overall response rates decreased across lines: 58% at 1st line, decreasing to 44% and 28% at 2nd and 3rd lines. Differences in median OS and PFS by treatment line were observed according to ISS stage and IMWG risk (Table). An analysis of pts (excluding those in clinical trials) receiving Ld 2nd to 4th line, showed median OS decreased at each subsequent line (2nd line 26.2 [21.7-30.8] and 3rd line 12.6 [11.4-13.7]), comparable to median OS in all patients. Median PFS was 7.5 (6.3-8.8); values at 2nd and 3rd lines were 8.7 (7.3-10.1) and 6.6 (5.3-8.0). Similar trends were seen in pts (excluding those in clinical trials) receiving bor in 2nd to 4th lines. At 2nd and 3rd lines, median OS values were 27.3 (22.7-31.8) and 16.2 (12.1-20.3); median PFS values were 11.3 (9.9-12.6) and 5.7 (4.7-6.8).

Table 1.

Line	ISS Stage I	ISS Stage II	ISS Stage III	IMWG Risk Low	IMWG Risk standard	IMWG Risk High
Median OS, months (95% CI)						
First	82.1 (Not Estimable)	46.9 (40.3–53.5)	29.9 (26.9–32.9)	Not reached	50.6 (40.6-60.6)	31.8 (27.1-36.5)
Second	46.7 (31.4–62.0)	28.6 (24.7–32.5)	19.3 (16.5–22.1)	57.3 (19.3-95.3)	35.5 (26.9-44.1)	14.9 (5.6-24.2)
Third	19.6 (14.2–25.0)	13.7 (10.9–16.4)	10.0 (7.6–12.4)	24.8 (14.7-35.0)	19.8 (14.5-25.2)	7.6 (3.3-11.9)
Median PFS, months (95% CI)						
First	23.2 (21.1–25.3)	17.2 (15.9–18.6)	11.6 (10.2–12.9)	37.5 (20.6-54.4)	17.3 (14.7-19.9)	14.2 (11.5-16.9)
Second	13.3 (12.2–14.5)	11.0 (9.6–12.4)	8.6 (7.4–9.9)	21.6 (5.9-37.3)	12.0 (9.7-14.2)	7.0 (5.0-9.0)
Third	7.3 (6.1–8.6)	5.7 (4.9–6.6)	5.5 (4.5–6.4)	5.9 (5.6-6.1)	7.1 (4.8-9.4)	4.4 (3.2-5.5)

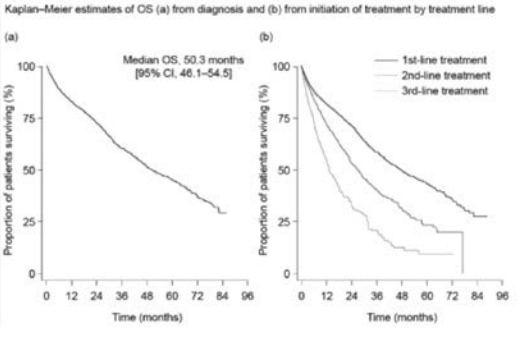

Figure 1.

Summary/Conclusions: Real-world outcomes in pts with symptomatic MM are considerably lower than those reported in clinical trials. OS and PFS decreased significantly with each successive treatment line. Despite availability of novel agents, MM remains a disease with high unmet need.

E1281

FC-GAMMA RECEPTOR POLYMORPHISMS AND PROGRESSION-FREE SURVIVAL: ANALYSIS OF THREE CLINICAL TRIALS OF ELOTUZUMAB IN MULTIPLE MYELOMA

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Background: Elotuzumab is a humanized IgG1 immunostimulatory monoclonal antibody that targets SLAMF7, a glycoprotein highly expressed by myeloma and natural killer (NK) cells. Elotuzumab works via a dual mechanism of action, both by directly activating NK cells and by facilitating antibody-dependent cell-mediated cytotoxicity (ADCC) to cause targeted myeloma cell death. Fcγ receptors, including CD16a (FcγRIIIa) and CD32a (FcγRIIa), bind the Fc portion of IgG1 antibodies to facilitate ADCC.[1] Multiple alleles exist for CD16a and CD32a

that confer affinity for Fc binding; therefore, differences in Fcγ receptor genotypes could affect responses to elotuzumab.[1] The high-affinity allele for CD16a is determined by a valine (V) residue at amino acid position 158, while a phenylalanine (F) residue determines the low-affinity variant. For CD32a, the high-affinity allele is a histidine (H) residue at position 131; arginine (R) determines the low-affinity variant.

Aims: To assess whether CD16a and CD32a polymorphisms affect progression-free survival (PFS) in patients receiving elotuzumab as monotherapy or as part of a combination therapy.

Methods: In ELOQUENT-2 (NCT01239797), patients with relapsed/refractory multiple myeloma (RRMM) and 1–3 prior therapies were randomized to elotuzumab (10 mg/kg IV) in combination with lenalidomide (Len) and dexamethasone (dex; ELd) or Len/dex alone (Ld). In Study 009 (NCT01478048), patients with RRMM and 1–3 prior therapies were randomized to elotuzumab (10 mg/kg IV) in combination with bortezomib (Btz) and dex (EBd) or Btz/dex alone (Bd). In Study 011 (NCT01441973), patients with high-risk (Mayo Clinic criteria) smouldering MM were treated with elotuzumab (10 mg/kg IV twice monthly or 20 mg/kg IV once monthly). Patients from ELOQUENT-2 and Study 009 were categorized by CD16a (VV *vs* FF) and CD32a (HH *vs* RR) genotype. Patients from Study 011 were categorized by the presence of ≥1 *vs* 0 high-affinity CD16a (VV/VF *vs* FF) or CD32a (HH/HR *vs* RR) alleles. PFS by genotype was assessed in the elotuzumab and control groups of ELOQUENT-2 and Study 009, and for all patients in Study 011 (combined-dose cohort).

Results: Genotyping was performed in 339/646 randomized patients in ELOQUENT-2, in all 152 patients in Study 009, and in 28/31 patients in Study 011. In ELOQUENT-2, neither CD16a genotype (VV *vs* FF) nor CD32a genotype (HH *vs* RR) was associated with a difference in PFS distribution in the ELd or Ld group (Table). In Study 009, CD16a VV *vs* FF status was associated with longer median PFS in the EBd arm, but not in the control arm. Given the available data for each estimate (n=15–19), CD32a genotype did not appear to be associated with a meaningful difference in median PFS in either the EBd or Bd group. In Study 011, median PFS was >20 months or non-estimable for all CD16a and CD32a genotypes. PFS was numerically higher for patients with ≥1 *vs* 0 CD16a V alleles; however, interpretation is limited by the small numbers of patients with each genotype.

Table 1. Median PFS (ITT definition) in ELOQUENT-2, study 009, and study 011, by FcγR genotype and treatment group.

Treatment arm	Median PFS (95% CI), months			
	ELOQUENT-2 (n=339)*			
	CD16aVV	CD16aFF	CD32aHH	CD32aRR
ELd group	n=29 22.3 (12.5–31.3)	n=60 22.2 (14.8–29.9)	n=59 18.5 (11.1–24.0)	n=40 19.5 (14.8–30.1)
Control (Ld)	n=18 18.4 (8.5–19.4)	n=69 19.4 (13.0–23.9)	n=62 18.4 (12.6–22.2)	n=33 14.4 (11.1–20.3)
	Study 009 (n=152)			
	CD16aVV	CD16aFF	CD32aHH	CD32aRR
EBd group	n=13 22.3 (3.8–33.2)	n=24 9.8 (5.7–14.3)	n=15 10.7 (4.5–17.0)	n=19 7.4 (3.7–18.2)
Control (Bd)	n=16 8.2 (1.7–10.2)	n=22 6.9 (3.8–10.6)	n=18 5.6 (2.8–13.1)	n=16 8.3 (3.5–15.2)
	Study 011 (n=28)			
	CD16aVV/VF	CD16aFF	CD32aHH/HR	CD32aRR
Dose cohorts combined	n=16 31.3 (12.3–NE)	n=12 22.1 (8.3–NE)	n=21 31.3 (12.9–NE)	n=7 NE (8.3–NE)

*PFS assessed by independent review committee and censored for subsequent therapy.

NE, not estimable.

Summary/Conclusions: The effect of Fcγ receptor polymorphisms on PFS may depend on elotuzumab treatment combinations, as CD16a genotype correlated with PFS in patients treated with EBd but not ELd. ELd benefits all patients, irrespective of genotype, as there is no evidence that high-affinity Fcγ alleles are associated with a differential benefit.

Reference

1. Weng W et al. *J Clin Oncol* 2003;21:3940-7.

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E1282

HIGH EXPRESSION OF AF1Q IS AN ADVERSE PROGNOSTIC FACTOR AND A PREDICTION MARKER OF EXTRAMEDULLARY DISEASE IN MULTIPLE MYELOMA

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Background: Multiple myeloma is an intractable hematological malignancy with various clinical manifestations, especially extramedullary disease (EMD). The prognosis of patients with EMD is extremely poor due to limited treatment options and aggressive nature of EMD, however the mechanism of the progression of EMD is not well known. AF1q is an oncogene which expressed in leukemia cells, located in 1q21. The gene is well known as one of the fusion partners of MLL, and as a poor prognostic factor in acute myeloid leukemia and myelodysplastic syndrome. Recently, we reported that high expression of AF1q results in an autonomous Wnt activity that promotes distant metastasis of breast cancer.

Aims: We hypothesized that high expression of AF1q is a poor prognostic factor of multiple myeloma and associated with EMD. To evaluate our hypothesis, we analyzed the expression of AF1q in patients with multiple myeloma and investigated the impact on the prognosis and the progression of EMD.

Methods: Newly diagnosed multiple myeloma patients in National Center for Global Health and Medicine hospital from January 2001 to March 2013 were studied. Patients were treated with vincristine-adriamycin-dexamethason or bortezomib-dexamethason induction therapy followed by autologous stem cell transplantation using high dose melphalan. The expression of AF1q was evaluated using the immunostaining of bone marrow clot samples at the diagnosis of multiple myeloma. The expression of AF1q was graded from "-" to "+++". EMD was diagnosed by pathological examination and/or CT/MRI/PET. The clinical response, progression free survival (PFS), and overall survival (OS) were analyzed using Kaplan Meier method with Log-rank test. Correlation between EMD and AF1q expression was tested by Chi-square test.

Results: Clinical data and bone marrow clot samples of 117 patients were analyzed. Mean age was 54.9 years old, and 51.9% was male. The grades of AF1q expression were 7 of "-", 33 of "+", 35 of "++", and 42 of "+++". We defined the cases with "-" and "+" as low expression, "++" and "+++" as high expression. Very good partial response or better was obtained after completion of autologous stem cell transplantation in 42.5% of patients with low AF1q expression and 37.7% of high expression. There was no statistically significant difference. Survival analysis using Log Rank method showed that high expression of AF1q was associated with significantly shorter progression free survival (3 year PFS was 56.2% in low AF1q expression *vs* 30.5% in high AF1q expression, p=0.009), but not overall survival (3 year OS was 89.6% in low AF1q expression *vs* 73.3% in high AF1q expression, ns.). EMD was found in 25.0% of patients with low AF1q expression and 44.7% of high expression. Chi-square test showed that the incidence of EMD was significantly higher in patients with high AF1q expression than low expression (p=0.045)

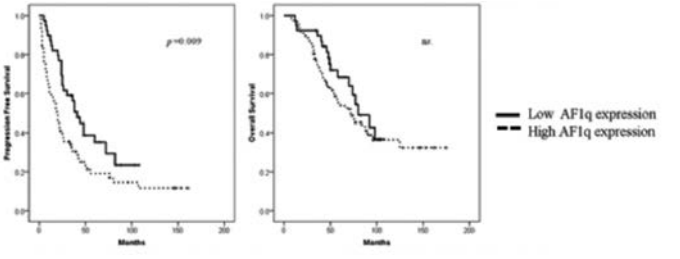

Figure 1.

Summary/Conclusions: To our knowledge, this is the first study that demonstrates a molecular marker associated with myeloma with EMD. We found that the high expression of AF1q was an adverse prognostic factor in multiple myeloma.

E1283

PHASE 1/2 TRIAL OF LENALIDOMIDE IN COMBINATION WITH CYCLOPHOSPHAMIDE AND PREDNISONE (REP) IN PATIENTS WITH LENALIDOMIDE-REFRACTORY MULTIPLE MYELOMA (REPEAT-STUDY)

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Background: The outcome of lenalidomide- and bortezomib-refractory multiple myeloma (MM) patients is very poor, with a median event-free survival of 5 months and median overall survival (OS) of 9 months. Therefore new treatment modalities are urgently needed. In this respect, several new drugs have recently

been approved for the treatment of double refractory MM (such as pomalidomide, carfilzomib, and daratumumab). However, next to the development of new drugs, also the strategy of combining drugs with synergistic activity may result in significant clinical benefit for patients with advanced myeloma. Lenalidomide has well-described immunomodulatory effects, even in the setting of lenalidomide-refractory disease. Similarly, administration of continuous low-dose cyclophosphamide (metronomically dosed) has direct anti-tumor activity, as well as several well-characterized immunomodulatory effects. We have previously shown in a retrospective study that lenalidomide combined with continuous low-dose oral cyclophosphamide (endoxan) and prednisone (REP) had remarkable activity in heavily pretreated, lenalidomide-refractory MM patients.
Aims: To further evaluate this combination, we initiated a prospective phase I/II study to determine the optimal dosing schedule of lenalidomide, low-dose oral cyclophosphamide and prednisone, and to assess its efficacy and safety in lenalidomide-refractory MM patients.
Methods: 82 lenalidomide-refractory MM patients were included in this trial; 21 were included in the phase 1 dose-finding part with a classic 3+3 dose-escalation design (5 dose-levels) and 61 patients were included in the phase II part of this trial in which all patients were treated at the maximum tolerated dose (MTD).
Results: The median age of the 82 patients in this phase I/II trial was 66 years (range 41-82); 71% were male. The median duration from diagnosis was 48 months (range 5-169), median number of prior therapies was 3 (range 1-6), and 50 patients (61%) had previously received autologous SCT. All patients were lenalidomide-refractory, 71 (87%) had prior bortezomib treatment, and 54 (66%) had both lenalidomide- and bortezomib-refractory MM. 41% of the patients had high-risk cytogenetic abnormalities as determined by FISH. The MTD was defined as 25 mg lenalidomide (days 1-21/28 days), combined with continuous oral cyclophosphamide (50 mg/day) and prednisone (20 mg/day). Sixty-seven patients were treated at the MTD (6 in dose-level 4 of phase I and 61 in phase II part of the study). Forty-four of these patients (67%) achieved a partial response or better, including 12 patients (18.2%) with a very good partial response and 3 (4.5%) with a complete remission. Eleven patients (16%) achieved a minimal response, translating to an overall 83% clinical benefit rate. After a median follow-up of 24.5 months median PFS and OS were 12.1 and 29.0 months respectively. Similar results were achieved in the subset of patients with lenalidomide- and bortezomib-refractory disease as well as in patients with high-risk cytogenetic abnormalities. At the MTD, neutropenia (22%) and thrombocytopenia (22%) were the most common grade ≥3 hematological adverse events. Infections (21%) were the most common grade ≥3 non-hematological adverse events.
Summary/Conclusions: REP is a well-tolerated regimen and induces a high response rate with prolonged PFS and OS in lenalidomide-refractory MM patients. REP should be considered a valuable salvage option for lenalidomide-refractory MM patients.
This trial was registered at www.clinicaltrials.gov as #NCT01352338.

E1284

MANAGEMENT OF ADVERSE EVENTS IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA TREATED WITH POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE: A POOLED SAFETY ANALYSIS OF 3 CLINICAL TRIALS

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Background: Patients with relapsed and refractory multiple myeloma (RRMM) who are heavily pretreated often have advanced disease and comorbidities, increasing their susceptibility to adverse events (AEs). The management of AEs is important to ensure that patients remain on the therapy for as long as needed to receive a clinical benefit.
Aims: To further characterize the safety profile of pomalidomide (POM)+low-dose dexamethasone (LoDEX) and management of AEs using pooled safety data from 3 clinical trials of POM+LoDEX (Richardson et al, *Blood,* 2014; San Miguel et al, *Lancet Oncol,* 2013; Dimopoulos et al, ASH 2015).
Methods: The 3 trials enrolled patients who provided informed consent and had ≥2 prior therapies, including lenalidomide and bortezomib, and had progressed on or within 60 days of their last therapy. Patients received POM 4 mg/day on days 1-21 of each 28-day cycle and LoDEX 40 mg (20 mg for those >75 years of age) weekly until disease progression or unacceptable toxicity. Thromboprophylaxis was required. Grouped AE terms were used for analysis.
Results: A total of 1088 patients from the 3 trials were included in the safety population; median age was 66 years (range, 34-88 years). Most patients were male (57%), had an Eastern Cooperative Oncology Group performance status of ≤1 (88%), were refractory to both lenalidomide and bortezomib (77%), and had prior stem cell transplant (68%). The most common grade 3/4 AEs were neutropenia (56%), infections (34%), anemia (32%), and thrombocytopenia (26%). The median time to onset was 20 days (range, 1-591 days) in the 693 patients with neutropenia, 15 days (range, 1-498 days) in the 393 patients with thrombocytopenia, and 41.5 days (range, 1-493 days) in the 748 patients with infections. AEs were managed by dose modifications and/or supportive care, including anti-infectives (88%), red blood cell transfusion (45%), granulocyte colony-stimulating factor (17%), and platelet transfusion (14%). The rate of grade 3/4 venous thromboembolic events was low (2%). Peripheral neuropathy (PN) of any grade occurred in 17% of patients; 1% experienced grade 3/4 PN. The median average POM dose was 4 mg (range, 1.6-4.2 mg). AEs leading to POM dose interruptions occurred in 66% of patients. AEs leading to dose reductions occurred in 24% of patients; the most common were neutropenia (8%), thrombocytopenia (5%), and infections (4%). AEs leading to discontinuation of POM were infrequent (7%).
Summary/Conclusions: In this large pooled safety analysis, POM+LoDEX showed an acceptable safety profile in patients with RRMM. AEs were manageable, and discontinuations due to AEs were uncommon.

E1285

MP0250, A BISPECIFIC VEGF- AND HGF-BLOCKING MULTI-DARPIN*, IN COMBINATION WITH BORTEZOMIB IN MULTIPLE MYELOMA: PRECLINICAL RATIONALE AND PHASE 2 STUDY OUTLINE

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Background: Despite advances in the treatment of multiple myeloma (MM), there is no cure. Patients eventually relapse, requiring multiple lines of treatment. Upregulation of both the vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) pathways has been implicated in loss of response to therapy. VEGF and HGF are increasingly overexpressed with MM progression and have been linked to poor prognosis: they are known to be able to stimulate neovascularization, bone destruction, and myeloma proliferation, migration, and adhesion in the bone marrow. Preclinical studies have shown that refractory, high HGF-expressing MM cell lines can be re-sensitized to treatment by inhibiting HGF/cMET signaling. MP0250 is a bispecific VEGF / HGF blocking multi-DARPin which is currently in a phase 1 study in solid tumors.
Aims: We are presenting the preclinical rationale for using MP0250 in combination with bortezomib (BTZ) in a planned phase 2 study in MM patients.
Methods: MP0250 and BTZ, alone and in combination, were tested in an orthotopic murine xenograft model in which human MM cells were implanted in murine tibial bone marrow. Tumor growth and muscle invasion was monitored by caliper measurements and CT scans, and bone destruction was analyzed by X-ray and microCT. Ex vivo analysis included the determination of M protein levels.
Results: MP0250 significantly inhibited bone lysis (total and cortical bone volumes) and tumor invasion in a dose-dependent manner. In both the MP0250 and MP0250+BTZ groups, tumor infiltration into the tibial muscle was significantly reduced while BTZ alone had no effect. M protein was reduced and inhibition of bone lysis was increased when the combination of BTZ and MP0250 was compared to BTZ alone. The results show that blockade of HGF and VEGF by MP0250 on its own has a beneficial effect and that enhanced effects can be achieved in combination with BTZ.
Summary/Conclusions: MP0250, a multi-DARPin specifically blocking HGF and VEGF, improves the efficacy of BTZ in a preclinical MM model. Consequent to this and a favorable phase 1 study, MP0250 will be evaluated in a phase 2 study in MM in combination with BTZ+DEX.
**DARPins are small repeat proteins, designed to bind targets with high specificity and potency, and which can be combined in a modular fashion.*

E1286

CAN CAPILLARY ZONE ELECTROPHORESIS MONITOR DARWINIAN EVOLUTION OF MULTIPLE MYELOMA CLONES?

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Background: At the time of diagnosis of multiple myeloma (MM) and similar disorders there are multiple clones in bone marrow or in extramedullary sites. Usually only a dominant clone is diagnosed and monitored in daily clinical practice before, during or after chemotherapy. Non dominant clones are not measured routinely nor their evolution under pressure of chemotherapy.We present identification and monitoring of multiple clones of multiple myeloma and similar

disorders by capillary zone electrophoresis (CZE) in routine clinical settings and evolution of these clones during a 5 year follow up and during third line chemotherapy by lenalidomide
Aims: Multiple myeloma is heterogenous disease with multiple clones. There is no techique available in routine clinical practice to monitor dominant and non-dominant clones in the same time. Chemotherapy bears a risk of supressing less aggressive dominant clone and enhancing evolution process of very aggressive and chemotherapy resistant clones. Our aim was to monitor all clones simultaneously during natural course of disease and during chemotherapy to understand evolution process in routine practice.
Methods: 1/CZE results captured from 1.1.2009 till 31.12.2009 in a single hospital pathology centre with a 5 year follow up correlated with clinical outcomes of patients. 2/Results of patients with mutliple myeloma on third line chemotherapy with lenalidomide in single centre from 1.1.2010 till 31.12.2015 monitored by CZE.
Results: 1/ All consecutive patients with any paraprotein visible in CZE within year 2009 in our centre: N=314. Break down these patients to a number of all patients with only one paraprotein on CZE is n= 277, a number of patients with two or three paraproteins is n= 37.Evolution of clones within one year (2009): from one paraprotein to two visible in CZE was in n=8 patients. One patient has experienced evolution from one clone to thee clones within one year. 5 year follow up data of all patients with more than 1 paraprotein are available only in 11 patients from original 37 patients.Their diagnoses were: MGUS n=2 with further increasing number of clones from 2 to 3 within 5 years; CLL n= 2, one patient with increasing number of paraproteins from 2 to 3, Waldenstroam Macroglobulinaemia n= 3, one patient with increasing number of paraproteins from 2 to 3. 2/ Patients on third line chemotherapy n=56 based on lenalidomide were monitored by CZE. Evolution of multiple clones is demonstrated by CZE
Summary/Conclusions: CZE is a very sensitive and unique method capable capturing even non-dominant minor clones and monitoring them during observation or chemotherapy and to assess response to chemotherapy in a deeper level in routine practice. CZE can describe selection of clones during chemotherapy. A high sensitivity of CZE can reveal paraprotein in patients with lymphopoliferative disorders. For better understanding of CZE in monitoring of non-dominant clones further correlation with flowcytometry and even with cytogenetic and genomic assays are needed.

E1287

THE CLINICAL COURSE OF RELAPSED OR REFRACTORY U.S. MULTIPLE MYELOMA (RRMM) PATIENTS RECEIVING TWO OR MORE LINES OF THERAPY

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Background: The introduction of novel agents, proteasome inhibitors (PIs) and immunomodulatory drugs (IMIDs), has led to an improvement in prognosis in multiple myeloma (MM). However, over the course of the disease, less durable remissions with each successive therapy have been noted in MM. In a retrospective single-institution, chart review study, patients experienced shorter overall survival (OS) as they progressed through each line of therapy, first-line (1LT) and second-line (2LT), OS of 28.4 and 17.1 months, respectively (Kumar 2004). Other evidence from a multi-center observational analysis of 383 MM patients (treated between 2007 and 2010) reported a median progression free (PFS) and OS from start of salvage therapy after first relapse of 13 and 35 months, respectively (Durie 2012); however, the clinical course of RRMM in the era of novel agents among pts treated in the real-world remains to be elucidated.
Aims: This study aims to describe the PFS and OS of RRMM patients receiving two or more lines of therapy in the era of novel agents in a large national database in the U.S.
Methods: In this retrospective cohort study using an electronic medical records (EMR) database from 38 states in the U.S, newly diagnosed, adult MM patients initiating 1LT between 1/2008 and 12/2014 were followed to identify 2LT accordingly: 1) retreatment after a treatment gap of >3 months of a prior LT, or 2) a switch to another drug combination after 1LT initiation. Subsequent lines of therapy, 3LT and 4LT, began with a switch in regimen compared to the previous line. Pts with salvage stem cell transplants (SCT) were excluded. Time to new treatment or death (TTNT) was used as a surrogate for PFS. Kaplan-Meier analyses were performed for OS/TTNT from start of each line of therapy. Observations were censored at time of loss to follow up or end of study period (6/30/2015).
Results: Among 628 patients initiating both 1LT and 2LT, mean age was 69.2 years (SD: 10.3) at start of 2LT; 51% were male; 9.6% had known high cytogenetic risk MM (del[17p] and/or t[4:14] and/or t[14:16]); 19.6% had a frontline SCT; 46.7% had a Charlson Comorbidity Index (CCI) score ≥2. PI-based regimens predominated in 1LT (44.2%), 3LT (43.1%) and in 4LT (41.2%) (Table 1). The most common regimen in 2LT was IMID-based (44.2%). PI+IMID-based combination therapy increased from 17.7% in 1LT to 40.2% in 4LT. As patients progressed through lines of therapy, median TTNT decreased from 15.1 months (95% CI: 13.4, 17.3) in 2LT to 7.8 months (95% CI: 6.1, 9.7) in 3LT and 6.9 months (95% CI: 5.1, 11.8) in 4LT. Median OS decreased from 41.0 months (95% CI: 32.1, 59.5) in 2LT to 30.3 months (95% CI: 20.1, 46.0) in 3LT and 24.8 months (95% CI: 12.8, 43.0) in 4LT.

Table 1. Treatment patterns by line of therapy among MM patients initiating 1st and 2nd line therapy.

Line of Therapy	1st Line N=628	2nd Line N=628	3rd Line N=218	4th Line N=102
Type of Therapy, %				
PI-based	41.6	33.3	43.1	41.2
Bortezomib ± non-IMID	41.4	28.0	30.7	11.8
Carfilzomib ± non-IMID	0.2	5.3	12.4	29.4
IMID-based	33.8	44.2	30.7	35.3
Lenalidomide ± non-PI	29.3	36.2	17.9	11.8
IMID (other than lenalidomide) ± non-PI	4.5	8.0	12.8	23.5
PI + IMID-based	17.7	8.9	22.5	40.2
Lenalidomide + bortezomib ± other	14.8	5.3	12.4	29.4
Bortezomib + IMID (other than lenalidomide) ± other	2.6	0.6	3.7	2.0
Carfilzomib + IMID ± other	0.3	3.0	6.4	8.8
Other, not categorized elsewhere	7.0	13.7	11.0	8.8

*measured from the index date which is the start date of each line of therapy.

Key: IMID – immunomodulator (lenalidomide, thalidomide, pomalidomide); OS – overall survival; PI – proteasome inhibitor (bortezomib, carfilzomib); Other – includes: cyclophosphomide, melphalan, vincristine, (liposomal) doxorubicin, interferon, panobinostat, bendamustine, vorinostat, single agent steroid; TTNT – time to next treatment

Summary/Conclusions: Median OS and time to progression from 2LT was longer than previously reported (Durie 2012; Kumar 2004). However, successive treatment regimens yielded progressively shorter TTNT and OS durations. These data may support treatment decision making by informing the trade-offs between prognosis, treatment effectiveness and toxicity.

Reference

1. Durie ASCO 2012; abs 8095 Kumar, et al. Mayo Clin Proc. 2004; 79(7):867-874.

E1288

PRACTICE PATTERNS AND OUTCOMES IN ELDERLY U.S. PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM)

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Background: Multiple myeloma (MM) is a disease of the elderly; median age at diagnosis is 70 yrs (Larocca 2015). The advent of novel therapies has improved survival (OS) among newly diagnosed MM (NDMM) patients (pts) (Kumar 2014). But, the link between therapeutic regimens and OS benefit in elderly pts is not clearly established (Kumar 2014; Verlest 2012). Real-world information regarding prescribing patterns and clinical outcomes in elderly pts with RRMM is limited.
Aims: To describe the prescribing patterns and clinical outcomes, and to evaluate the impact of age on progression-free survival (PFS) and OS in elderly RRMM pts initiating second line therapy (2LT) in a large, national database in the U.S.
Methods: In this retrospective cohort study of an electronic medical records (EMR) database, NDMM adult pts initiating first-line therapy (1LT) between 1/2008 and 12/2014 were included and 2LT was identified accordingly: 1) retreatment after a treatment gap of >3 months of 1LT, or 2) a switch to another drug combination after starting 1LT. Third-line therapy (3LT) began with a switch in regimen post 2LT. Pts with salvage stem cell transplants (SCT) were excluded. Time to new line of therapy (TTNT) or death was used as a surrogate for PFS. Cox proportional hazard models for TTNT and OS were conducted from start of 2LT. Pts were censored at loss to follow up or the end of study period (6/30/2015).
Results: Among 628 pts with 1LT and 2LT, 37.1% were ≥75 yrs of age (yo) at start of 2LT, 51% male; 2% of those ≥75 yr *vs* 30% of those <75 yr underwent frontline SCT. Pts ≥75 yo had a higher comorbidity burden (mean Charlson Comorbidity score [CCI]: 2.1 *vs* 1.6), and a higher proportion had cardiovascular disease (CVD [38.6% *vs* 27.3%]) than those <75 yo at start of SLT. Median follow-up from start of 2LT was 13.0 mos (IQR: 5.2, 23.3).Younger pts were 2-fold more likely to receive more intensive therapy with a proteasome inhibitor+immunomodulatory drug (PI+IMID)-based combination both in 1LT

(21.8% *vs* 10.8%) and in 2LT (11.1% *vs* 5.2%) compared to ≥75 yo (Table). In 1LT, older age was associated with an increased use of IMID-based therapies (42.1% *vs* 28.9% among ≥75 *vs*<75 yo). In 2LT, older age was associated with a higher frequency of PI-based therapies, 39.5% *vs* 29.6% in the younger cohort. OS probabilities at 1 yr and 2 yrs were 84.6% and 68.0% for those<75 yrs and 72.3% and 57.4% for those ≥75 yrs. Adjusting for observed confounders (gender, race, cytogenetic risk, CCI, CVD, yr of diagnosis, 1LT and 2LT regimen type), older age was associated with a significantly higher mortality risk after initiation of 2LT (HR: 1.57 [95% CI: 1.2, 2.1], *P*<0.01, for age ≥75 *vs*<75 yrs) (Figure). Median TTNT was 16.6 mos (95%CI: 13.5, 19.1) for pts<75 yrs and 13.8 mos (95% CI: 11.7, 17.8) for pts ≥75 yo. Age was not significantly associated with TTNT in 2LT in multivariate analyses.

Table 1. Drug therapy by age among MM patients (n=628).

Age Cohort, years	<75 N=395		≥75 N=233	
Line of Therapy	1st Line	2nd Line	1st Line	2nd Line
≤Doublet vs ≥Triplet therapy, %				
≤2 drug combination	55.4	75.7	75.1	85.0
≥3 drug combination	44.6	24.3	24.9	15.0
PI-based	43.0	29.6	39.1	39.5
Bortezomib ± non-IMID	42.8	24.3	39.1	34.3
Carfilzomib ± non-IMID	0.3	5.3	0	5.2
IMID-based	28.9	44.8	42.1	42.9
Lenalidomide ± non-PI	25.3	36.5	36.1	35.6
Other IMID[a] ± non-PI	3.5	8.4	6.0	7.3
PI + IMID-based	21.8	11.1	10.8	5.2
Lenalidomide + bortezomib ± other[b]	17.7	6.1	9.9	3.9
Bortezomib + other IMID[a] ± other[b]	3.5	1.0	0.9	0
Carfilzomib + IMID ± other[b]	0.5	4.0	0	1.3
Other[b], not categorized elsewhere	6.3	14.4	8.2	12.5

[a]IMID other than lenalidomide (ie, thalidomide or pomalidomide); [b]Other includes cyclophosphomide, melphalan, vincristine, (liposomal) doxorubicin, interferon, panobinostat, bendamustine, vorinostat, single agent steroid.

Key: IMID – immunomodulator (lenalidomide, thalidomide, or pomalidomide); OS – overall survival; PI – proteasome inhibitor (bortezomib or carfilzomib); TTNT – time to next treatment

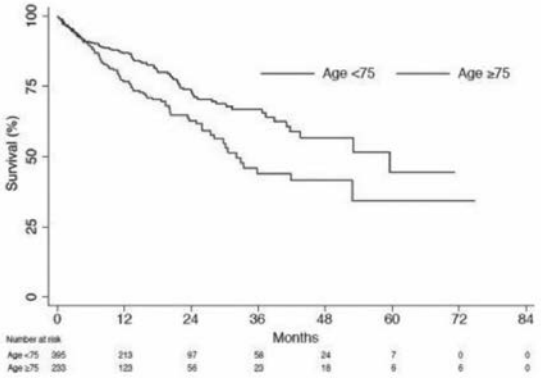

Figure 1. Adjusted overall survival by age from 2LT initiation.

Summary/Conclusions: In 1LT and 2LT in MM, older age appeared to impact treatment choice with less intensive therapy. The majority of patients received ≥1 novel agent irrespective of age; however, elderly pts were more likely to initiate IMID-based therapy in 1LT and PI-based therapy in 2LT compared to younger pts. Older age was associated with significantly worse OS outcomes after start of 2LT in the real-world.

References

1. Larocca et al. *Blood.* 2015;126:2179-85;
2. Verlest et al. *Haematologica.*2012; 97:242;
3. Kumar et al. *Leukemia.* 2014;28:5:1122-1128.

E1289

COMPARISON OF FOUR NEUROPATHY ASSESSMENT SCALES IN EVALUATION OF TREATMENT EMERGENT NEUROPATHY IN NEWLY DIAGNOSED PATIENTS OF MULTIPLE MYELOMA TREATED WITH BORTEZOMIB BASED REGIMEN

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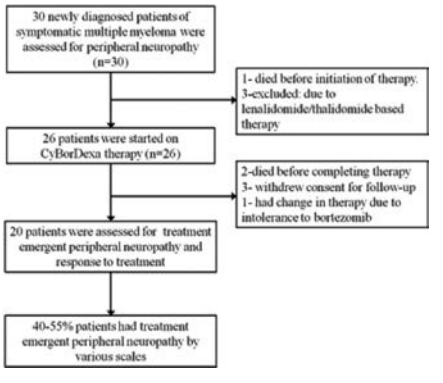
Background: Treatment emergent peripheral neuropathy (TEPN) is a distressing and potentially dose limiting side-effect of bortezomib containing regimen for multiple myeloma (MM). The reporting of extent and severity of TEPN is variable due to use of different neuropathy scales. Presently, most investigators use National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) for reporting of TEPN.
Aims: We aimed to compare the utility of different neuropathy scoring scales for detection of TEPN and to evaluate the incidence, severity and electrophysiological characteristics of TEPN after bortezomib based regimen in newly diagnosed patients of MM.
Methods: We prospectively evaluated the incidence of TEPN in treatment naïve patients of MM receiving cyclophosphamide, bortezomib and dexamethasone (CyBorD) on days 1, 8, 15 and 22 of each 28-day cycle, by clinical evaluation, nerve conduction study (NCS) and four different neuropathy scores- NCI CTC v4.0, Total neuropathy score reduced (TNSr) and clinical (TNSc) and Numerical Response Scale (NRS) for neuropathic pain. TNSr includes results of nerve conduction study in addition to the clinical parameters used in TNSc. Post-treatment increment by one grade for NCI-CTC, by score of ≥2 for TNSr and TNSc, and by score of ≥1 for NRS defined TEPN. Informed consent was obtained from each patient and the study was approved by institutional review board.
Results: A total of twenty six patients received CyBorD regimen. Twenty patients completed follow-up. After a median of 7 (range: 3.5-11) months, the rates of occurrence of TEPN differed when the four scales were used; viz. NCI CTC=45% (n=9), TNSr=55% (n=11), TNSc=40% (n=8) and NRS=40% (n=8). All four scales showed worsening following treatment with CyBorD, especially with regard to sensory symptoms (p<0.01 for all scales). TNSr could pick up higher number of cases with TEPN in comparison to any other score, most notably in comparison to NCI CTCAE. Among 12 patients who did not have TEPN by NCI CTC scale, 41.7% (n=5), 16.7% (n=2) and 8.3% (n=1) patients satisfied the criteria for TEPN by TNSr, TNSc and, NRS, respectively. When compared against TNSr, sensitivity for detecting TEPN by NCI CTCAE, TNSc and NRS were 77.8%, 88.9%, and 77.8%, respectively. NCI CTCAE and TNSc, both showed specificity of 63.3% for detection of TEPN. Specificity with NRS was found to be 54.5%. The higher detection rate of neuropathy by TNSr is probably due to wider range of scores given for graded sensorymotor impairment and incorporation of electrophysiological parameters which increases its sensitivity. On NCS, three patients (42.9%) had sensorimotor and four (57.1%) patients had motor nerve conduction abnormalities. Five patients (71.4%) had axonal neuropathy while 2 (28.6%) patients had evidence of demyelination on NCS.

Table 1.



Summary/Conclusions: We suggest that, NCI-CTCAE may be suboptimal in comparison to TNSr in assessment of TEPN in patients with MM on bortezomib based therapy. It is desirable to use better scoring systems like TNSr as they may be more sensitive and they improve our understanding of bortezomib induced neuropathy. NRS is a simple screening tool that can be used in routine clinical practice for detecting bortezomib induced neuropathy. Further studies with large sample size including intraepidermal nerve fiber density testing on skin biopsy are required to better define TEPN in patients receiving bortezomib.

E1290

SERUM B-CELL MATURATION ANTIGEN IS A BIOMARKER FOR PATIENTS WITH B-CELL DISORDERS

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Background: Serum B-cell maturation antigen (sBCMA) is a tumor necrosis factor receptor family member that is expressed on normal and malignant B-cells including plasma cells.
Aims: We determined sBCMA levels in large cohorts of patients (pts) with multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and Waldenström's macroglobulinemia (WM) and immune deficiency (ID) including common variable immune deficiency (CVID) and X-linked agammaglobulinemia (XLA).
Methods: Serum was obtained on healthy individuals and pts with B-cell disorders following informed consent. Enzyme-linked immunosorbent assay (ELISA) was used to determine sBCMA levels (R&D Systems, Minneapolis, MN). Kaplan-Meier survival analysis and log-rank comparison tests were used to determine the association between sBCMA levels and time to first treatment

(TTFT), overall survival (OS) and progression-free survival (PFS). Similarly, sBCMA levels were correlated with bone marrow (BM) and PET scan findings for non-secretory disease (NSD) MM pts. Proportional Hazard Regression Analysis and a multivariate analysis were utilized to determine the predictive ability of sBCMA on OS and other prognostic factors including: age, serum creatinine, serum hemoglobin, and ISS staging. One-way univariate analysis of variance was performed to study the relationship of sBCMA with bone disease status, based on presence of osteopenia, osteolytic lesions and fractures as well as the clinical status. All of the statistical analysis was performed using JMP Pro for Windows by SAS and P values<0.05 are reported as statistically significant.

Results: Compared to healthy donors (n=43), pts with untreated active MM (n=44) showed increased levels of sBCMA (*P*<0.0001). sBCMA levels correlated with changes in BM plasma cell involvement (Pearson r=0.609; p<0.0001) and current clinical status (n=164, *P*-values: 0.0045 CR *vs* PR;<0.0001 CR *vs* NR) for MM pts. Those with other untreated B-cell malignancies showed increased levels (CLL: n=94, WM: n=29, both *P*<0.0001). sBCMA correlated with changes in M-protein levels for MM and WM pts and changes in clinical status among both MM and CLL pts. Furthermore, sBCMA also correlated with PET scan and BM findings among MM pts with NSD. Kaplan-Meier analysis demonstrated that sBCMA levels predict PFS (*P*=0.0004) and OS (*P*=0.0043) among pts with MM (n=242). sBCMA levels correlated with depth of response (*P*-values: 0.05 CR *vs* PR, 0.03 CR *vs* SD, and 0.004 CR *vs* PD). Among CLL pts (n=171), sBCMA levels predicted TTFT (*P*<0.0001) and OS (*P*=0.02), and correlated with WBC count, serum ß2-microglobulin level, IgVH mutational status, ZAP-70 scores, and chromosome 13 deletion. Additionally, sBCMA did not correlate with renal function or presence of the bone disease or other prognostic factors and maintained independent significance when tested in cohorts of MM (n=188, Pairwise R=0.18) or CLL (n=155, Pairwise R=0.001) pts. Compared to healthy donors (n=104), pts with an ID showed lower levels of sBCMA (CVID: n=46; XLA: n=8; both *P*<0.0001). Among pts with CVID, sBCMA correlated with complications from the disease (*P*=0.01).

Summary/Conclusions: We have identified sBCMA as a novel independent diagnostic and prognostic biomarker that can monitor and predict outcomes for pts with MM, CLL and WM which show elevated levels whereas those with ID including CVID and XLA show decreased levels. Levels of this marker among pts with B-cell malignancies also correlate with clinical status, predict TTFT, PFS and OS, and provide MM pts with NSD a way to follow their disease course.

E1291

IMPACT OF IMMUNOPARESIS AND ALTERED SERUM FREE LIGHT CHAIN RATIO ON THE PROGRESSION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) IN A DISTRICT GENERAL HOSPITAL IN UNITED KINGDOM

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Background: Monoclonal gammopathy of undetermined significance (MGUS) occurs in 3% of people >50 y and up to 10% in those >70 years.The risk of progression is heterogenous.The numbers are expanding and the existing risk stratification models needs to be dynamically improved to ensure the clinical monitoring to be more cost effective.The impact of altered serum free light chain ratio (ASFLCR) and immunoparesis (IP) as tools on MGUS progression been analyzed in the current study, in addition to the already known prognostic factors: type of M protein and the amount of monoclonal component.

Aims: In general, we aimed to identify the impact of immunoparesis and skewed SFLCR as prognostic factors for progression of MGUS to multiple myeloma in a district general hospital in UK.The specific objectives were to determine the socio-demographic characteristics of study population;to identify proportion of de novo myeloma at presentation and MGUS progressing to myeloma; to determine the impact of immunoparesis at presentation on progression of MGUS;to identify any association between abnormal SFLCR and progression of MGUS.;to find any association between the type of light chains with the risk of progression.

Methods: A Retrospective cohort. All patients with MGUS and Myeloma from 2000 to 2013 included in the study population. Baseline immunoglobulin, paraprotein and light chain levels are the main parameters. Data sources are medical records of hematology out patients' clinic department & electronic hematology data base. All patients with monoclonal proteins referred to Department of Hematology recruited. Other disorders contributing to immunodeficiency: hypogammaglobulinaemia, lymph proliferative disorders, autoimmune disorders, stage 4 CKD, nephrotic syndrome, and generalized hypoproteinaemic conditions, Patients on disease modifying drugs and immunosuppressive medications systemic steroids, chemoimmunotherapy within previous 1 year of diagnosis, Patients having paraproteins together with polyclonal increase in immunoglobulines on presentation were excluded from the study.

Results: Here, we have assessed the incidence of immunoparesis (IP) and abnormal serum free light chain ratio (ASFLCR) in a cohort of 653 patients with MGUS.They were 323 (49.2%) males and 333 (50.8%) females, with their ages ranging from 34 to 94y, having the median age of 71y. The median time from the diagnosis to the time of observation was 35 months. The paraproteins: IgG in 57.8%, IgM in 23.3% and IgA in 17.3%. About 1.2% of were light chain (LC) MGUS.Approximately, 10% of the myelomas were LC secreting. Kappa LC identified in 60% and Lambda in 37.6%.SFLCA was not available for the hospital before 2005. Even later, subjected to selection bias, as it is not an in house facility.Out of, 437 patients who had the assay 255 were having ASFLCR. Immunoparesis is seen in 254 (38.7%) and 402 (61.3%) have normal immunoglobulin levels. There is a significantly positive correlation with the incidence densities of myeloma in the group with >/=2 immunoparesis. The relative risk is 4.662 (95% CI 1.847-11.772) and the (p 0.001). We have identified positive correlation of relative risk of MGUS progression to Myeloma in the presence of abnormal SFLCR.(Rate ratio 2.531).

Summary/Conclusions: It is apparent that immunoparesis and altered SFLC ratio play a pivotal role in accelerating the MGUS progression to myeloma. Our current study shows that there a statistically significant evidences that immunoparesis and serum free light chain ratio correlates positively with MGUS progression.

E1292

OVERALL SURVIVAL IN PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA IN THE REAL-WORLD SETTING: A RETROSPECTIVE ANALYSIS OF THE PHAROS REGISTRY IN THE NETHERLANDS

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Background: Survival rates have improved for patients with multiple myeloma (MM), yet relapse remains common. Data on real-world outcomes in these patients in Europe is limited.

Aims: To provide insights into the survival of patients with symptomatic MM in the Netherlands through a retrospective analysis of data from the PHAROS registry, a Dutch population-based database.

Methods: The PHAROS registry included patients with MM diagnosed between 2004 and 2012 (aged ≥18 years). The study population consisted of 1522 patients starting first-, second- or third-line therapy in 2008 or later (not all patients in the analysis on second- and third-line therapy were included in the analysis of previous lines). The primary endpoint was overall survival (OS); secondary endpoints included progression-free survival (PFS) and healthcare resource utilisation (HRU).

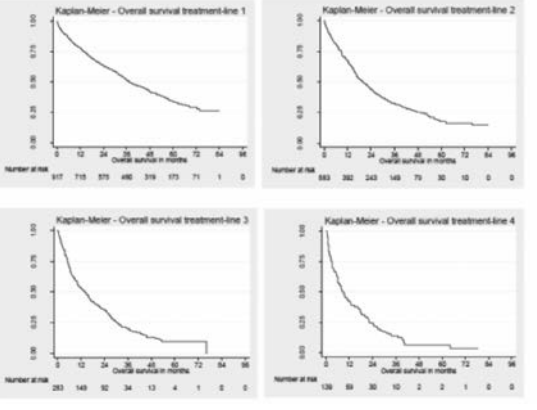

Figure 1. Kaplan-Meier curves of OS from first-, second-, third- and fourth-line therapy.

Results: Median patient age was 70, 71, 71 and 72 at the initiation of first-, second-, third- and fourth-line therapies, respectively. Median follow-up for the primary endpoint (OS from first-line therapy) was 62.4 months (95% confidence interval [CI]: 60.6, 64.3). First-line thalidomide-based regimens were prescribed to 66% of patients (n=608) and bortezomib-based regimens to 15% (n=139). In the second-line setting (n=583), the majority of patients received bortezomib- (41%, n=239) or lenalidomide- (27%, n=159) based regimens. Median OS (95% confidence interval [CI]) in first- (n=917), second- (n=583) and third-line (n=283) were 37.5 (34.8-41.8), 19.7 (17.2-22.9) and 13.9 (10.5-16.6) months, respectively. Median PFS (95% CI) were 18.0 (16.3-18.9), 8.9 (7.9-9.7) and 6.4 (5.5-7.2) months, respectively. Large differences were seen between subgroups in all treatment-lines, e.g. median OS from first-line therapy was 31.9 (29.1-35.4) and 64.6 (53.2-not reached) months for patients >65 years old and patients ≤65 years old, respectively, and was 32.2 (29.2-35.5) for patients without prior stem cell transplantation (SCT) (median OS not reached for patients

with prior SCT). Similarly, median PFS from first-line therapy was 16.2 (14.5-17.9) and 22.6 (19.8-26.5) months for patients >65 years old and patients ≤65 years old, respectively and 15.2 (13.6-17.0) and 32.0 (26.2-36.5) months for patients without or with prior SCT, respectively. Mean (standard deviation [SD]) number of inpatient days per month (excluding intensive care unit) were 1.7 (3.4), 1.4 (3.2) and 2.3 (4.6) in first, second and third-line, respectively.
Summary/Conclusions: In patients receiving first-line therapy for MM between 2008 and 2012 in The Netherlands, median OS was approximately three years. A difference in OS was observed for SCT *versus* non-SCT patients and resource use (inpatient stays) increased with later lines. Large differences in OS were also observed depending on a patient's age.

E1293

COMPARISON BETWEEN 8-COLOR MULTIPARAMETER FLOW CYTOMETRY AND NEXT-GENERATION SEQUENCING TO DETECT MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA PATIENTS WHO UNDERWENT AUTO-SCT

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Background: Autologous stem cell transplantation (ASCT) in conjunction with novel therapeutic drugs can dramatically improve response rates and the prognosis of patients with multiple myeloma (MM). However, most patients with MM are considered to be incurable, and relapse owing to minimal residual disease (MRD) is the main cause of death among these patients. Therefore, new technologies to assess deeper responses are required. Next-generation sequencing (NGS) and multiparameter flow cytometry (MFC) methods have been used to assess MRD. However, the lack of standardization of conventional MFC approaches has had a negative impact on its reproducibility. Recently, a next-generation MFC method (EuroFlow) has been developed by the EuroFlow Consortium and the International Myeloma Foundation (IMF) for a highly sensitive and standardized detection of MRD in MM.
Aims: To compare the prognostic value of MRD detection in autografts in MM between NGS and EuroFlow.
Methods: A total of 22 newly diagnosed MM patients who underwent ASCT were included in this study. Median age 60 (range 41-65); males 14, females 8; ISS 1 (n=4), 2 (n=12), 3 (n=6). 6 patients showed high-risk chromosomal abnormalities (t(4;14) (n=6), del17p (n=1)). The induction regimen was bortezomib-based chemotherapy. All patients received melphalan 200 mg/sqm conditioning regimen before ASCT. 18 of 22 (82%) patients received maintenance therapy using lenalidomide or thalidomide. The best response post-ASCT was as follows: 10sCR, 1CR, 9VGPR, 2PR. 22 autografts, one from each MM patient, were analyzed using EuroFlow and NGS methods. The EuroFlow method was based on a standardized lyse-wash-and-stain sample preparation protocol, the measurement of high numbers of cells (≥5x10^6 cells/tube) and an optimized 8-color, 2-tubes, antibody panel, for accurate identification of plasma cells (PCs) and discrimination between phenotypically aberrant (aPC) and normal PC (nPC): tube 1: $CD138_{BV421}$ /$CD27_{BV510}$ /$CD38_{FITC}$ /$CD56_{PE}$ /$CD45_{PerCP-Cy5.5}$ /$CD19_{PE-Cy7}$ /$CD117_{APC}$ /$CD81_{APC-C750}$; and tube 2: $CD138_{BV421}$ /$CD27_{BV510}$ /$CD38_{FITC}$ /$CD56_{PE}$ /$CD45_{PerCP-Cy5.5}$ /$CD19_{PE-Cy7}$ /cytoplasmic (Cy) Immunoglobulin (Ig) κ_{APC} /Cyl$\lambda_{APC-C750}$. NGS-based MRD assessment was performed using the immunosequencing platform (Adaptive Biotechnologies, South San Francisco, CA) (Martinez-Lopez et al Blood 2014).

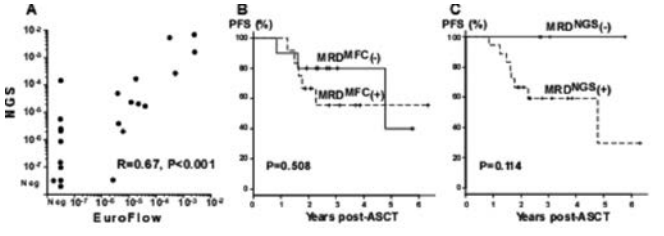

Figure 1.

Results: We identified abnormal plasma cells (aPC) in autografts based on multivariate analysis of individual cells from each patient (e.g. CD56+, CD19-, Cylgκ+, CD117+). For the MM MRD in autografts, the events from tube 1 and tube 2 were combined and a median of 5.6×10^6 (range: 1.3×10^6-11.7×10^6) events was acquired. 12 of 22 (55%) cases were MRD positive by 8-color MFC while 18 of 22 (82%) cases were MRD positive by NGS. The correlation of MRD level between 8-color MFC and NGS was relatively high (Fig. A). There was no significant difference in PFS between MRD negative cases by EuroFlow (MRD^{MFC} (-)) and MRD^{MFC} (+) cases (Fig. B) (P=0.508). However, MRD negative cases by NGS (MRD^{NGS} (-)) tended to show better PFS than MRD^{NGS} (+) (P=0.114) (Fig. C). There was no significant difference in overall survival between the MRD positive and negative groups.
Summary/Conclusions: In this comparison study of MRD assessment in autografts using Euroflow and NGS approaches, the NGS platform showed higher sensitivity and prognostic value than EuroFlow.

E1294

RENAL AND HEMATOLOGIC OUTCOMES OF BORTEZOMIB-BASED TREATMENT IN PATIENTS WITH LIGHT CHAIN DEPOSITION DISEASE

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Background: Light chain deposition disease (LCDD) is characterized by amorphous deposits of monotypic immunoglobulin light chains in the kidneys leading inevitably to end-stage renal disease (ESRD) if untreated. LCDD is one of the recently defined monoglonal gammopathies of renal significance (MGRS) and less often is associated with an underlying symptomatic myeloma. However, no optimal treatment or response criteria for the evaluation of treatment outcomes have been established although bortezomib-based regimens have been recommended based on small patient series.
Aims: to evaluate the outcomes of LCDD after bortezomib-based treatment, the prognostic effect of clinicopathological characteristics and the impact of hematologic response to renal survival.
Methods: 18 consecutive patients with renal biopsy-proven LCDD who received primary therapy with bortezomib were included in the analysis.
Results: by IF all biopsies had linear staining exclusively for κ or λ chains along the TBM and/or GBM; other findings included interstitial fibrosis (78%), mesangial expansion/proliferation (72%), tubular atrophy (67%) and nodular mesangial sclerosis (56%). Median age was 65.5 (range: 46-85) years and 10(55%) patients were >65 years; 89% presented with hypertension, median 24h proteinuria was 3.3 gr (range 0.4-10.2), 13/18 patients had eGFR<60 ml/min and 9/18 (50%) had eGFR<30ml/min. Fourteen patients (78%) had measurable FLCs (dFLC≥50mg/l and abnormal ratio). Median bone marrow infiltration was 15% but no patient had symptomatic MM by the CRAB criteria. Bortezomib with dexamethasone (VD) was given in 12/18(67%) and VD plus cyclophosphamide (VCD) in 6/18 (33%) patients for a median of 4 cycles; 10/18(56%) received bortezomib SC. Among evaluable patients 11/14(79%) had a hematologic response (PR, VGPR or CR), including 5(36%) with CR, 1(7%) with VGPR and 5(36%) with PR. At the time of diagnosis or during follow-up, 6/18 (33%) patients progressed to ESRD. Three of the 9 patients improved their eGFR from<15 to 15-29ml/min and 3 patients from 15-29 to 30-59ml/min while 9/18 patients achieved a 50% reduction of proteinuria. Improvement in eGFR was observed only in patients (6 /14) who achieved a hematologic CR or VGPR, and no patient who achieved a VGPR or CR developed ESRD. In contrast, 5/8 patients who achieved a PR or less progressed to ESRD (P=0.031). Improvement of proteinuria was observed in all patients who achieved a CR and in 2/9 patients who achieved a hematologic PR. In univariate analysis baseline eGFR<30ml/min was associated with increased risk for ESRD (OR=8.75, p=0.086); no pathology feature was associated with renal response or risk of ESRD. Median follow-up is 39 months and 13/18 patients remain alive; only 3 (17%) had a hematologic relapse/progression. Two patients died on dialysis and median survival from initiation of dialysis was 5 (range:1-13) months. Three patients received ASCT as consolidation after bortezomib induction. Bortezomib-related peripheral neuropathy occurred in 67% (grade 2 in 7 and grade 3 in 2 patients); in 9/18 patients a dose reduction of bortezomib was required but no patient discontinued bortezomib due to neuropathy. Neuropathy grade ≥2 occurred in 7/8(87.5%) *vs* 2/10(20%) patients who received IV *vs* SC bortezomib (P=0.041).
Summary/Conclusions: bortezomib-based treatment is active and safe for patients with LCDD but at least a hematologic VGPR is required in order to improve renal prognosis. Prospective studies are needed to determine the optimal management for LCDD and identify further predictors for renal prognosis.

E1295

A POOLED ANALYSIS OF THE IMPACT OF AGE ON OUTCOMES IN PATIENTS WITH REFRACTORY OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA TREATED WITH POMALIDOMIDE+LOW-DOSE DEXAMETHASONE

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Background: The survival duration of patients with multiple myeloma is inversely proportional to their age (Pulte et al, *Oncologist*, 2011). Pomalidomide (POM), a distinct immunomodulatory agent with tumoricidal and immunoregulatory effects, plus low-dose dexamethasone (LoDEX) is approved in the United States and European Union for the treatment of patients with relapsed and refractory multiple myeloma (RRMM) who have had ≥2 prior therapies, including lenalidomide and a proteasome inhibitor (bortezomib in the European Union). Approval was based on 2 phase 3 studies (Richardson, *Blood,* 2014; San Miguel, *Lancet Oncol,* 2013), and the regimen has been further evaluated in a large confirmatory study (Dimopoulos ASH 2015).
Aims: To conduct a pooled analysis of the impact of age on outcomes in patients treated with POM+LoDEX in 3 large trials.
Methods: Patients who provided informed consent, had ≥2 prior therapies, including lenalidomide and bortezomib, and progressed on or within 60 days of their last therapy were enrolled in the 3 trials. Patients received POM 4 mg/day on days 1-21 of each 28-day cycle and LoDEX 40 mg (20 mg for those >75 years of age) weekly until disease progression or unacceptable toxicity. Thromboprophylaxis was required. Outcomes analysis was conducted based on patient age subgroup (≤65, >65, ≤75, and >75 years).
Results: In total, 1097 patients were assigned to receive POM+LoDEX in the 3 trials and were included in the intent-to-treat population. Baseline demographic and disease characteristics were similar regardless of age (Table). Overall response rate (range, 32%-34%) and median progression-free survival (range, 4.2-4.6 months) were also similar across the age groups. Median overall survival was slightly longer in the ≤65 *vs* >65 subgroups (13.4 *vs* 11.9 months) but was similar in the ≤75 and >75 subgroups (12.3 months). The median duration of response was slightly longer in the >65 and >75 subgroups (7.7 and 9.0 months, respectively) compared with the ≤65 and ≤75 subgroups (7.0 and 7.1 months, respectively). In the 1088 patients who received at least 1 dose of POM+LoDEX (safety population), the median relative dose intensity was 0.9, and the median treatment duration was 4.8 months in each age subgroup. Grade 3/4 treatment-emergent adverse events (TEAEs), including neutropenia (range, 47%-50%), anemia (range, 29%-32%), thrombocytopenia (range, 17%-25%), and infections (range, 32%-35%) were similar across the age groups. Additionally, similar rates of TEAEs leading to dose reduction (range, 23%-26%) and interruption (range, 64%-69%) were observed in the four subgroups. POM discontinuation due to TEAEs was infrequent (range, 5%-8%).

Table 1.

	≤ 65 years	> 65 years	≤ 75 years	> 75 years
Intent-to-treat population	n = 542	n = 555	n = 971	n = 126
Median age, years	58	72	64	78
Median prior anti-MM regimens, n (range)	5 (2-14)	4 (2-18)	5 (2-14)	4 (2-18)
ECOG PS 0/1, %[a]	89.1	86.3	87.8	86.5
ECOG PS 2/3, %[a]	10.9	13.3	11.9	13.5
ISS I/II/III/missing, %	23.1/35.2/ 26.2/15.5	18.4/34.6/ 33.5/13.5	21.2/35.0/ 29.6/14.2	16.7/34.1/ 32.5/16.7
Refractory to LEN and BORT, n (%)	418 (77)	424 (76)	739 (76)	103 (82)
ORR, n (%)	178 (33)	178 (32)	313 (32)	43 (34)
Median PFS, months	4.4	4.4	4.2	4.6
Median OS, months	13.4	11.9	12.3	12.3
Median DOR, months	7.0	7.7	7.1	9.0
Safety population	n = 537	n= 551	n = 962	n = 126
Median relative dose intensity	0.9	0.9	0.9	0.9
Median Tx duration, months	4.8	4.8	4.8	4.8
Grade 3/4 TEAEs ≥ 10%, n (%)				
Neutropenia	269 (50)	258 (47)	464 (48)	63 (50)
Anemia	173 (32)	173 (31)	310 (32)	36 (29)
Thrombocytopenia	136 (25)	114 (21)	229 (24)	21 (17)
Leukopenia	44 (8)	48 (9)	79 (8)	13 (10)
Infections[b]	190 (35)	177 (32)	325 (34)	42 (33)
Pneumonia	71 (13)	80 (15)	137 (14)	14 (11)
Grade 3/4 TEAEs of interest, n (%)				
DVT/PE[b]	9 (2)	9 (2)	18 (2)	0
Peripheral neuropathy[b]	5 (1)	6 (1)	11 (1)	0
≥ 1 TEAE leading to dose reduction, n (%)	124 (23)	134 (24)	225 (23)	33 (26)
≥ 1 TEAE leading to dose interruption, n (%)	342 (64)	380 (69)	636 (66)	86 (68)
≥ 1 TEAE leading to discontinuation, n (%)	28 (5)	46 (8)	64 (7)	10 (8)

[a] ECOG status of 2 patients (> 65 and ≤ 75 years) is missing.
[b] Grouped term.
BORT, bortezomib; DOR, duration of response; DVT, deep vein thrombosis; ECOG, Eastern Cooperative Oncology Group; ISS, International Staging System; LEN, lenalidomide; MM, multiple myeloma; ORR, overall response rate; OS, overall survival; PE, pulmonary embolism; PFS, progression-free survival; TEAE, treatment-emergent adverse event; Tx, treatment.

Summary/Conclusions: POM+LoDEX demonstrated efficacy in patients with RRMM, with similar progression-free survival and response rates regardless of age; safety and efficacy in patients >75 years was comparable to that in patients<75 years. Similarly, the exposure and safety profile were similar across the age groups. These results support POM 4 mg as an effective starting dose and use of POM+LoDEX, regardless of age, in patients with RRMM.

E1296

PROSPECTIVE FUNCTIONAL GERIATRIC ASSESSMENT (CF-GA) IN MULTIPLE MYELOMA (MM) PATIENTS (PTS): CHANGES FROM BASELINE (T0) TO FOLLOW UP ASSESSMENT (T1)

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Background: Multiple myeloma (MM) is a hematologic malignancy, its incidence increasing with age. MM patients (pts) present with heterogeneous health status, whereby defined tests help to objectively define biological fitness, rather than considering pts' chronological age. This is specifically relevant, since treatment options are numerous today. For the discrimination of current therapies, a variety of MM- and patient-related risk factors, such as comorbidities (CM) and frailty can be considered. Therefore, the careful assessment of individual conditions before treatment and with subsequent, defined follow-up seems relevant. Therapy-related complications and early mortality may substantially increase in pts with CM, but may also change during treatment.
Aims: Our aim is to establish a prospective comorbidity and functional geriatric assesment (CF-GA) for MM pts and to show whether the GA shows substantial changes from baseline (T0) to follow-up (T1 and subsequently T2) and which tests are most indicative. This has - to the best of our knowledge - never been assessed in MM.
Methods: This prospective intervention trial performed a simple, defined GA in consecutive MM pts prior to initiation of antimyeloma treatment and during subsequent follow-up (6-12 [T1] and 24 months [T2]). The GA included the Karnofsky Performance Status (KPS), pain scale, rating of fitness, IADL, ADL, malnutrition, geriatric depression scale (GDS), mini-mental status (MMS) and established comorbidity (CM) scores: Charlson Comorbidity Index (CCI), Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI), Myeloma Comorbidity Index (initial-MCI [I-MCI] and revised-MCI [R-MCI]; http://www.myelomacomorbidityindex.org) and Kaplan Feinstein (KF). The trial was approved by the ethics committees of the Freiburg University. All patients provided written informed consent and all procedures were conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization, and Guidelines for Good Clinical Practice.
Results: Currently a total of 97 consecutive pts have been included in this GA, all receiving both T0- and T1-assessment (median 12 months after treatment initiation). The median pt age was 60.3 years (27-83). Between T0 and T1, 54 pts had received stem cell transplants (n=49: ASCT, n=1: allo-SCT, n=4 auto-allo-SCT); and the other half of pts novel agent-containing, standard chemotherapy and/or regular surveillance. Significant changes between T0- to T1-assessments were observed for various GA tests as summarized in Table 1. Of note, all mean test and score results improved, most notably the I-MCI, R-MCI-, IMWG- and CCI-scores, whereas the Kaplan Feinstein and HCT-CI remained almost those of baseline levels. Moreover, the KPS, ADL, IADL, fitness as rated both by physicians and patients as well as MMS improved. Of interest, the fitness rating by physicians seemed to improve more substantially than that given by pts, demonstrating again that a brief GA assessment with simple tests is more objective than clinical judgement alone. Pain, depression and malnutrition also improved, albeit to lesser and non-significant extends.

Table 1. Ga via CM tests and scores: changes from baseline (T0) to follow-up (T1) (n=97 MM pts).

Comorbidity Tests / Scores	Mean T0 values	Mean T1 values	Mean changes from T0 -> T1	p-values
Functional comorbidity tests				
Karnofsky Performance Status (0%-100%)	73.9	87.7	13.8	**<0.0001**
Activity of Daily Living (0-6)	4.6	5.7	1.1	**<0.0001**
Instrumental Activity of Daily Living (0-8)	7.2	7.5	0.3	**0.0094**
Fitness by Physician (1-6)	3.5	3.0	-0.5	**<0.0001**
Fitness by Patient (1-6)	3.4	3.0	-0.4	**0.0040**
Mini Mental Status (0-30)	27.5	28.0	0.5	**0.0483**
Pain Scale Visual analogue Scale (0-10)	2.5	2.2	-0.3	0.22
Geriatric Depression Scale (0-15)	3.3	2.9	-0.4	0.1669
Comorbidity scores				
Revised Myeloma Comorbidity Index (0-9)	4.6	3.3	-1.3	**<0.0001**
Inital Myeloma Comorbidity Index (0-3)	0.6	0.3	-0.3	**<0.0001**
International Myeloma Working Group Score (0-5)	1.3	0.7	-0.6	**<0.0001**
Charlson Comorbidity Index (0-33)	2.0	1.5	-0.5	**<0.0001**
Kaplan Feinstein (0-3)	1.4	1.3	-0.1	0.1222
Hematopoietic Cell Transplant Comorbidity Index (0-26)	1.9	1.9	0.0	0.9756

Summary/Conclusions: Our CF-GA contains simple, reliable tests, which consistently and objectively test MM pts' fitness before and after treatment. Our results impressively demonstrate that MM pts' general and specific fitness

- as measured via defined GA tests - may improve during follow-up. This was induced by the implemented antimyeloma treatment, best supportive care measures and lower disease-specific symptoms after therapy. We continue this GA in an even larger prospective multicenter cohort to determine each test's predictive power for survival and treatment toxicity.

E1297

IMPROVEMENT OF OVERALL SURVIVAL IN DANISH MULTIPLE MYELOMA PATIENTS AFTER 2008; A POPULATION-BASED STUDY FROM THE DANISH NATIONAL MULTIPLE MYELOMA REGISTRY

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Background: Improvement of overall survival in multiple myeloma (MM) after 2000 has been reported and it has been associated with introduction of new therapeutics (Kumar, *Blood* 2008,111,2516-20 & Kumar, *Leukemia* 2014,28,1122-28). The Danish National Multiple Myeloma Registry (DMMR) was established in 2005 and has registered all patients with newly diagnosed multiple myeloma in Denmark since 1 January 2005. The Danish Myeloma Study Group (DMSG) published in 2009 the first National evidence-based guideline for treatment of MM in Denmark. The guideline recommended bortezomib based induction treatment for younger MM patients prior to HDT and for elderly patients with aggressive disease presentation and adverse cytogenetics. Moreover, lenalidomide was included in the algorithm for treatment of relapsed/progressive disease.
Aims: We aimed to analyse population-based overall survival in Danish MM patients in relation to shift in National treatment guidelines in 2009.
Methods: At 30 June 2015, the database had registered a total of 2907 patients, 991 patients<65 years and 1916 patients >65 years, with newly diagnosed treatment-demanding MM. Age-adjusted survival analyses were done per 1 September 2015.
Results: Comparing OS for patients diagnosed in the two time periods 2005-2008 and 2009-2015, we found a highly significant improved age-corrected OS in patients >65 years, increasing from median 24.7 months (2005-08) to median 32.9 months (2009-15) (p=0.003). For patients<65 years at diagnosis the overall survival also improved from median 67 months (2005-2008) to above 74 months (median OS not yet reached) (2009-2015) (p=0.02). In patients >80 years at diagnosis the survival is poor and has not improved significantly between the 2 time periods (median 11.4 *vs* 14.9 months), and the early mortality rate in these very elderly is high (about 30% die within 6 months). The prognosis has particularly improved for MM patients that present with renal insufficiency. The database documents a shift in chosen therapies according to the National guidelines. Only, novel agents seem to have been less chosen for the most elderly patients.
Summary/Conclusions: Our "real life data" documents improved survival of MM patients in Denmark after 2008 and demonstrates a significant impact of National DMSG guidelines on treatment of multiple myeloma.

E1298

ASSESSING MYELOMA BONE DISEASE WITH WHOLE-BODY DIFFUSION-WEIGHTED MRI: COMPARISON WITH FDG PET-CT AND ASSESSMENT OF ADC VALUE

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Background: The recent International Myeloma Working Group (IMWG) consensus statement recommends whole-body magnetic resonance imaging (MRI) for patients with suspected smoldering multiple myeloma (SMM) and solitary plasmacytoma (Dimopoulos et al. 2015). Whole-body diffusion-weighted MRI (WB-DWI) is a promising technique with higher sensitivity for bone lesions and lower cost. However, little is known about its sensitivity compared to ^{18}F-fluorodeoxyglucose positron emission tomography (FDG PET-CT).
Aims: The aim of this study is to compare the sensitivity of myeloma-related bone disease by WB-DWI and FDG PET-CT, and to compare the apparent diffusion coefficients (ADC) of myelomatous lesions with normal healthy controls.
Methods: We identified patients with monoclonal plasma cell disorders that underwent WB-DWI between July 2015 and February 2016 at Kameda Medical Center, Kamogawa-shi, Japan. We compared disease burden by matched simultaneous assessments of WB-DWI and FDG PET-CT using a previously described scoring system based on the number and pattern (focal/diffuse) of disease in each body region (C-spine, T-spine, L-spine, pelvis, ribs/other, long bones). Whole-body score was calculated as the sum of each body lesion (Sharon et al. 2014). Scans performed in patients in different clinical status were excluded. We also analyzed ADC values of WB-DWI in comparison to eight healthy volunteers. Minimal ADC values were measured in each body region (C-spine, T-spine, L-spine, pelvis, ribs/other) and whole-body ADC value was defined as the mean ADC value of each body region. Inversion grayscale maximum intensity projection images of whole-body diffusion-weighted and ACD maps with *b* value=900s/mm^2 were assessed in this study. All statistical analyses were performed with EZR, which is a graphical user interface for R ver. 3.2.1.
Results: We identified 64 patients that underwent WB-DWI during the study period. Fifty patients underwent FDG PET-CT in the same disease status. Median age of the patients was 71 years (range: 53-89) and 23 (44.8%) were male. Three patients had monoclonal gammopathy of undetermined significance (MGUS), five patients had SMM, and 42 patients had symptomatic MM, including five with plasmacytoma. Diffuse lesions were found in 15 (30%) patients by WB-DWI, while FDG PET-CT detected two (4%) diffuse lesions. The detection ratio of focal lesions was the same for both procedures. The whole-body score was significantly higher in WB-DWI than FDG PET-CT (WB-DWI: mean 5.61±8.56 *vs* FDG PET-CT: mean 1.08, *P<0.001*). In each body region, scores for WB-DWI were significantly higher than those for FDG PET-CT. ADC values were analyzed in 64 patients that underwent diffusion-weighted WB-MRI and compared with eight healthy volunteers. Median age and number of male patients was 71 years (range 47-89) and 25 (39.6%), respectively. Four patients were diagnosed as MGUS, eight patients had SMM, and 51 patients had MM, including five with plasmacytoma. The median ADC value of the whole body was significantly lower in patients with monoclonal plasma cell disorders than in the healthy volunteers (680mm^2/s x10^{-6} *vs* 765.5 mm^2/s x10^{-6}, *P*=0.02). In each body region except C-spine and ribs/other, the ADC value was significantly lower in patients with monoclonal plasma cell disorders than in the healthy volunteers.

Table 1.

Score of WB-DWI vs FDG PET-CT

N=50	Score Mean (±SD)		P-value
	FDG PET-CT	WB-DWI	
WholeBody	1.08(±3.29)	5.61(±8.56)	<0.001
Region			
C spine	0.10(±0.59)	0.83(±1.49)	<0.001
T spine	0.24(±0.66)	1.02(±1.60)	0.001
L spine	0.10(±0.59)	0.73(±1.41)	0.002
Pelvis	0.26(±0.84)	0.91(±1.45)	<0.001
Long bones	0.10(±0.59)	0.65(±1.40)	0.005
Ribs/other	0.20(±0.41)	1.10(±1.66)	<0.001

Wilcoxon matched-pairs signed rank test

Summary/Conclusions: In patients with monoclonal plasma cell disorders, WB-DWI is more sensitive than FDG PET-CT for detection of bone lesions. Assessment of ADC value is an effective method for detecting bone lesions of monoclonal plasma cell disorders.

E1299

IMPROTANCE CD138 IMMUNOSTAINING FOR ASSESSMENT OF BONE MARROW PLASMA CELL INFILTRATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Estimation of plasma cell (PC) infiltrates in bone marrow (BM) is integral to the diagnosis and monitoring of patients with multiple myeloma (MM). Traditionally, quantification of BM PCs has been performed by differential counting of May-Giemsa-stained aspirate smears. Although previous studies consistently showed a higher % of PCs in BM biopsy sections compared to BM aspirate smears, BM biopsy with CD138 immunostaining has not been widely adopted in clinical practice.
Aims: This study was performed to clarify the importance of CD138 immnostainig for quantification of BM PC by comparing the different methods [BM aspirate clot, BM aspirate smear, and multicolor flow cytometry (FCM)] in patients with MM and monoclonal gammmopathy of undetermined significance (MGUS). In addition, to clarify the clinical relevance of quantifying BM PCs by CD138 immunostaining, 116 patients with monoclonal gammopathy who did not fulfill the criteria of smoldering MM (sMM) based on aspirate smears were reexamined for its diagnosis by BM clots stained with anti-CD138 monoclonal antibody.
Methods: A total of 150 samples of BM biopsy and smears from 100 patients with MGUS and MM, at diagnosis or following therapy were selected. Percentages of PC of aspirated marrow was quantified by May–Giemsa staining, multicolor flowcytometry (MFC), and CD138 immunostianing of BM clot section and biopsy section. PC identification by FCM required at least two markers, *i.e.*, CD38 and either CD45 or CD138. The percentages of PC were calculated relative to the total nucleated cell population. We also investigated the clinical relevance of CD138 immunostaining in the differentiation of MGUS and MM. BM slide sets from 116 untreated patients with M-protein level <3000mg/dL in

serum or 500mg/day in urine and without organ damage were re-assessed with based on the % of BM PCs by CD138 immunostaining.
Results: Median % of PC measured by BM biopsy, BM clot, BM smear, and FCM were 13.3%, 12.8%, 3.7%, and 2.4%, respectively. There were significant correlations between BM biopsy and the three other measurement methods, and BM clot showed the strongest correlation with BM biopsy (r=0.94). In correlation analysis, BM biopsy and BM clot showed significant and very strong correlations in both NCC ≥40×10^3/μL and NCC<40×10^3/μL (r=0.95 and r=0.91, respectively). Bland-Altman plots showed that BM biopsy agreed well with BM clot but not with BM smear or FCM. There was a significant fixed bias between BM biopsy *vs* BM smear and BM FCM with mean difference of 15.0% and 16.4%, respectively, indicating that both of these measurements significantly underestimated PCs compared to BM biopsy. However, no significant fixed bias between BM biopsy and BM clot was observed (mean difference: 1.1%). Proportional bias by regressing the differences in values on means of values with linear regression analysis showed significant proportional bias between BM biopsy and BM smear and FCM, but not between BM biopsy and BM clot. As CD138 immunostaining consistently yielded a higher percentage of BM PCs than aspirate smear, it is possible that a considerable portion of patients diagnosed as MGUS by aspirate smear alone could be reclassified as sMM by CD138 immunostaining of BM clot or biopsy. We retrospectively reanalyzed the 116 consecutive patients with monoclonal gammopathy and <3000 mg/dL or 500 mg/dL M-protein in serum or urine at our hospital. Among the 116 patients that met the above criteria, 13 patients (11%) were classified as sMM as PCs exceeded 10% on aspirate smears. Among the remaining 103 patients, 59 patients (51%) showed >10% PCs by CD138 immunostaining and was reclassified as sMM and the remaining 44 patients (38%) were still classified as MGUS.

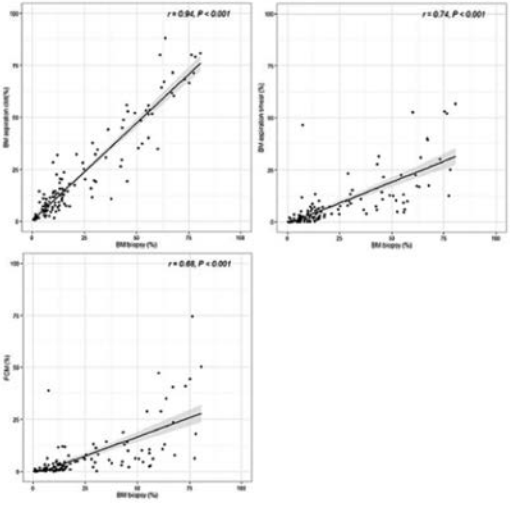

Figure 1.

Summary/Conclusions: Our data showed that considerable portion of sMM patients could be misclassified as MGUS by bone marrow smear alone and highlight the necessity of CD138 immunostaining of BM biopsy/clot specimens for correct diagnosis.

E1300

UPDATED RESULTS OF A SYSTEMATIC REVIEW OF THE RELATIVE EFFECTIVENESS OF TREATMENTS IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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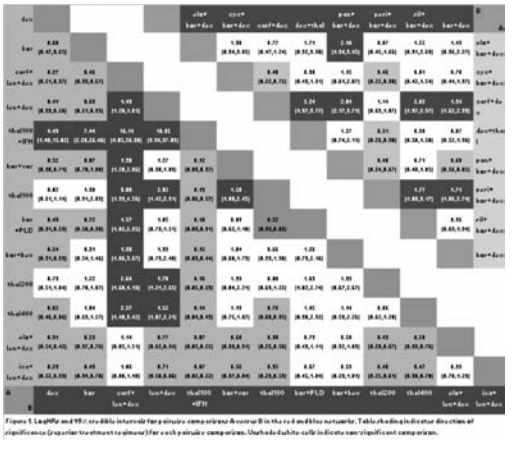
Background: Outcomes for multiple myeloma (MM) have improved with the introduction of novel therapies, and ongoing trials suggest positive outlooks for those with relapsed/refractory MM (rrMM). However, head-to-head clinical trials across treatment options are limited, and as such the evidence on relative effectiveness to inform best practice is lacking. A recent systematic review[1] attempted to address this via a mixed treatment comparison using data from randomised control trials (RCTs) published before 2015. A network meta-analysis (NMA) was developed to estimate relative treatment effectiveness in this review, and two evidence networks were compiled, but no comparisons could be drawn across networks.
Aims: This systematic review is an update to a previously published study and aims to estimate the relative effectiveness of treatments for rrMM using the most up to date information available.
Methods: The literature search for the previous analysis was conducted in August and repeated again in December 2014. The search was then repeated January 2016 to identify further RCTs which might have been published in the interim. Data was extracted from all RCTs that reported median duration of progression-free survival (PFS), overall survival (OS) or time to progression (TTP) as a primary or secondary treatment outcome. A Bayesian NMA using non-informative prior distributions and assuming fixed effects was fitted due to scarcity of data in the networks. The number of prior treatment lines among patients recruited into trials in rrMM varied (1-5) and trials conducted in heavily pre-treated and refractory patient populations (a median of 3 or more prior lines of therapy) were excluded from the presented analysis in an attempt to reduce some heterogeneity.
Results: 14 RCTs were included in the initial analysis[1]. These 14 trials assessed 16 different treatment regimens. From the updated review, 5 new RCTs which presented PFS or TTP data for 5 additional treatment regimens were identified to be included into the existing NMA. The combined trials form two separate evidence networks; all pairwise comparisons in each network were estimated in the analysis (figure 1). No relative effects between the two networks could be estimated. In the larger evidence network, novel agents combined with len+dex had superior outcomes with carf+len+dex being the most effective treatment, followed by ixa+len+dex, and then elo+len+dex. In the other, smaller evidence network, bort+dex+pan was the most effective treatment, followed by bort+dex+cyc.

Table 1.



Summary/Conclusions: The availability of treatments for rrMM is rapidly increasing. Within a year of conducting an initial systematic review the PFS and TTP outcomes of five additional RCTs of treatments for rrMM were released. Results of the larger network suggest that novel agents combined with len+dex have superior outcomes compared to other treatment options in the network. Less inferences can be drawn from the smaller network; of the newly included RCTs bort+dex+cyc ranked second in the network, whilst new treatment regimens elo+bor+dex and carf+dex ranked lower, and demonstrated no significant superiority relative to all other treatments in the network. The networks are not connected and as such it is impossible to make comparisons between the two regarding overall effectiveness of treatments. More RCTs comparing existing treatment regimens would greatly supplement these comparisons and allow for greater certainty. Nonetheless, the presented analysis is a significant step in evidence-based assessment for treatment in rrMM which indicates that novel agents combined with len+dex substantially improve treatment outcomes.

E1301

DURATION OF THERAPY IN U.S. PATIENTS TREATED FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) IN THE REAL-WORLD

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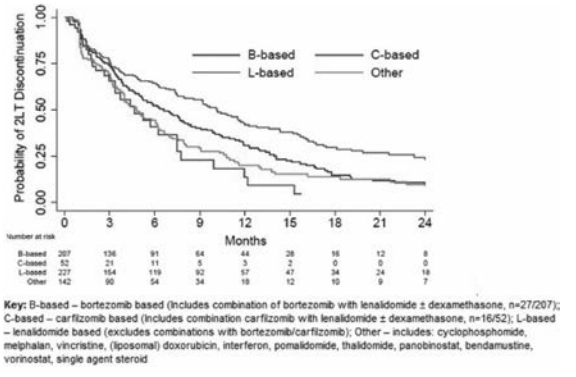
Background: Extended duration of therapy (DOT) is associated with better clinical outcomes in clinical trials in patients (pts) with multiple myeloma (MM)

(Palumbo 2015; Mateos 2015). Many of the factors that influence DOT are more prevalent outside of clinical trials, including older age, and concomitant comorbidities. Real-world data related to DOT among RRMM pts are limited.
Aims: This study aims to 1) describe DOT of second line (2LT) and third line (3LT) therapy in RRMM, and 2) evaluate the associations between 2LT DOT and age; comorbidities; and regimen type.
Methods: In this retrospective cohort study, newly diagnosed adult MM pts initiating first-line therapy (1LT) between 1/2008 and 12/2014 were followed in a large U.S. national electronic medical records (EMR) database to identify 2LT accordingly: 1) retreatment after a treatment gap of >3 months of 1LT, or 2) a switch to another drug combination after starting 1LT. 3LT began with a switch to another regimen after 2LT. Pts with salvage stem cell transplants were excluded. Kaplan-Meier analyses were performed to calculate DOT from start of both 2LT and 3LT. Observations were censored at time of loss to follow up/end of study period (6/30/2015). A two-tailed log-rank test was used to test for statistical significance between groups.
Results: Among 628 pts, 37.1% were ≥75 years of age; 51.0% were male; 66.4% had ≥1 of the following comorbidities of interest at initiation of 2LT: diabetes, renal insufficiency, thromboembolic disease, cardiovascular disease, peripheral neuropathy. In 2LT, pts were most commonly treated with lenalidomide (L)-based regimens (36.2%) without bortezomib (B), followed by B-based therapies (33.0%, with/without L). Carfilzomib (C)-based treatments were least common (8.3%, with/without L). The remaining regimens (Other) comprised of MM-therapies without L, B, or C (22.6%). Overall, the median DOT in 2LT and 3LT was 6.9 months (mos) (95% CI: 5.9, 7.7) and 5.5 mos (95% CI: 4.0, 6.2), respectively. DOT in 2LT was similar for those <75 *vs* ≥75 years, median: 6.3 mos (95% CI: 5.2, 7.6) and 7.4 mos (95% CI: 5.9, 9.2), *P*>0.05, respectively (Table). Pts with baseline comorbidities of interest were significantly more likely to have a shorter DOT in 2LT, median: 6.2 mos (95% CI: 5.0, 7.4) compared to pts without, median: 9.2 mos (95% CI: 7.0, 11.9), *P*=0.02. In 2LT, the drug composition of the regimen was significantly associated with DOT (*P*<0.0001). L-based therapies had the longest DOT (median: 10.1 mos; 95% CI: 7.9, 11.9) compared to B-based (median: 6.6 mos; 95% CI: 5.1, 8.0), C-based (median: 4.6 mos; 95% CI: 3.0, 7.5) and Other regimens (median: 4.8 mos; 95% CI: 3.9, 6.2) (Figure).

Table 1. DOT of 2LT by age and comorbidity.

		Treatment Duration (in months)				
		Mean	SE	Median	Lower 95% CI	Upper 95% CI
2nd Line	*Overall*	11.71	0.68	6.90	5.90	7.67
By Age	*Age <75*	11.68	0.81	6.27	5.17	7.57
	Age ≥75	11.22	0.99	7.43	5.90	9.20
By Comorbidity	*Present*	11.07	0.83	6.17	4.97	7.40
	Absent	12.82	1.07	9.23	6.97	11.90

Key: CI – confidence interval; SE – standard error



Key: B-based – bortezomib based (includes combination of bortezomib with lenalidomide ± dexamethasone, n=27/207); C-based – carfilzomib based (includes combination carfilzomib with lenalidomide ± dexamethasone, n=16/52); L-based – lenalidomide based (excludes combinations with bortezomib/carfilzomib); Other – includes: cyclophosphomide, melphalan, vincristine, (liposomal) doxorubicin, interferon, pomalidomide, thalidomide, panobinostat, bendamustine, vorinostat, single agent steroid

Figure 1. Treatment duration by regimen type in 2LT.

Summary/Conclusions: DOT wanes with subsequent lines of therapy. Baseline comorbidities and regimen type in 2LT are significantly associated with DOT. The longest median DOT was observed with L-based therapies and the shortest DOT with C-based therapies. Tailored therapy choices that account for pts' baseline comorbidities may mitigate the observed variation in DOT.

References

1. Palumbo et al. JCO 2015;33:3459-66;
2. Mateos et al. Am J Hematol.2015;90:314-9.

E1302

THE SERUM HEAVY/LIGHT CHAIN IMMUNOASSAY SERVES AS AN ADDITIONAL VALUABLE TOOL FOR SENSITIVE PARAPROTEIN ASSESSMENT, RISK STRATIFICATION AND DISEASE MONITORING IN PATIENTS WITH MONOCLONAL GAMMOPATHY

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Background: A novel polyclonal immunoassay specific for the different light chain types of intact immunoglobulins (Ig) enables measurement of changes in the production of clone specific Ig and of the non-involved polyclonal Ig of the same isotype. This serum heavy/light chain immunoassay (HLC) was developed to recognize and separately quantify light chain types of each intact Ig class. Prior studies have demonstrated that immunofixation (IFE) and HLC-assay are complementary methods, and that HLC-values and -ratios serve as additional parameters for evaluation of response and early relapse recognition in patients with monoclonal gammopathies.
Aims: We aimed to a) investigate the HLC-assay compared to standard Multiple Myeloma (MM)-diagnostics in consecutive MM, smoldering MM (SMM) and Monoclonal gammopathy of undetermined significance (MGUS) patients, b) assess HLC results in MM *vs* MGUS and SMM patients, c) define a possible HLC cut-off for high-risk SMM patients and d) decipher the role of the HLC-assay for response, stage groups, progression free (PFS) and overall survival (OS) in MM.
Methods: We investigated the HLC [Hevylite™]-assay and compared its results to standard diagnostics, such as the capillary zone electrophoresis (CZE) and serum free light chain (SFLC)-assay: SFLC and HLC (IgGκ/IgGλ, IgAκ/IgAλ, IgMκ/IgMλ) measurements were performed using polyclonal antisera Freelite™ and Hevylite™ assays. Standard diagnostics for MGUS, MM and Waldenström's Macroglobulinemia (WM) patients also included IFE and bone marrow aspiration/biopsies as well as stage (Durie and Salmon and International Staging System [ISS]) and EBMT response assessment.
Results: We assessed 146 consecutive patients with abnormal M-spike via CZE: 57 had IgGκ-MM, 24 IgGλ-MM, 8 IgAκ-MM, 7 IgAλ-MM, 5 SMM, 5 light-chain-only MM, 28 MGUS and 12 had IgM WM. HLC-values and -ratios significantly correlated with the respective Ig values, isotype-matched SFLCs, M-gradient, ISS and remission status. Of note, with measurement of HLCs, up to 33% more pathological serum values were detected than via SFLC-assay alone. Moreover, due to pathological HLC-values, 57% of patients with MGUS were reclassified as SMM or MM, suggesting that the assay may assist in recognition of true MGUS, SMM *vs* symptomatic MM patients. Furthermore, patients with WM-M-spikes - the latter often difficult to quantify - revealed a clearly abnormal median IgMκ/λ ratio of 231 (normal range: 1.0-2.4). PFS in patients with extreme (<0.5/>50) HLC-values *vs* those without (0.5-50) was significantly impaired.
Summary/Conclusions: The HLC-assay is of particular value to monitor slow or indolent disease progression. Moreover, in WM, it can be a helpful additional technique to quantify the amount of monoclonal Ig clone and to more reliably decipher MGUS from SMM/MM patients.

E1303

ASSESSMENT OF FRAILTY IN A MULTIPLE MYELOMA (MM) SERIES

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Background: An increasing number of articles overflow literature concerning the importance of frailty assessment in elderly MM patients in treatment decisions making. Nevertheless, little is known about frailty assessment influence/importance in younger MM patients.
Aims: To assess frailty and investigate its impact in MM patients of any age.
Methods: We studied 409 MM patients, diagnosed and followed –up in our department. Median age was 69 years (31-90) while 55% were males. Twenty-eight percent, 25% and 47% were Durie-Salmon staged 1, 2 and 3 respectively, while 32%, 26% and 42% were ISS staged I, II and III respectively. Twenty-seven percent were asymptomatic (Smoldering MM). MM type was IgG in 66%, IgA in 22% and light-chain in 11% of the population, while 4 patients (1%) were biclonal. Frailty score was estimated following the Geriatric Assessment formula that involves Renal score, Katz andAkpom's basic activities of daily living (BADL) scale, Lawton and Brody's instrumental scale (IADL), and the Charlson Comorbidity Index (CCI). Statistical analysis was performed conventionally with SPSS v22.0 software.
Results: Of the 409 patients 24% were fit (frailty score 0), 39% were unfit (frailty score 1) and the rest were frail (14%, 19%, 3% ans 1% with frailty scores 2, 3,4,5 respectively). Frailty score was correlated to OS in the whole cohort (p<0,0001), being more significant in the symptomatic (p<0, 0001) than in the asymptomatic group (p=0,01). In the symptomatic patients, all the parameters examined were significantly correlated to OS [IADL (p<0,0001), BADL (p<0,0001), Renal score (p<0,0001), Performance status (PS) (p<0,0001), ISS (p=0,01), abnormal Ca (p=0,006) and abnormal LDH (p=0,007)]. In the Multiple regression analysis, only ISS (p=0,009), PS (p<0,0001) and level of fitness (p=0,018) preserved their prognostic value. However, in the patient group >70 years, parameters proven statistically important were GFR (p<0,0001) and CCI (p=0,04), while, in the age group >75 years, only CCI was correlated to OS (p=0,002). Finally frailty parameters proved to be more powerful in younger patients (<65 years), for whom GFR (p<0,0001), CCI (p=0,001), IADL (p=0,009) and level of fitness (p=0,002) were correlated to OS.
Summary/Conclusions: Intriguingly, frailty score better predicted OS in young MM patients than in elderly ones.

E1304

OVERALL RESPONSE RATE OF PATIENTS WITH REFRACTORY MULTIPLE MYELOMA TREATED WITH POMALIDOMIDE AND LOW DOSE DEXAMETHASONE AFTER LENALIDOMIDE FAILURE: INTERIM RESULTS OF THE POSEIDON STUDY

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Background: The management of patients with primary refractory or relapsed and refractory multiple myeloma (RRMM) who failed lenalidomide and bortezomib treatment poses a clinical challenge. Prognosis for these patients is poor. The pivotal MM-003 trial demonstrated a benefit of the oral regimen pomalidomide (POM) in combination with low dose dexamethasone (LoDEX) for RRMM patients who had failed lenalidomide and bortezomib treatment. With a median follow-up of 10 months, POM+LoDEX showed an overall response rate (ORR; ≥partial response [PR]) of 31% *vs* 10% (p<0.001), a significantly longer progression-free survival (PFS) and a significant improvement in overall survival (OS) compared to high-dose DEX. Efficacy of POM+LoDEX was consistent across various age groups and tolerability was unaffected by age.
Aims: POSEIDON is a non-interventional, prospective, multicenter study designed to further evaluate effectiveness (ORR, PFS, OS) and safety of POM+LoDEX in routine clinical practice. It is carried out in 55 private practices and outpatient clinics in Germany.
Methods: Transplant-ineligible patients with RRMM who have received at least two prior lines of therapy including both lenalidomide and bortezomib, and who have demonstrated disease progression or were refractory to their last line of treatment could be enrolled. Patients received POM+LoDEX according to SmPC and were treated until disease progression or the development of unacceptable toxicity. At enrollment, patients were prospectively assigned to two subgroups with regard to lenalidomide in the preceding treatment line (subgroup A) or no lenalidomide in the preceding treatment line (subgroup B). The interim analysis was based on the modified intention-to-treat population including all patients who received at least one dose of POM.
Results: At data cut-off for this interim analysis (December 1st, 2015), 138 patients have been enrolled; 126 patients were evaluable with a median follow-up of 6.9 months. 66 and 60 patients were assigned to subgroup A and subgroup B, respectively. Median age was 74 years (range 46-87) with 49% and 43% of patients >75 years of age in subgroups A and B, respectively. 59% were male. The median time from diagnosis was 4.8 years (range 0.4-18.2). Median number of prior treatment lines was n=3 (range 2-9), with n=2 in subgroup A (range 2-9), and n=4 in subgroup B, respectively (range 2-9). For the 99 patients (55 patients in subgroup A, 44 patients in subgroup B) who received POM+LoDEX and had at least one response evaluation documented at data cut-off, the ORR was 26% in subgroup A and 52% in subgroup B, respectively. Additionally, 13% (subgroup A) and 9% (subgroup B) of these patients achieved a minor response. The most common ≥grade 3 adverse events were leukopenia (10%) and anemia (7%), respiratory tract infections (10%) and vascular disorders (3%).
Summary/Conclusions: The results of this interim analysis confirm the beneficial effectiveness and safety of POM+LoDEX in heavily pretreated RRMM patients in a real-life setting. Even lenalidomide and bortezomib refractory patients with lenalidomide in the preceding treatment line and heavily pretreated patients >75 years of age do benefit from POM+LoDEX treatment. The interim data of the non-interventional POSEIDON study are encouraging and highlight the potential clinical value of POM+LoDEX in these poor prognosis patients.

E1305

IXAZOMIB PLUS LENALIDOMIDE-DEXAMETHASONE (IRD) *VS* PLACEBO-RD IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): CHINA CONTINUATION OF TOURMALINE-MM1

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Background: The global, randomized, double-blind, placebo-controlled, phase 3 TOURMALINE-MM1 study (NCT01564537) demonstrated 35% improvement in PFS (HR 0.74, p=0.012) with IRd *vs* placebo-Rd in pts with RRMM (Moreau et al, ASH 2015).
Aims: This continuation study assessed the efficacy and safety of IRd *vs* placebo-Rd in pts with RRMM in China as a separate regional expansion of the global study.
Methods: Eligibility criteria and study design were per the global study, except high-risk cytogenetic and patient-reported outcome endpoints were not assessed. The primary endpoint was PFS, assessed by the same independent review committee (IRC) used in the global study. Pts were analyzed separately from the global study. Sample size was not based on a formal statistical hypothesis.
Results: 115 pts were randomized and treated (57 IRd, 58 placebo-Rd). Compared to the global study, pts had more advanced disease (63% ISS stage II/III *vs* 46% in the global study), were more heavily pretreated (60% *vs* 41% had 2 or 3 prior therapies), had more frequently received prior thalidomide (84% *vs* 45%), and more frequently had refractory MM (43% *vs* 11%). At data cut-off (12 July 2015; median follow-up 8.0 *vs* 7.8 mos for IRd *vs* placebo-Rd), PFS was significantly improved with IRd *vs* placebo-Rd: median PFS 6.7 *vs* 4.0 mos; HR 0.598; 95% CI 0.367–0.972; p=0.035. This benefit was seen across most prespecified subgroups including those with ISS stage I/II at screening, pts who had received 2–3 prior therapies, or prior proteasome inhibitor/IMiD compound therapy. A prespecified sensitivity analysis of PFS according to the EMA censoring rules was consistent with the primary analysis: median PFS 5.8 mos *vs* 4.0 mos; HR 0.543; 95% CI, 0.340–0.869; p=0.009. TTP was also significantly improved with IRd *vs* placebo-Rd: median TTP 7.3 *vs* 4.1 mos; HR=0.583; p=0.032. OS data were not yet mature; 6 (11%) IRd and 16 (28%) placebo-Rd pts have died. The study remains blinded and is ongoing with a final analysis for mature OS data planned. Overall response rate was 56% *vs* 31% (odds ratio=2.84, p=0.007); the ≥VGPR rate was 25% *vs* 12%. Among responders, the median duration of response was 7.4 mos with IRd *vs* 5.6 mos with placebo-Rd. Pts had received a median of 7 and 5 cycles of IRd and placebo-Rd, and 59% and 41% of pts remained on treatment. The median relative dose intensities of all drugs were high (>95%), similar between arms, and consistent with the global study. The overall safety profile was similar in both treatment groups: 56% *vs* 62% had grade ≥3 AEs, 23% *vs* 26% had SAEs, 5% *vs* 12% discontinued treatment due to AEs, and 4% *vs* 5% died on treatment. Common grade ≥3 AEs with IRd *vs* placebo-Rd included thrombocytopenia (23% *vs* 13%), neutropenia (23% *vs* 19%), anemia (12% *vs* 26%), and pneumonia (16% *vs* 10%). Rash was seen in 18% *vs* 19% of pts (no grade ≥3 events); 7% of pts in each group had peripheral neuropathy (no grade ≥3 events). There was no evidence of cardiac or renal toxicity with the addition of ixazomib, and no new primary malignancies. Based on an observed higher incidence of herpes zoster reactivation in the IRd treatment group (18%) *vs* placebo-Rd (0%), the IDMC recommended an antiviral prophylaxis requirement for the entire study population still on treatment; the China continuation protocol was amended accordingly.
Summary/Conclusions: In Chinese pts with RRMM, IRd was associated with a significant improvement in PFS, with limited additional toxicity, demonstrating the consistent relative benefit of IRd *vs* placebo-Rd in this distinct Chinese population and the global study.

E1306

PROSPECTIVE EVALUATION OF PROGNOSTIC AND PATHOPHYSIOLOGIC IMPLICATIONS OF BLOOD PRESSURE MONITORING AND BARORECEPTOR REFLEX SENSITIVITY (BRS) IN PATIENTS WITH AL AMYLOIDOSIS

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Background: cardiac dysfunction is the major determinant of prognosis in patients with AL amyloidosis. Elevated levels of cardiac biomarkers (troponin and of NTproBNP) identify patients at high risk (stage 3 per Mayo stage) but low blood pressure (BP) is independently associated with poor prognosis especially in patients with Mayo stage-3 disease (Wechalekar et al Blood 2013). Cardiac output and autonomic nervous system (ANS) are major regulators of BP and both are affected in AL amyloidosis.
Aims: to prospectively evaluate the prognostic role of BP by using standard and 24 hour BP measurements and evaluate the importance of the deregulation of ANS in AL amyloidosis by assessing Baroreceptor reflex sensitivity (BRS).
Methods: Newly diagnosed patients, with biopsy confirmed AL amyloidosis, were prospectively evaluated. All patients underwent standard office BP office BP measurements (three BP measurements taken at a 1-min intervals, at sitting position, averaged to obtain a single systolic and diastolic office BP value), 24h ambulatory BP monitoring and a simultaneously electrocardiographic and

non-invasive BP monitoring (Finometer), under standardized conditions for 15 min. Ambulatory BP monitoring was performed on a usual working day. BP recordings were obtained automatically at 15-min intervals throughout the 24h period. BRS was expressed as the alpha-index (a-index), which was estimated by means of power spectral analysis.
Results: we evaluated 68 consecutive patients (median age 65, range 40-84 years, 50% males). Heart was involved in 65%, kidneys in 70% and nervous system in 23%, while 10% were Mayo stage-1, 60% stage-2 and 30% stage-3 and 14% had NTproBNP≥8500 ng/L. Median eGFR was 63 ml/min/1.73 m2. Primary treatment was bortezomib-based (VD, VCD or BMDex) in 80% and MDex in 20%. Median office systolic BP (SBP) was 118 mmHg and median diastolic BP (DBP) was 72 mmHg. SBP was lower in stage 2 *vs* stage 1 and stage 3 *vs* either stage 2 or stage 1 patients (p=0.026) but there was no difference in the DBP between groups. The median of mean 24h ambulatory SBP was 112.5 mmHg and for DBP was 69.5 mmHg. Advanced Mayo stage was associated with lower mean 24h SBP (p=0.048). None of the patients with Mayo stage 1, 13% of those with stage-2 and 33% of those with stage-3 had office SBP<100 mmHg. For 24h mean SBP, none with stage 1, 23% with stage 2 and 42% with stage 3 had mean ambulatory SBP<100 mmHg. Lower levels of SBP were associated with inferior survival: either office SBP<100 mmHg (6 months *vs* not reached) (p<0.001) or mean 24h SBP<90 mmHg (2 months *vs* not reached) (p<0.001) were associated with poor survival and early death. Further analysis of 24h ambulatory BP recordings indicated higher BP fluctuations among patients with less severe or no heart involvement, while more often patients with Mayo stage 3 had higher nighttime *vs* daytime SBP values than stage 2 or stage 1 patients. Median a-index was 2.85 (range 0.4-5.85) but it was lower in patients with advanced cardiac involvement and was also lower in patients who had nerve involvement (median 1.6 *vs* 3.3, p=0.016), reflecting both cardiac and nerve involvement by AL amyloidosis. In addition, low a-index was associated with early death.
Summary/Conclusions: Low BP, either measured in a sitting position as in standard office visits or by means of 24h ambulatory measurement, is associated with poor prognosis in patients with AL amyloidosis. Impaired BRS is associated with advanced cardiac and nerve involvement and risk of early death.

E1307

PREVALENCE AND PROGNOSTIC IMPACT OF OLIGOCLONAL BANDS IN PATIENTS WITH AL AMYLOIDOSIS

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Background: The emergence of oligoclonal bands (OB) in patients with multiple myeloma (MM) achieving a complete response (CR) is a well-recognized event after autologous stem-cell transplantation (ASCT) and the use of novel agents. The presence of OB is considered a benign phenomenon, associated with favorable outcome due to a deeper humoral immune reconstitution. However, the frequency of clinical impact and outcomes of the emergence OB in patients with AL amyloidosis have never been described.
Aims: The objective of this study is to determine the prevalence, natural history and prognostic impact of OB in patients with AL amyloidosis who achieved at least a partial response (PR) either after upfront ASCT or after conventional treatment in patients ineligible for transplant at Hospital Clínic of Barcelona.
Methods: We reviewed the clinical records of patients with AL amyloidosis from January 1996 to September 2015. Fifty-six patients (27F/29M; median age at diagnosis 59 years; range 39 to 82) with PR or better after different induction regimens and/or ASCT were found. Initial baseline demographics, clinical and laboratory data, treatment and follow-up were collected. Median follow-up was 3.6 years. An OB was defined by the presence of a serum and/or urine immunofixation monoclonal spike different either in heavy and/or light chain component from the original amyloid protein.
Results: Fifty-three percent (30) of the patients were transplant ineligible while 46.4% (26) that had received an ASCT. The distribution of the immunoglobulin isotypes at diagnosis was as follows: light chains only (53.6%), IgG (35.7%), IgA (5.4%), IgM (3.6%), and one patient biclonal (1.8%). The light chain type was lambda in 43 patients (76.8%). Response achieved was: CR in 50% of patients, very good partial response (VGPR) 48.2% and PR in one patient. Three-year survival rate was 84%. We observed OB in 59% of the patients. The median number of bands per patient was 2 (range 1 to 5). The most frequents isotypes were IgG-kappa (31%) and IgG-lambda (22%). The oligoclonal phenomenon was more prevalent in patients in CR compared to the other degrees of response (82.1% *vs.* 35.7%; p=0.0001). There were no statistical differences between the emergence of OB and type of treatment received (50% chemotherapy alone *vs.* 69.2% ASCT; p=0.145) or isotype of involved light chains (46.2% for kappa *vs.* 62.8% for lambda; p=0.29). However, the presence of OB was more frequent in the group of patients who received induction chemotherapy (mainly bortezomib-based; 75%) before ASCT as compared to those who received upfront ASCT (91.7% *vs.* 50%; p=0.02). Duration of oligoclonality for more than one year was more prevalent in patients who underwent ASCT than in those ineligible for transplant (42.3% *vs.* 16.7%; p=0.034). Regarding its prognostic value, the presence of oligoclonality lasting for more than one year resulted in a significantly longer overall survival (OS) as compared to patients without OB or with only a transient phenomena (p=0.012), (Figure 1).

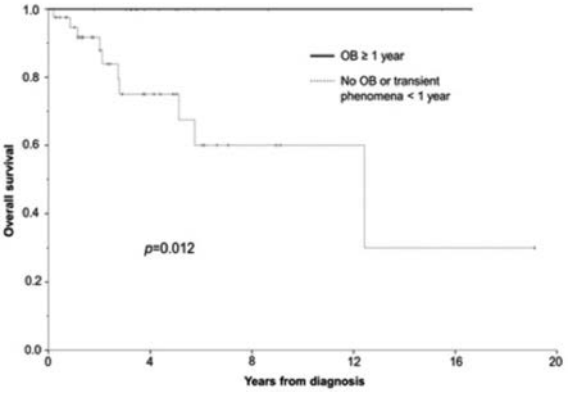

Figure 1. Overall survival according to the presence of oligoclonality lasting for more than one year compared with those without oligoclonal bands (OB) or with only a transient phenomena.

Summary/Conclusions: This is the first report describing the prevalence of OB in patients with AL amyloidosis after first-line therapy, which is even higher than that observed in patients with MM. The oligoclonal humoral phenomenon was more prevalent in patients who achieved CR as well as in those who received induction therapy prior to ASCT. Patients with oligoclonal humoral response lasting for more than one year had a significantly longer OS than those with shorter duration, likely reflecting a more robust humoral immune response.

E1308

COMPARISON OF PERIPHERAL BLOOD STEM CELL (PBSC) MOBILIZATION WITH G-CSF ALONE *VS* G-CSF WITH CYCLOPHOSPHAMIDE POST VCD INDUCTION IN MULTIPLE MYELOMA

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Background: Bortezomib, cyclophosphamide and dexamethasone (VCD) combination is often used as induction therapy in transplant eligible patients with multiple myeloma (MM). However, the optimal PBSC mobilization strategy in this context is not established.
Aims: To review the efficacy of G-CSF alone (G-alone) *versus* G-CSF and cyclophosphamide (G-cyclo) PBSC mobilization strategies in MM patients who had only received VCD induction prior to autograft.
Methods: Retrospective review of PBSC mobilization strategies in patients from six apheresis centres from November 2012 to March 2015 after receiving VCD induction. Extended data were collected from a single centre to January 2016. Prior radiotherapy was not an exclusion criterion. Successful mobilization was defined as achieving physician-determined target PBSC yield, which in some older patients was ≥2x10^6/kg but in most patients was ≥4x10^6/kg to allow for two autografts (≥90% of both groups). Plerixafor was used in some centres as a rescue strategy if PB CD34 ≤10x10^6/L on the day of planned collection or PBSC yield was <1x10^6/kg after first apheresis. Univariate analysis was performed using chi-squared test for categorical data and Mann-Whitney-U test for continuous variables.
Results: Table 1 summarizes the results of 192 patients. Baseline characteristics and cumulative exposure to bortezomib and cyclophosphamide were similar in both groups. G-alone strategy consisted of G-CSF at 10μg/kg/day (n=120) or 20μg/kg /day (n=16), administered for a median of 5 days. G-cyclo comprised of intravenous cyclophosphamide (1g/m^2 (n=1), 1.5g/m^2 (n=14), 2g/m^2 (n=25), 3g/m^2 (n=4), 4g/m^2 (n=12)) with either G-CSF 5-10μg/kg/day for a median of 4 days (n=35) or single-dose 6mg pegfilgrastim (n=21). Overall, the rates of successful mobilization and the proportion of patients achieving a total PBSC yield of ≥4x10^6/kg were comparable between G-alone and G-cyclo with no significant difference in the median number of aphereses to reach this target. However the G-cyclo group had a significantly higher median PBSC yield per apheresis and a higher proportion of patients attaining a PBSC yield of ≥4x10^6/kg with ≤2 apheresis without rescue plerixafor. Ten (18%) G-cyclo patients had unplanned hospitalization, of which 8 had febrile neutropenia. In the G-alone group, age ≤60 and G-CSF doses of 20μg/d were significantly

associated with a higher median PBSC yield. In the G-cyclo group, higher cyclophosphamide doses for mobilization (3-4g/m^2 *vs* 1-2g/m^2) resulted in a significantly higher PBSC yield. Cumulative bortezomib doses, pre-mobilization platelet count and prior radiotherapy did not significantly impact on PBSC yield in either group.

Table 1. PBSC mobilization outcomes with G-alone *vs* G-cyclo.

	G-alone (n=136)	G-cyclo (n=56)	P-value
Median age (years; range)	61 (36-72)	59 (35-75)	0.28
Male	80(59%)	35(63%)	0.63
Median cumulative bortezomib dose (g/m^2)	21 (14-31)	21 (14-29)	0.19
Median cumulative cyclophosphamide dose (g/m^2)	3.6 (0.9-7.2) (116 patients evaluable)	3.7 (1.6-5.5) (48 patients evaluable)	0.88
Prior radiotherapy exposure	20 (15%)	8 (14%)	0.94
Target yield ≥2x10^6/kg ≥4x10^6/kg	 11 (8%) 125 (92%)	 3 (6%) 53 (94%)	0.51
Outcomes of collection Successful • required plerixafor rescue Target yield not reached but deemed sufficient* Failed	 118 (87%) 9 (6%) 8 (6%) 10 (7%)	 51 (91%) 1 (2%) 2 (4%) 3 (5%)	0.70
No. of patients who achieved PB CD34+ of ≥18x10^6/L**	114 (84%)	50 (89%)	0.33
Median total PBSC yield (10^6/kg; range)	5.4 (1-18)	8.4 (1-41)	<0.01
Median D1 apheresis PBSC yield	3.6 (0.6-13)	4.3 (0.8-28)	<0.01
No. of patients who achieved PBSC yield ≥4x10^6/kg with ≤2 apheresis, without plerixafor use***	89/125 (71%)	45/53 (85%)	0.03
No. of apheresis required to achieve CD34+ ≥4x10^6/kg***			
1	60 (48%)	34 (64%)	0.25
2	37 (30%)	12 (23%)	
3	12 (10%)	3 (6%)	
Failed to achieve PBSC yield ≥4x10^6/kg	16 (13%)	4 (8%)	0.07

*Deemed sufficient by clinician to proceed to transplant without further collections (median CD34+ yield: 3.5x10^6/kg; range: 1.4-3.9)
**The threshold above which apheresis were generally performed
***Excluding 11 and 3 patients respectively with target yield <4x10^6/kg

Summary/Conclusions: Despite a significantly higher PBSC yield with G-cyclo mobilization, the G-alone strategy is a reasonable first approach to mobilizing stem cells in the majority of patients receiving VCD induction.

E1309

ROLE OF PET/CT IN PROGNOSTICATING POST-TRANSPLANT OUTCOMES BASED ON A NEW SCORING SYSTEM: RESULTS OF PIPET-M TRIAL

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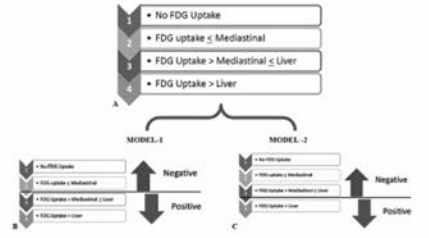
Background: Multiple myeloma (MM) is a heterogeneous disorder with varied responses to transplantation. ^{18}F-FDG-PET/CT has a role in prognosticating post-transplant outcomes, but the PET positivity has been reported in non-homogenous fashion leading to difficulty in interpretation and inter-trial comparisons. We defined a new staging system for PET/CT reporting in MM and validated it for predicting post-transplant outcomes, first in the world literature.
Aims: Aim: To assess the role of 18 FDG-PET/CT scan in prognostication of MM autologous stem cell transplantation (ASCT) candidates.
Objectives: To correlate pre-transplant FDG-PET/CT avidity with (a) Post transplant outcomes (b) Biochemical Markers (c) MRD as assessed by the flow cytometry (FCM).
Methods: It is a prospective systematic protocol based single centre observational study. As part of CTRI registered PIPET-M Trial, all autologous transplant MM patients underwent 18F-FDG-PET/CT during pre-transplant workup. The conditioning and treatment protocols were not modified based on PET/CT findings. PET positivity was defined as per a newly defined PIPET-M staging in lines with modified Deauville's staging based on target lesion SUVmax (Fig 1A). This staging was further classified into two models for defining PET positivity based on background physiological FDG uptake as an internal control (In model 1 - mediastinal SUVmax and in model 2 - Liver SUVmax was taken as physiological SUVmax) (Fig 1B, C). Progression was defined using revised IMWG criteria. Using SPSS ver.16.0, OS/PFS was assessed by Kaplan-Meier/Cox-regression survival analysis.
Results: A total of 43 patients underwent pre-transplant PET/CT evaluation. The staging was validated in this cohort. Further using model-1, PET negative patients had better PFS (88.2 *vs* 69.2%, p-0.65) and better OS (94.1 *vs* 65.4%, p-0.01). The hazard of all-cause mortality was 22.445 times higher for PET positive individuals (p-0.02). Using model-2, only statistically significant result was delayed neutrophil engraftment in PET-positive patients (11.69 *vs* 10.5d, p-0.02). There was good agreement between two models, but model-1 had significantly higher sensitivity and slightly lower specificity as compared to model-2 for predicting survival. Inter-observer variability was 0.02 for PIPET staging. Also, there was good agreement between the PIPET model -1 and MRD by FCM with Cohen's Kappa - 0.599,(p<0.0005). Levels of β2M and LDH were higher in the patients with PET positivity defined based on PIPET model 1, (Abnormal β2M-χ2 (1, 42)-0.842, p-0.017, Abnormal LDH - χ2 (1, 42)-0.762, p-0.037). There was no correlation of PET positivity with pre-transplant SPEP/UPEP/SIFE/SFLCA total of 43 patients underwent pre-transplant PET/CT evaluation. The staging was validated in this cohort. Further using model-1, PET negative patients had better PFS (88.2 *vs* 69.2%, p-0.65) and better OS (94.1 *vs* 65.4%, p-0.01). The hazard of all-cause mortality was 22.445 times higher for PET positive individuals (p-0.02). Using model-2, only statistically significant result was delayed neutrophil engraftment in PET-positive patients (11.69 *vs* 10.5d, p-0.02). There was good agreement between two models, but model-1 had significantly higher sensitivity and slightly lower specificity as compared to model-2 for predicting survival. Inter-observer variability was 0.02 for PIPET staging. Also, there was good agreement between the PIPET model -1 and MRD by FCM with Cohen's Kappa - 0.599, (p<0.0005). Levels of β2M and LDH were higher in the patients with PET positivity defined based on PIPET model 1, (p-0.017 and 0.037 respectively). There was no correlation of PET positivity with pre-transplant SPEP/UPEP/SIFE/SFLC.

Table 1.



Summary/Conclusions: Utilizing PET stages based on this newly proposed staging model is clinically relevant to predict post-transplant OS.

E1310

THE EFFECT OF LEVEL OF RESPONSE TO TREATMENT ON ASSOCIATED COSTS AND HEALTHCARE RESOURCE UTILIZATION: A RETROSPECTIVE CHART REVIEW STUDY IN PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA

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Background: An increase in incidence rates, treatment lines and use of novel agents, with improved efficacy, has impacted costs associated with treating multiple myeloma (MM). Real-world data are needed to accurately assess resource use and costs.
Aims: To evaluate how response to treatment affects associated costs and healthcare resource utilization (HRU) in the UK, Italy and France.
Methods: Physicians (n=189) retrospectively completed case report forms for patients (pts) with symptomatic MM who, in the 3 months before study start, had experienced disease progression after receiving specific treatment regimens (those most commonly prescribed) or received best supportive care (BSC) and died. Pt characteristics, treatment outcomes, costs and HRU were documented from beginning of last completed treatment line onwards. Pts had received: 2nd-line bortezomib (bor) or lenalidomide (len); 3rd-line bor, len, pomalidomide (pom) or bendamustine (ben); 4th-line bor, len, pom or ben; any regimen at 5th line or above; or BSC only. Costs of medication and resources were estimated based on standardized schedules and national databases.
Results: Data were collected for 1156 pts on active treatment and 126 pts receiving BSC. In all countries, mean monthly costs were lower in pts achieving deeper responses. In the UK, mean monthly costs from start of treatment line until progression were significantly lower (p≤0.05) for pts achieving a very good partial response or complete response (≥VGPR) than for pts achieving a partial response (PR) and for pts with stable or progressive disease (SD+PD) (Table 1). These costs were also numerically lower in Italy. In France, costs for pts with ≥VGPR were lower than for pts with PR and significantly lower (p≤0.05) than for pts with SD+PD (Table 1). Medications accounted for 92% of mean monthly costs in the UK, 88% in Italy and 83% in France. In all countries, when excluding cost of medication on a monthly basis, achievement of ≥VGPR was still associated with lower costs. In terms of healthcare resource use, the proportion of pts hospitalized increased with lesser response to treatment: in the UK, 11%, 16% and 28% of pts with ≥VGPR, PR and SD+PD, respectively, were hospitalized, and similar trends were observed in Italy (13%, 26% and 28%) and France (16%, 24% and 27%). In terms of overall costs for each country, the achievement of ≥VGPR was associated with higher mean total costs than the achievement of ≤PR. Total costs were significantly higher for ≥VGPR compared with costs for all pts combined (UK €71 414 *vs* €57 717; Italy €51 359 *vs* €34 496; France €45 831 *vs* €37 009; all p≤0.05). When medication cost was excluded, there was still a trend for pts with ≥VGPR to incur higher costs. These findings are linked to duration of treatment (DoT) and length of treatment-free interval (TFI). Mean DoT for pts with ≥VGPR was 10 months in the UK and 12 months in Italy and France. In all countries this was significantly longer than for pts with PR and twice as long as that for pts with SD+PD. Sim-

ilarly, in all countries, TFI until progression for pts with ≥VGPR was significantly longer than for pts with other response levels.

Table 1.

Multiple myeloma treatment costs by level of response in France, Italy and the UK

			Costs from beginning of line to progression* (€)		Costs from beginning of line to progression* excluding medication costs (€)	
Country	Level of response	N	Total Mean (SD)	Per month Mean (SD)	Total Mean (SD)	Per month Mean (SD)
France	Total	502	37 009 (31 530)	3904 (3272)	5193 (6006)	653 (1088)
	≥VGPR	163	45 831 (28 687)	3131 (2383)	5923 (6622)	405 (615)
	PR	153	41 758 (36 398)	3975 (3261)	5620 (6234)	627 (1003)
	SD+PD	177	25 855 (25 883)	4586 (3818)	4029 (4969)	886 (1380)
Italy	Total	393	34 496 (40 305)	2963 (2239)	3203 (3230)	349 (544)
	≥VGPR	140	51 359 (53 015)	2814 (1996)	3503 (3464)	205 (234)
	PR	120	32 437 (33 108)	2910 (2369)	3180 (3175)	310 (355)
	SD+PD	118	18 847 (18 320)	3007 (2247)	3058 (3073)	556 (837)
UK	Total	387	57 717 (52 432)	5504 (5189)	3467 (4378)	461 (720)
	≥VGPR	114	71 414 (67 140)	4342 (4466)	4082 (3605)	271 (310)
	PR	124	64 404 (49 374)	6031 (5735)	2931 (3984)	410 (469)
	SD+PD	138	32 518 (26 518)	5924 (5004)	3521 (5232)	736 (1108)

*BSC patients who did not progress before death were not included in analysis

BSC, best supportive care; PR, partial response; SD, standard deviation; SD+PD, stable disease and progressive disease; ≥VGPR, very good partial response or complete response.

Summary/Conclusions: Most of the cost of managing pts with relapsed MM is medication-related. Although overall costs are higher due to longer treatment duration, mean monthly costs for pts achieving a deeper response to treatment were lower compared with those pts who had a lesser response. These findings may be explained by hospitalization rates, as pts who exhibited deeper and better responses to treatment were hospitalized less frequently than those with a lesser response.

E1311

SEVERE DECREASE OF BONE MATERIAL STRENGTH IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE PATIENTS DETECTED BY OSTEOPROBE® MICROINDENTATION METHOD

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Background: Monoclonal gammopathy of undetermined significance (MGUS) affects over 3% of adults over 50 years old. Previous studies show that MGUS patients experience a significant bone fracture risk increase and that MGUS prevalence is higher in osteoporotic patients (1). The microindentation for *in vivo* measurement of bone tissue method (2,3) has shown to detect decrease in Bone Material Strength (BMS) even at early stages. These findings correlate with bone fragility and risk of fracture independently of bone mineral density (BMD) (4) measured by bone densitometry (DXA).
Aims: To analyze the BMS by Osteoprobe® microindentation method and the BMD by densitometry in MGUS patients and compare with an age control matched group.
Methods: We have included 22 patients diagnosed with MGUS. An informed consent was obtained and a general laboratory workup was undertaken. BMD measurement at the lumbar spine and hip using DXA (Hologic QDR 4500 SR™, Hologic, Bedford, MA) was performed. BMS determination by inserting a probe assembly through the skin covering the anterior midtibia and applying 20 indentations with an Osteoprobe® (Active Life Scientific Sta Barbara CA) device (3) was done. The results were compared with controls adjusted by sex and age.
Results: MGUS patients present low values of BMS (68.3±5) compared with control group values (83±4, p<0.001). No significant difference was observed in BMD between controls and patients (0.977 *vs* 0.929 p :0,288 at lumbar spine; 0.783 *vs* 0.730 p: 0,179 at femoral neck; 0.902 *vs* 0.898 p: 0,157at total femur).
Summary/Conclusions: These results suggest that microindentation method directly measures bone mechanical properties at the tissue level and can detect significant decrease of BMS in MGUS patients, which correlates with a greater risk of fracture. This could be useful to identify MGUS patients that would benefit from early antiresorptive treatment despite a normal DXA.

References

1. Drake MT Unveiling skeletal fragility in patients diagnosed with MGUS: no longer a condition of undetermined significance? J Bone Miner Res. 2014:2529-33.
2. Daniel Bridges, Connor Randall, and Paul K. Hansma. A new device for performing reference point indentation without a reference probe. Review of Scientific Instruments.2012;83,044301
3. Randall C, Bridges D, Guerri R, Nogues X, Puig L, Torres E, Mellibovsky L, Hoffseth K, Stalbaum T, Srikanth A, Weaver JC, Rosen S, Barnard H, Brimer D, Proctor A, Candy J, Saldana C, Chandrasekar S, Lescun T, Nielson CM, Orwoll E, Herthel D, Kopeikin H, Yang HT, Farr JN, McCready L, Khosla S, Diez-Perez A, Hansma PK. Applications of a New Handheld Reference Point Indentation Instrument Measuring Bone Material Strength. J Med Device. 2013:410051-56;
4. Mellibovsky L, Prieto-Alhambra D, Mellibovsky F, Güerri-Fernández R, Nogués X, Randall C, Hansma PK, Díez-Perez A. Bone Tissue Properties Measurement by Reference Point Indentation in Glucocorticoid-Induced Osteoporosis.J Bone Miner Res. 2015;30:1651-6.

E1312

REAL-WORLD PRESCRIBING PATTERNS IN U.S. MULTIPLE MYELOMA (MM) PATIENTS REFRACTORY TO LENALIDOMIDE IN THE FRONT-LINE

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Background: The introduction of novel agents into the treatment paradigm of MM has improved outcomes; however, MM remains incurable with patients requiring subsequent lines of therapy post-relapse. Patients with relapsed MM who are refractory to bortezomib and refractory to or ineligible for lenalidomide have a median overall and event-free survival of 9 and 5 months, respectively (Kumar 2012). There is a scarcity of data regarding real-world treatment patterns and clinical outcomes in previous lenalidomide-exposed patients with relapsed / refractory MM (RRMM).
Aims: This study aims to describe the patient characteristics and treatment patterns among MM patients treated in the real-world who relapsed or were refractory to lenalidomide in first-line treatment (1LT) within the U.S.
Methods: This was a retrospective cohort study using a large national EMR database. Newly diagnosed MM patients initiating a lenalidomide-based 1LT between 1/2008 and 12/2014 were followed 1 year prior to and up to 7 years after diagnosis. Maintenance therapy, if given, was included within 1LT. Patients were required to be ≥18 years of age with evidence of starting second-line therapy (2LT), which was identified accordingly: 1) retreatment after a treatment gap of >3 months of 1LT, or 2) a switch to another drug combination after starting 1LT. All patients were followed until death/loss to follow up or the end of study period (6/30/2015). Based on the International Myeloma Workshop Consensus Panel 1 and using initiation of 2LT as a surrogate marker for non-response or progressive disease, lenalidomide-refractory patients, for the purpose of this study, were defined as those who initiated 2LT within 60 days of 1LT discontinuation (Rajkumar 2011). Relapsed patients were those with a >60 day gap between end of 1LT and 2LT initiation.
Results: There were 279 RRMM patients who received lenalidomide in 1LT (n=89 (31.9%) refractory; n=190 (68.1%) relapsed); overall, mean age was 69.9 years (standard deviation (SD): 9.9) and 49.5% were male. More patients in the refractory population had known high cytogenetic risk disease (presence of any: del[17p], t[4:14], t[14:16]) than in the relapsed population (16.9% *vs* 3.2%), and refractory patients were more likely to have renal impairment (52.8% *vs* 37.9%), anemia (65.2% *vs* 45.3%), hypercalcemia (15.7% *vs* 3.7%) and bone disease (30.3% *vs* 23.7%) at initiation of 2LT than relapsed patients. Refractory patients were more likely than relapsed patients to receive only PI-based therapy (57.3% *vs* 19.5%) and less likely to receive only immunomodulatory drug (immunomod)-based therapy (16.9% *vs* 62.6%) in 2LT (*P*<0.001 for both; chi-square test). In addition, use of ≥3 drug-therapy in 2LT was higher in the refractory population than in the relapsed population (40.5% *vs* 8.4%) (*P*<0.001; chi-square test).

Table 1. 2LT by Lenalidomide refractory *vs* relapsed status.

Type of Regimen Utilized for 2LT, %	Lenalidomide Refractory N=89	Lenalidomide Relapsed N=190
PI-based	57.3	19.5
Bortezomib + steroid	27.0	13.2
Bortezomib + cytotoxic ± steroid	21.3	3.2
Carfilzomib ± steroid	7.9	2.6
Carfilzomib + cytotoxic ± steroid	1.1	0.5
Immunomod-based	16.9	62.6
Other immunomod (other than lenalidomide) + steroid	11.2	5.3
Lenalidomide + panobinostat or cytotoxic ± steroid	4.5	0.5
Lenalidomide ± steroid	1.1	56.3
Other immunomod (other than lenalidomide) + cytotoxic + steroid	0	0.5
PI + Immunomodulatory Drug-based	14.6	3.7
Bortezomib + lenalidomide ± steroid	6.7	2.1
Carfilzomib + lenalidomide ± steroid	4.5	0.5
Carfilzomib + other immunomod ± steroid	2.2	0
Bortezomib + other immunomod + steroid	1.1	1.1
Cytotoxic ± steroid	11.2	5.3
Steroid monotherapy	0	8.9

Key: Cytotoxic – includes any of the following: cyclophosphamide, melphalan, (liposomal) doxorubicin, and bendamustine; Immunomod – immunomodulator (lenalidomide, thalidomide, or pomalidomide); PI – proteasome inhibitor (bortezomib or carfilzomib)

Summary/Conclusions: RRMM patients refractory to lenalidomide in 1LT presented with a higher disease-related symptom burden at start of 2LT, were treated more aggressively in 2LT, and were more likely to switch to PI-based therapy than those defined as relapsed. One limitation to this real-world study is that patients may have stopped 1LT due to factors other than nonresponse or progressive disease.

References
1. Kumar et al. *Leukemia*. 2012 January; 26(1): 149-157;
2. Rajkumar et al. *Blood*. 2011;117(18):4691-4695.

E1313

DETERMINATION OF PROTHROMBOTIC PHENOTYPE IN PATIENTS WITH MULTIPLE MYELOMA ON IMMUNOMODULATORY THERAPY BY THE CALIBRATED AUTOMATED THROMBOGRAM

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Background: Patients with multiple myeloma (MM) have an increased risk of venous thromboembolism (VTE), particularly when immunomodulatory therapy is used. It is of great importance to choose proper thrombosis prophylaxis in these patients. Resistance to activated protein C (APCR) is proposed to be one of the pathogenic mechanism underlying thrombotic complications in MM patients. The Calibrated Automated Thrombography (CAT) determines the action of both procoagulant and anticoagulant factors and is now considered to reflect patient's phenotype better than traditional coagulation tests. When thrombomodulin (TM) is used CAT becomes sensitive to all disorders of the protein C (PC) system. Clinical utility of CAT in MM patients needs further elucidation.
Aims: Aim of our study was to evaluate the prevalence of APCR and determine prothrombotic phenotype in MM patients by an integrated approach with CAT made in parallel with and without TM.
Methods: The study involved 63 patients (M/F 23/40, mean age 62,0±14,0 yr) with MM and 30 age and sex matched controls. Patients and controls gave informed consent. We divided patients in 3 groups: 30 patients with IgA associated MM, 20 patients with IgG associated MM and 13 patients with MM associated with IgG on lenalidomide treatment with prophylaxis of VTE by aspirin. CAT was done according to Hemker et al. at 5 pM TF and 4 μM phospholipids in platelet poor plasma (PPP) with PPP plasma+/-TM reagent. The procedure was carried out on an automated fluorometer (Fluoroscan Ascent, Thermolab system, Finland). Thrombin generation curves were calculated using the Thrombinoscope software (Thrombinoscope BV, The Netherlands). Lupus anticoagulants (LA), activities of FVIII, protein C and S and antithrombin were also measured. STATISTICA 6.1 was used.
Results: Importantly endogenous thrombin potential (ETP) and peak thrombin (PT) in MM patients on lenalidomide were within the normal range. Increased ETP were defined as values above 95th percentile measured in controls (i.e. >2114 nMmin in the absence of TM and >1433 nMmin in the presence of TM). Normal ranges of ETP and PT inhibition calculated in controls were 22-62% and 15-51% respectively, means values of ETP and PT inhibition were 45% and 33% respectively. Abnormalities of inhibition were more pronounced in IgA associated MM compared to IgG group without lenalidomide (41% *vs* 64% for ETP and 25% *vs* 53% for PT). The most significant changes in the protein C system activities were found in IgG group on lenalidomide (mean inhibition of ETP 36% and 24% of PT). Values below 22% for ETP inhibition and/or 15% for PT inhibition (i.e. APRC) were found in 3 (23%) of patients on lenalidomide. Though ETP and PT inhibition showed significant correlation with PC activity (R=0,51 and R=0,63 respectively), all these patients had protein C and S activities as well as FVIII activity within the normal range and no one demonstrated LA.
Summary/Conclusions: An integrated approach defining an individual's phenotype could help in identifying patients with higher risk for thrombotic event. We suggest APCR detected by CAT to be important marker of thrombotic risk in MM patients. Whether these patients would benefit from more active primary thromboprophylaxis need further elucidation.

E1314

IN MULTIPLE MYELOMA, T REGULATORY CELLS ARE SIGNIFICANTLY REDUCED AFTER TREATMENT WITH LENALIDOMIDE BUT NOT BORTEZOMIB AND CORRELATE WITH THE ACHIEVEMENT OF AT LEAST VERY GOOD PARTIAL RESPONSE

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Background: Immune dysfunction is an important feature of Multiple Myeloma (MM) and has been associated with reduced survival. Studies have shown that T-regulatory cells (Tregs) implicated in immune surveillance are expanded in tumors, including MM. Data regarding alterations of Tregs during therapy with novel agents (NA) *i.e.* bortezomib and lenalidomide, are limited.
Aims: Our aim was to explore possible alterations of Tregs and lymphocyte subpopulations (T4, T8, B, NK, NK-like), as well as changes in the levels of cytokines related to Tregs function and MM biology (IL-6, IL-2, IL-17, TGF-β) during treatment with NA and to seek for correlations with disease characteristics and response parameters.
Methods: We evaluated 29 patients with symptomatic MM at diagnosis or relapse (M/F: 15/14, median age: 61 years, range: 39-77) and 20 healthy volunteers (HV). Eleven patients received bortezomib-dexamethasone (BD) (group A) and 18 patients received lenalidomide-dexamethasone (Rd) (group B). The median number of previous treatment lines was 2(0-3). The detection of Tregs and lymphocyte subpopulations was performed in peripheral blood samples using flow cytometry analysis. The cytokines were measured in serum samples using the enzyme-linked immunosorbent assay ELISA. The statistical analysis was performed with the appropriate methods; $p<0.05$ was considered statistically significant.
Results: In group A, no significant alterations of Tregs%, lymphocyte subpopulations or cytokines were observed during treatment. In group B, there was a significant reduction of Tregs% ($p<0.001$) and this was more profound in those who achieved ≥vgPR ($p=0.04$). No alterations regarding T subpopulations or cytokines were observed during treatment with NA in either group of patients. Patients had significantly higher median Tregs% compared to HV ($p<0.001$). There were no significant correlations between disease characteristics and Tregs in either group of patients. In the cox regression analysis, Tregs% did not correlate with progression-free survival (PFS).
Summary/Conclusions: Herein, we have demonstrated that Tregs% were significantly reduced after treatment with Rd especially in patients with ≥vgPR, suggesting a possible relation of immune surveillance with quality of response. However, PFS was not affected in the current study. Bortezomib-based treatment had no impact on Tregs number or function. Patients with myeloma had higher Tregs% compared to HV, confirming the implication of immune impairment in the biology of this disease. No relation between Tregs and disease characteristics was observed in this study nor between Tregs and relative cytokines, indicating that immune mechanisms underlying MM remain unexplored.

E1315

THE IMPACT OF THE TYPE OF MYELOMA-DEFINING EVENT ON EARLY MORTALITY AND SURVIVAL

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Background: Multiple myeloma (MM) is a complex disease with several levels of heterogeneity. The impact of the clinical presentation of MM is probably underestimated in daily clinical practice. The relationship between the initial clinical presentation in MM and the outcome in terms of overall survival (OS) has been recently highlighted (Greenberg et al, 2014). In this study, patients were divided into four groups: renal failure, anemia, lytic bone disease and a mixed group. They showed that patients with isolated RF as myeloma-defining event (MDE) had the worst OS, whereas patients with bone disease had the best outcome. However, there are many other well-established MDE that can be further analyzed in this regard. On the other hand, little is known about the impact of MDE on early mortality.
Aims: The aim of this study is to assess the impact of the key MDE on the outcome of MM patients, in terms of early mortality and OS.
Methods: All newly diagnosed MM patients recorded in our population-based registry for which the MDE is known were analyzed. Patients were divided into nine groups according with the key MDE: bone pain, pathological fracture, anemia, constitutional syndrome (weight loss, decreased appetite, malaise), infection, other, tumor, renal failure and mixed group (a combination of pain, renal failure, anemia and other). Median OS was estimated in months by the Kaplan-Meier method. Log-rank test was used to compare OS curves. Early mortality was analyzed at two, six, and twelve months.
Results: The MDE was available in 398 patients. Bone disease is the most frequent MDE (49% isolated bone pain, 8% pathological fracture), followed by anemia (9.5%). A constitutional syndrome was present in 7.5%, the same percentage as the mixed group. Isolated renal failure was detected in 6.3%, infection in 5.3%, other in 4.5% and a tumor in 2.3%. Median OS for the whole cohort was 32.7 months (IC 95%, 25-40.4). There were statistically significant differences in OS between subgroups (p=0.004). The subgroup with the best OS was anemia (77.6 months) and the worst was constitutional syndrome (8.9 months). The mixed group and the renal failure group had 20.4 and 21.8 months,

respectively. In patients with bone disease, OS of those with isolated bone pain reach 40.4, but falls to 18 months for those with pathological fracture. Early mortality at two months was observed in 53 patients (13.5%), whereas 87 patients (22.7%), and 116 (31.9%) had mortality at six and twelve months, respectively. In relation to the group constitutional syndrome, 24.1, 40.7, and 60% died at 2, 6, and 12 months. For those with pathological fracture, the percentages were 12.9, 25.8 and 45.2%, respectively. The mixed group had 13.3, 28.6 and 44.4%. Patients with renal failure had 21.7, 34.8 and 36.4% mortality, respectively.
Summary/Conclusions: The importance of clinical evaluation at diagnosis in MM should be emphasized. Patients presenting with isolated anemia as the MDE have the best survival, whereas those with constitutional syndrome have the worst prognosis. Patients having constitutional syndrome, pathological fracture, renal failure or those in the mixed group as the key MDE have a poor outcome in terms of early mortality and OS. The potential association between the MDE and other prognostic factors should be analyzed in depth.

E1316

REVLIMID, BENDAMUSTINE AND PREDNISOLONE (RBP) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: FINAL RESULTS OF A PHASE II CLINICAL TRIAL; OSHO-#077

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Background: While the role of lenalidomide monotherapy in the treatment of relapsed/refractory patients with multiple myeloma (MM) is well established, combination therapies with lenalidomide are still under investigation in many phase 2/3 studies.
Aims: In the current study, a combination therapy of lenalinomide (Revlimid®), bendamustine and prednisolone (RBP) was tested in patients with relapsed or refractory MM.
Methods: In the previously completed phase 1 study RPB with a dose of 25 mg lenalidomide d 1-21 and 75 mg/m² bendamustine d 1-2 was well tolerated in patients with relapsed/refractory MM (Pönisch et al. 2013). The treatment was repeated every 28 days for a maximum of eight cycles. Thereafter, patients received a maintenance therapy with 10 mg lenalidomide over 10 cycles.
Results: 25 patients (19 patients of the phase 2 study and additional 6 patients from the highest dose level of the phase 1 study) were included in this analysis. Twenty two patients (88%) responded after at least two cycles of RBP with 1 sCR, 5 nCR, 8 VGPR and 8 PR. Due to increased haematological toxicity a dose reduction was required in subsequent therapy cycles in the most of all patients. 19 patients discontinued the treatment prematurely due to a stem cell transplantation (n=7), hematological toxicity (n=7), non hematological toxicity (n=3) or progress (n=2). The median progression-free and overall survival was 22 and 38 months, respectively.
Summary/Conclusions: RBP is a highly effective therapy for patients with relapsed or refractory MM. However, dose reduction is necessary in many patients because an increased haematological toxicity rate.

E1317

BETTER OUTCOME IN AL AMYLOIDOSIS (ALA) TREATED WITH MULTIPLE MYELOMA-ORIENTED REGIMENS COMPARED WITH CYCLOPHOSPHAMIDE/BORTEZOMIB/DEXAMETHASONE (CYBORD) IN A SINGLE CENTER EXPERIENCE

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Background: Treatment of AL amyloidosis (ALA) is based on anti-myeloma therapy but there is no standard. Treatment-related toxicity is deemed to be higher in pts with ALA compared with patients with multiple myeloma (MM). Recently, the combination of cyclophosphamide-bortezomib-dexamethasone (CyBorD) showed high rates of haematologic response, although the outcome of high risk patients remained poor.
Aims: We compared the outcome of patients treated at our center with CyBorD with patients receiving more intensive therapy, including MM-type chemotherapy regimens followed or not by high doses of melphalan (HD-Mel) and autologous stem cell transplantation (ASCT) or upfront HD-Mel with ASCT.
Methods: Of 50 ALA patients (median age 62 years) referred to our center, 48 of whom newly diagnosed between 2007 and 2015, 25 patients received CyBorD, and 5 of them (20%) subsequently underwent ASCT. By intention to treat, 18 patients (control group) received more intensive treatment (upfront ASCT: 6 (33%); conventional myeloma induction therapy (VAD, VTD, VMP): 12 (67%), followed by ASCT in 5 of them, 42%). Seven patients receiving less intensive therapy were excluded from analysis. Risk groups were identified as follows: cTnI >0.1ng/ml and/or ECOG PS ≥3: high risk; age ≤65 years with normal cTnI levels, ECOG PS<3 and eGFR >50ml/min: low risk. Intermediate risk patients were defined if not meeting criteria for high or low risk. MM diagnosis was made according to IMWG criteria. Haematological and organ response, overall survival (OS) and event free survival (EFS: time to 2nd line therapy or death) were analyzed.
Results: The proportion of high risk patients was 32% in the CyBorD and 28% in the control group. Median age was 62 and 61 years in the CyBorD group and in the control group, respectively. Concomitant MM was present in 52% CyBorD and in 94% control patients. Treatment results of CyBorD patients were in line with those recently reported (Palladini et al, Blood 2015). Compared to the control group, no difference was observed in terms of haematologic response. Overall response rate (ORR) and complete remission (CR) were 72% and 20% in the CyBorD group and 89% and 39% in the control group, respectively. However, organ response rate was significantly lower in the CyBorD group (32% *vs* 75%, p 0.01). Toxicity was manageable in both groups. After a median follow up of 33 months no significant difference was seen between CyBorD and control group in terms of EFS (48% *vs* 61% at 2 years, respectively; HR 1.5, 95% CI 0.66-3.41), whereas OS was significantly inferior in the CyBorD group (47.6% *vs* 80% at 4 years, p 0.027, HR 3.45, 95% CI 1.15-10.36, see Figure 1). Excluding high risk patients in both groups, OS resulted similar, although a trend toward better long term survival was seen in the control group.

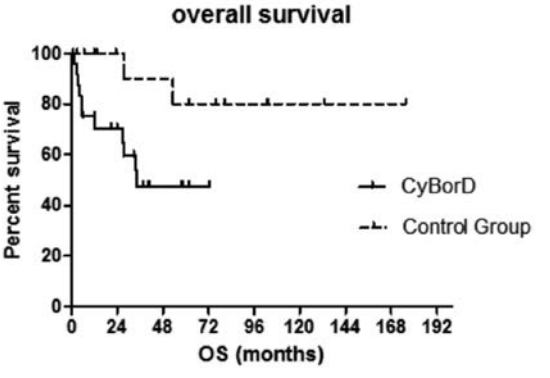

Figure 1. Overall Survival according to treatment: CyBorD *vs* MM-type chemotherapy (control group).

Summary/Conclusions: despite the high haematologic response rates, CyBorD regimen seems not to overcome the long term poor prognosis associated with organ damage in ALA. of high risk patients. Selected patients, even in the high-risk group may have a survival advantage with MM conventional treatment, including ASCT whenever possible.

E1318

PROGNOSTIC SIGNIFICANCE OF THE REVISED INTERNATIONAL STAGING SYSTEM IN MULTIPLE MYELOMA

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Background: New prognostic markers are currently in the focus of investigation in order to personalize treatment approach in multiple myeloma (MM).
Aims: The aim of this study was to analyze the prognostic significance of the newest Revised International Staging System (R-ISS) in MM patients ineligible for autologous stem cell transplantation (ASCT).
Methods: A total of 92 newly diagnosed MM patients (median age 67 years, range 35-80 years; 44 male/48 female) were analyzed in the study, with following distribution: IgG myeloma had 55 patients (59.8%), IgA 18 (19.6%), light chains 16 (17.4%), and IgD 2 patients (2.2%). According to the clinical stage (CS, Durie&Salmon), advanced III CS was found in 68 patients (73.9%), II in 18 (19.6%), and symptomatic I CS in 6 (6.5%) patients. The ISS score 1 had 24 (26.1%) patients, 28 (30.4%) ISS 2, and 40 patients (43.5%) had ISS 3. Elevated LDH level was present in 18/92 patients (19.6%). High risk chromosomal abnormalities (CA, iFISH): t(4; 14), del(17p), or t(14;16) were detected in 14 patients (15.2%). Renal impairment existed in 16 patients (17.4%). New R-ISS scoring system based on LDH level, ISS and CA defines three risk categories with the following distribution: RSS I 21 patients (22.8%), II 63 (68.5%), and III 8 patients (8.7%). Renal impairment existed in 16 patients (17.4%).

Thalidomide based combinations were applied in 77 patients (83.7%) while 15 patients (16.3%) were treated with bortezomib based combinations. The patients were ASCT ineligible due to the age, high comorbidity index, progressive disease or personal attitude.
Results: Overall treatment response (CR/VGPR/PR/MR, IMWG criteria) was achieved in 77 patients (83.7%). In the group treated with thalidomide based combinations, 65 patients (84.5%) achieved favourable treatment response, in comparison to the 12/15 patients treated with bortezomib based combinations. According to the R-ISS score, overall treatment response was achieved in 20/21 patients (95.2%) with R-ISS I; in 54/63 (85.7%) with R-ISS II; and in 3/8 (37.5%) with R-ISS III. There wasn't observed correlation between R-ISS and therapy response neither in the thalidomide treated group (p=0.47) nor in the bortezomib treated group (p=0.149). The median follow up of analyzed group was 27 months (range 4-42 months). The components of R-ISS score as: ISS, elevated LDH and the presence of high risk CA, was found to be the factors of prognostic significance on PFS (Log Rank=4.19, p=0.041; Log Rank=12.23, p=0.001; Log Rank=4.94, p=0.026, respectively) and OS (Log Rank=4.34, p=0.04; Log Rank=13.34, p=0.001; Log Rank=5.08, p=0.024, respectively). In analyzed group, age didn't have impact on the PFS or OS (p>0.05). The R-ISS was highly statistically relevant regarding PFS (Log Rank=10.79, p=0.005) and OS (Log Rank=14.41, p= 0.001). Similarly, in the group treated with thalidomide based combinations, the R-ISS retained prognostic significance on OS (Log Rank=17.54, p=0.0001) and PFS (Log Rank=12.56, p=0.002). Cox regression analysis confirmed that R-ISS was the most important prognostic parameter that influenced PFS in thalidomide treated patients (HR, 5.1; 95% CI, 2.02-12.85; p=0.001).
Summary/Conclusions: Incorporating the findings of molecular genetics along with parameters of disease activity, the R-ISS score represents currently most sensitive prognostic tool in multiple myeloma with consequent implications to personalized treatment approach.

E1319

SHORT SYSTEMIC TREATMENT CONCURRENT WITH INTENSITY-MODULATED RADIOTHERAPY BY TOMOTHERAPY (IMRT) SHOWS IMPROVEMENT OF PROGRESSION-FREE SURVIVAL IN SOLITARY PLASMACYTOMA OF THE BONE (SPB)

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Background: SPB is a rare plasma cell dyscrasia characterized by a single bone tumor without evidence of multiple myeloma. Standard treatment is currently local 40Gy radiotherapy. Local control is achieved in more than 90% of patients. However, approximately half of the patients develop further disease progression, be it a new plasmacytoma or multiple myeloma.
Aims: To determine the impact and tolerance of short-term treatment with Immunomodulatory Drugs (ImIDs) or proteasome inhibitors (PI) in association with 40 Gy IMRT on the progression-free survival of SPB patients.
Methods: We retrospectively reviewed the medical and dosimetric records of all patients treated for solitary plasmacytoma of the bone at a single institution between 2004 and 2013. All patients had histologically proven plasmacytoma of the bone, without bone marrow involvement. Initial evaluation included clinical examination, biological tests and radiological exams. The absence of additional lesions was assessed by standard radiographies associated with a more sensitive technique such as total body MRI, CT-scan and/or PET-CT. Radiotherapy modalities were reviewed. Additional treatment was assessed. Toxicity was evaluated weekly during radiotherapy, then at 6 weeks, 4 months and 1 year using CTCAEv3.0. Local control of the lesion was defined as negativation of PET-CT. Progression was defined as the incidence of a new plasmacytoma or a multiple myeloma. Statistical analysis: Progression-Free Survival (PFS) was defined as the period from the beginning of radiotherapy to the first documentation of progression or death. Patients alive without progression were censored at the date of last known contact. PFS was estimated using the Kaplan Meier method and compared with the log-rank test.
Results: 28 patients were analyzed. Median age was 53.5y. Median follow-up was 52.2 months (7-99). 15 patients received the standard treatment, a local 40Gy radiotherapy (group 1); 13 patients (46%) received treatment with IMiDs or PIs, in addition to the 40 Gy local radiotherapy delivered by IMRT (group 2). In group 2, 9 patients (69%) received 4 cycles of lenalidomide+dexamethasone, of whom 2 also received bortezomib (15%); 3 received bortezomib+dex for 4 cycles (23%); and 1 received thalidomide for 12 months (7.5%) There was no significant difference between subgroups by conventional prognostic factors such as age, localization, M-Spike and ECOG. Toxicity was mostly hematological and manageable with standard approaches. 1 patient developed profound venous thrombosis on lenalidomide treatment in spite of aspirin prophylaxis. All patients achieved local control. Overall survival was more than 95% at 4 years. 1 patient died of disease progression (MM) in group 1. In group 1, 5 patients (33%) developed SPB and 5 patients developed multiple myeloma (33%) during follow-up, *versus* 1 SPB (7.5%) and 1 MM (7.5%) in group 2. PFS rates at 48 months were 50.3% CI95% [29.8 ;84.6] in group 1 and 80.0% CI95% [58.7 ;100] in group 2. PFS was significantly different between groups (p=0.032).
Summary/Conclusions: An additional treatment with IMiDs or PI to 40 Gy IMRT is well tolerated and allows prolonged progression-free survival for solitary plasmacytoma of the bone patients. These results need to be confirmed in a larger, standardized clinical trial.

E1320

POLYCLONAL SERUM IGM AS A PROGNOSTIC MARKER IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: Normal serum IgM plasma levels have been reported as an independent marker of good prognosis in patients with multiple myeloma (MM) but their role has been poorly investigated
Aims: We aimed to evaluate the role of polyclonal serum IgM levels as a surrogate marker of normal residual bone marrow function in newly diagnosed patients with multiple myeloma (NDMM).
Methods: Data were collected from the enrolling Hematology Centers. Patients with NDMM were evaluated for polyclonal IgM levels and their correlation with clinical and laboratory parameters of MM, at diagnosis and up to one year after standard treatment. Baseline data were retrospectively analyzed between January 2012 and June 2015; analyzed parameters were taken at the following time-points: diagnosis, after standard treatment and 1 year after diagnosis. The one-year evaluation was performed at least 3 months after the last autologous stem cell transplant (ASCT) and at least 2 months after the end of a first-line treatment in patients not eligible for ASCT. A dedicated database was created. Statistical analysis was performed with Medcalc 12.5.00. Wilcoxon test for paired samples was applied when indicated Patients were divided into two groups based on the serum IgM levels at diagnosis (IgM<0.3 g/L and IgM ≥0.3 g/L,respectively).

Table 1.

Characteristics at diagnosis	IgM<0,3 g/L (n=120)	IgM≥0,3 g/L (n=50)	p-Value
Male (%)	59,1%	40,9%	0,4901[ns]
Age (yr)	65,3 (38,8-83,8)	64,4 (47,3-91,2)	0,9681[ns]
Subtype of myeloma (%)			0,2009[ns]
IgG	63/110 (57%)	33/45 (73%)	
IgA	25/110 (23%)	5/45 (11%)	
Light chain	19/110 (17%)	5/45 (11%)	
Non secretory	3/110 (3%)	2/45 (4%)	
ISS (%)			0,0024[**]
I	7/74 (9%)	10/26 (38%)	
II	29/74 (39%)	5/26 (19%)	
III	38/74 (51%)	11/26 (42%)	
ZI-S stage			0,1588[ns]
I-II	25/90 (28%)	15/35 (43%)	
III	65/90 (72%)	20/35 (57%)	
β2-microglobulin (mg/L)	5,3 (2,0-20,2)	3,8 (1,7-14,7)	0,0012[*]
Albumin (g/dL)	3,8 (2,0-4,8)	3,4 (2,4-4,2)	0,8321[ns]
Hemoglobin (g/dL)	9,90 (3,0-13,7)	12,0 (7,4-13,9)	<0,0001[**]
Platelets (x10⁹/L)	193,0 (44-416)	252,0 (5-426)	0,0001[**]
Calcium (mg/dL)	9,1 (7,0-13,8)	9,4 (7,2-14,8)	0,2529[ns]
Creatinine (mg/dL)	1,09 (0,35-11,07)	0,90 (0,50-7,81)	0,0209[*]
ClCr (mL/min)	69,90 (3,0-152,0)	84,0 (3,20-143,0)	0,0906[ns]
IgM suppression	at diagnosis (n=83)	at first revaluation (n=83)	
IgM (g/L)	0,16 (0,0-1,81)	0,29 (0,0-1,90	0,0023[**]

*Results are expressed as mean and SD.

Results: At diagnosis, we evaluated 170 NDMM (not IgM myeloma) patients and divided them into two groups based on polyclonal serum IgM levels (Table). M protein type frequency was similar between both groups. We found that patients with polyclonal serum IgM level higher than 0,3g/L had higher hemoglobin levels (median 12,0 *vs* 9,90g/dL; p<0,0001) and platelet count (252x10^9L^{-1} *vs* 193x10^9L^{-1}; p=0,0001), lower β2-microglobulin (3,6 *vs* 5,3mg/L; p=0,0012), lower creatinine levels (0,90mg/dl *vs* 1,09mg/dl; p=0,0209), higher creatinine clearance (ClCr, 84mL/min *vs* 69,90ml/min; p=0,0906), lower frequency of ISS stage III (42% *vs* 51%; p=0,0024). No significant statistical correlation was found between IgM plasma levels and LDH, serum calcium and albumin. At regression analysis, a strong correlation between polyclonal serum IgM level and serum beta-2 microglobulin (negative; p=0,034) and hemoglobin (positive; p<0,001) was observed.At the first revaluation, data on 98 patients were available. When comparing serum IgM polyclonal levels between diagnosis and first re-evaluation, IgM at diagnosis resulted lower (0,16g/L *vs* 0,29g/L; p=0,0023).Overall

survival was similar between both groups (82,8 *vs* 82 months; p=0,9118) and it was unrelated to the type of treatment administered.
Summary/Conclusions: In newly diagnosed MM, normal IgM levels can be evaluated as a surrogate marker of good bone marrow residual function in NDMM. After treatment, IgM do not statistically correlate with any of the clinical and laboratory prognostic markers examined.

E1321

NEW IMWG COMPARED TO CRAB CRITERIA TO PROPERLY DEFINE START-TIME THERAPY IN MULTIPLE MYELOMA: A RETROSPECTIVE SINGLE-CENTER ANALYSIS OF 180 PATIENTS

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Background: The diagnosis of Multiple Myeloma (MM) traditionally requires the evidence of signs of end-organ damage like hypercalcemia, renal failure, anemia and osteolytic bone lesions,usually referred by the acronym "CRAB". The International Myeloma Working Group (IMWG) recently updated the criteria for the diagnosis of MM,including biomarkers that are considered myeloma defining events (MDEs).They are: clonal bone marrow plasma cells >60%,serum free light chain (FLC) ratio >100 and the presence of more than one focal lesion on magnetic resonance imaging (MRI).
Aims: In this paper we'd like to share our experience regarding the recent IMWG criteria in a group of 180 newly diagnosed MM patients, to discuss strengths as well as caveats and uncertainties. Ninety were young and treated with transplant procedure and ninety were old (more than 65-year-old)and were treated with different regimens,including new drugs.
Methods: We performed a retrospective analysis of these MM cases with MDEs diagnosed from 1999 to 2014 in our Department, comparing traditional CRAB *versus* recent IMWG criteria. We retrospectively looked for the new defining characteristics published by Rajkumar (Lancet Oncol 2014) during the disease course before both the development of CRAB events and beginning of treatment.

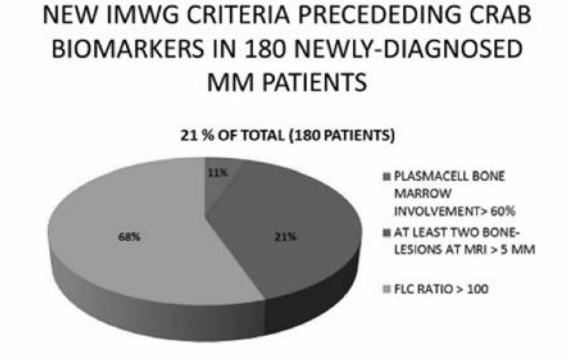

Figure 1.

Results: We found the occurrence of IMWG new criteria before clear manifestation of the CRAB markers in thirty-eight patients (21% of total). In particular the majority of this group has shown a FLC ratio>100 (14% of total),5% presented injuries detected by MRI and only 2% had a bone marrow plasma cells involvement >60%. Most of them (92%) were followed by development of CRAB features after a medium time of seven months (range 3-15) and progressed to active MM requiring therapy in less than one year. In the category of patients carrying the new criteria 71% were old (median age 70 years, range 53-89), 29% were young. Of them 56% had IgG MM, 26% IgA MM, 18% micromolecular MM; prevalent light chain was kappa (65%). The majority of these cases (76%) had a previous history of MGUS and smouldering MM. Only two patients among 180 started therapy not according to classic criteria, but on new IMWG biomarkers, associated with deterioration of clinical condition and increase in monoclonal component. However three young patients presented new IMWG events associated with a stable monoclonal protein, good clinical status. Clinical judgement was most important in this setting, as we decided not to start therapy and to maintain close follow-up. In our view it is likely that earlier treatment of these patients would not be beneficial,but might instead result in greater toxicity. On the other hand patients without IMWG 2014 preceding CRAB criteria,often identify a high-risk subgroup with a severe and faster biological behavior. The largest part of them indeed (57%) presented markers of aggressive biological profile like IgD paraprotein, extramedullary involvement or unfavorable cytogenetic abnormalities such as deletion of chromosome 17. Probably a well defined temporal difference between development of new IMWG and the old CRAB characteristics could be present only for intermediate-risk MM patients with a slower kinetics of disease. In most high-risk patients probably new IMWG and CRAB markers present all together simultaneously.
Summary/Conclusions: Our single-center study confirms the pivotal role of new IMWG criteria to optimize management of patients with smouldering MM and to antedate start-time of effective treatment before end-organ damage, expecially for elderly patients.In our experience the most powerful biomarker of progression is FLC ratio>100. Neverthless particular caution should be used in youth patients to avoid too much toxicity of a earlier intervention,that can also lead to selection of fitter clone and accelerated progression.Finally these new biomarkers should be critically validated prospectively in future clinical trials to allow an early treatment in patients without CRAB features with high-risk markers.

E1322

SPECIAL IMMUNE SIGNATURE OF MM PATIENTS IN LONG TERM COMPLETE RESPONSE (LTCR) WITH NEGATIVE MINIMAL RESIDUAL DISEASE (MRD) AFTER AUTOLOGOUS TRANSPLANT (ASCT): A SINGLE CENTRE EXPERIENCE

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Background: The immune profile in MM patients in long term complete remission (LTCR) could be relevant in MM control and minimal residual disease (MRD) monitoring.
Aims: To evaluate this hypothesis, we have analyzed the immune profile and the MRD status of a group of LTCR-MM patients after autologous transplant (ASCT) and we have compared the results with a healthy control group.
Methods: 13 MM patients, median 60 y (46-71) in LTCR for >7 years, median 9 y (7-19) after ASCT were included. Only 2 patients had received thalidomide as induction. The rest only chemotherapy schemes. No other patient had received new drugs neither maintenance. 7 patients received conditioning with HD Melphalan and 6 with Busulphan+Melphalan. Only 2 patient showed high hisk iFISH, one with del(17p) and other with t(4;14). Control arm included 15 age-matched healthy blood donors (HD). Peripheral blood was immunophenotyped by multiparametric flow cytometry with 8 colours and $CD4^+$ and $CD8^+$ T-cells, B lymphocytes and NK cells were quantified. Serum immunoglobulins, free light chains and heavy/light chains (HLC) were quantified. MRD was evaluate with bone marrow multiparametric cytometry study (8 colours). Response was evaluated by IMWG Criteria.
Results: The percentage of $CD4^+$ T-cells in the LTCR group was significantly lower than in HD (p=0.0002), whereas no differences were observed in the proportion of total $CD8^+$ T-cells (p=0.3046). Conversely, the relative values of NK-cells (CD3-CD56+CD16+) were increased in the LTCR group (p=0,0514). The percentage of total B-cells ($CD19^+CD20^+$) in the patients was within normal range and no significant differences were found when compared to HD. Remarkably, the normalization of HLC measurements confirmed a complete reconstitution of the immune paresis in these patients, and a negative MRD by multiparametric flow cytometry ratified its usefulness at demonstrating a sustained complete response.
Summary/Conclusions: LTCR-MM MRD neg patients could express a particular immune signature which could reflect a "high quality" immune reconstitution in terms of anti-tumor immunological surveillance and the recovery of the humoral immunity. More studies are needed to confirm these results, with patients treated with new drugs combinations including immunomodulatory ones.

E1323

DURATION OF SECOND LINE TREATMENT AND SURVIVAL IN MULTIPLE MYELOMA

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Background: Evidence from post-hoc analyses of clinical trials supports the paradigm of extended treatment leading to an overall survival (OS) benefit in relapsed/refractory multiple myeloma (RRMM).
Aims: This observational study evaluated whether longer 2nd line treatment (SLT) duration is associated with improved OS among patients with RRMM.
Methods: In this retrospective cohort study of a US national EMR database, newly diagnosed adults with MM initiating 1st line therapy (FLT) and SLT between 1/2008 and 12/2014 were followed through earliest of either 1 year or death/loss to follow up/end of study period (12/31/2014) after start of SLT. FLT began with the first claim for MM-directed therapy following diagnosis (dx). SLT was identified by: 1) retreatment with follow-up regimen with gap of >3 months

after end of FLT, or 2) switch to another drug combination after FLT. A logistic marginal structural model was used to estimate the causal effect of SLT duration on 1-year OS probability, adjusting for time-dependent confounders and baseline covariates. Immortal time bias was addressed by estimating the effect of SLT duration on outcome within each monthly interval from start of SLT.
Results: Among 340 patients, mean age was 70 years (standard deviation (SD), 10); 51% were male; 9% had known high risk MM; 46% received immunomodulatory (IMID-), 35% received proteasome inhibitor (PI-), and 6% received PI+IMID-based SLT. Median duration of SLT was 6.87 months (95% CI: 6.17, 8.40). The odds of 1-year OS were 1.17 times higher for each additional month of SLT (OR: 1.17 [95% CI: 1.10, 1.25] *P*<0.001), controlling for age, gender, cytogenetic risk, comorbidities, race, ethnicity, region, insurance type, treatment-free interval prior to SLT, FLT/SLT regimen type, year of diagnosis (dx), time since initial dx, time-dependent treatment- and MM-related symptoms, and post SLT regimens (Table 1).

Table 1. Adjusted predicted 1-year OS probability based on DOT in SLT.

Duration of SLT (months)	1-yr OS
1	0.61
3	0.68
4	0.72
6	0.78
7	0.81
8	0.83
9	0.85
10	0.87
12	0.90

Key: OS – overall survival; SLT – second-line therapy; yr – year

Summary/Conclusions: Longer duration of SLT was significantly associated with longer OS. Despite substantial heterogeneity in patient/disease characteristics and treatment patterns, the clinical benefit of continued longer term therapy at relapse appears to be generalizable to patients receiving care in the real-world setting.

E1324

AGE-RELATED HEALTHCARE DISPARITIES IN MULTIPLE MYELOMA
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Background: Age is a well-known factor in solid tumor linked to a lower adherence to guidelines. Scarce data exist for hematologic malignancies like Multiple Myeloma (MM), a disease that affects primarily elderly patients.
Aims: The aim of the study was to investigate the relationships between age, adherence to guidelines in MM and overall survival.

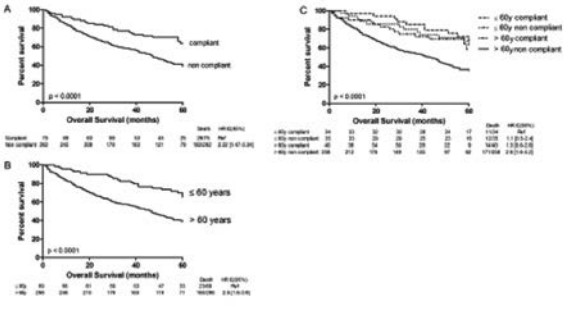

Figure 1.

Methods: The Poitou-Charentes cancer registry has exhaustively registered the incident cases of MM from 2008 to 2010. Provided care (diagnosis, staging, prognosis and first-line treatment) was compared to international guidelines.
Results: Three hundred and sixty seven patients aged 36 to 93y were included. Compliance to diagnostic procedure was 98%, staging 62%, prognosis 30% and first-line treatment 89%. Cytogenetic analysis was compliant in 37% (74%<66y, 31% between 66-74y and 13% for the oldest, *P*<0.001). Age was the strongest factor associated to compliant provision of care (OR 14.4 [6.1-33.8] for<66y, and 2.3 [0.9-6.1] for 66-74y; *P*<0.0001). The second independent factor was the diagnosis of multiple myeloma (OR 3.5 [1.6-7.3]; *P* =0.0009). Adherence to guidelines increased overall survival after adjustment on age HR: 1.8 [1.2-2.7], *P*=0.008.

Summary/Conclusions: Age is linked with inadequate provision of care in MM, particularly prognosis and first-line treatment. Compliance to guidelines improves OS. Future guidelines should stress that age and frailty should be taken into account at Myeloma care, eventually with specific guidelines for this population.

E1325

THE FIRST TRIAL: ANALYSIS OF THE ASIAN SUBGROUP OF TRANSPLANT-INELIGIBLE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH CONTINUOUS LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE
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Background: The incidence of multiple myeloma (MM) in Asian countries is increasing; therefore, effective treatment options for these patient (pt) populations are needed (Kim et al, *Am J Hematol,* 2014). The pivotal phase 3 FIRST trial investigated continuous treatment with lenalidomide plus low-dose dexamethasone until disease progression (Rd continuous) in pts with newly diagnosed MM (NDMM) who were ineligible for autologous stem cell transplant (ASCT) from 18 countries, including China, South Korea, and Taiwan. Treatment with Rd continuous in the FIRST trial improved progression-free survival (PFS; hazard ratio [HR]=0.72; *P*<.001) and overall survival (OS; HR=0.78; *P* =.02) compared with melphalan-prednisone-thalidomide (MPT) (Benboubker et al, *N Engl J Med,* 2014).
Aims: To examine the efficacy and safety of Rd continuous in the Asian population of the FIRST trial.
Methods: Pts with NDMM aged ≥65 years or ineligible for transplant were randomized to 3 treatment arms: Rd continuous, Rd for 18 cycles (Rd18; 72 weeks), or MPT for 12 cycles (72 weeks). The primary endpoint was PFS in pts treated with Rd continuous *vs* those treated with MPT (primary comparators). Data cutoff was May 24, 2013; response and progression were assessed by an independent response adjudication committee. Data cutoff for OS was extended to March 3, 2014. All pts provided informed written consent.

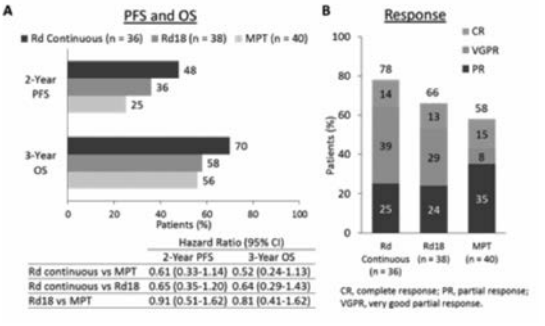

Figure 1.

Results: In the 114 pts enrolled in Asia, median age (68 yrs [range, 43-86 yrs]) was similar across the Rd continuous (n=36), Rd18 (n=38), and MPT (n=40) arms but lower than that of the overall study population (73 yrs [range, 40-92 yrs]). Pts in Asia also had a higher rate of Eastern Cooperative Oncology Group performance status ≥2 (28% *vs* 22% overall), a higher rate of International Staging System stage III disease (45% *vs* 41% overall), and double the rate of severe renal insufficiency (creatinine clearance<30 mL/min; 18% *vs* 9% overall), the latter of which was more frequent in the MPT (23%) and Rd18 (24%) arms *vs* the Rd continuous arm (8%). Median treatment duration was 18.4 mos (range, 0.5-35.9 mos) for Rd continuous, 11.0 mos (range, 0.6-19.6 mos) for Rd18, and 11.1 mos (range, 0.3-19.1 mos) for MPT. Treatment with Rd continuous *vs* MPT resulted in a 39% reduction in the risk of progression or death (Figure A). Rates of 2-year PFS were nearly doubled with Rd continuous (48%)

vs MPT (25%). Rd continuous also resulted in a 48% reduced risk of death *vs* MPT. Overall response rate was greater in the Rd continuous arm (78%) *vs* the Rd18 (66%) and MPT (58%) arms (Figure B). Median duration of response was not reached for Rd continuous and was 17.2 and 13.8 mos for Rd18 and MPT, respectively. The most frequent grade 3/4 adverse events with Rd continuous, Rd18, and MPT were neutropenia (25%, 34%, 44%), anemia (19%, 5%, 15%), pneumonia (6%, 24%, 15%), and thrombocytopenia (14%, 5%, 5%). Deep vein thrombosis was reported in 1 pt in the MPT arm, and pulmonary embolism was reported in 1 pt in each treatment arm. There were no reports of second primary malignancies in the Asian population.
Summary/Conclusions: Rd continuous treatment resulted in numerically larger PFS and OS benefits and higher response rates compared with MPT in the Asian subgroup of the FIRST trial, although pt numbers were small. Results were consistent with those in the overall population, with no unexpected safety signals, a low rate of thromboembolic events, and no second primary malignancies as of data cutoff. These findings support Rd continuous as a standard treatment for pts with NDMM who are ineligible for ASCT, including Asian populations.

E1326

DIAGNOSTICS AND PROGNOSTIC STRATIFICATION OF PATIENTS WITH WALDENSTRÖM'S MACROGLOBULINEMIA-SINGLE CENTRE EXPERIENCE
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Background: Waldenström's macroglobulinemia (WM) is a rare B-cell neoplasm defined as lymphoplasmacytic lymphoma with bone marrow infiltration and monoclonal IgM in the serum. More than 90% of WM patients carry a point mutation L265P in the MYD88 gene and concurrently, almost one third of MYD88^{L265P}-positive patients harbor frameshift (WHIM-FS) or non-sense (WHIM-NS) mutation in gene CXCR4. The mutations in CXCR4 result in premature stop codons and in shortening of CXCR4 protein product. Incomplete C-terminal domain of CXCR4 chemokine receptor is known to hyperactivate CXCR4-mediated signalization. The presence and type of mutation in genes MYD88 and CXCR4 appears to be significant in diagnostics and prognostic stratification of WM patients and it also influences the clinical manifestation of the disease.
Aims: To analyze mutational status of MYD88 and CXCR4 genes in patients with WM, to compare our results with laboratory parameters and to evaluate the prognostic stratification of the patients according to MYD88 and CXCR4 mutational status.
Methods: Analyzed DNA was isolated from mononuclear fraction of bone marrow cells at the time of diagnosis. Mutational status of analyzed genes was determined using allele-specific PCR (in the case of MYD88) and using direct Sanger sequencing (in the case of CXCR4). All found mutations were confirmed by specific cleavage with restriction endonucleases at defined conditions.
Results: We analyzed a group of patients with WM (*n=16*). We identified 15 MYD88^{L265P}-positive patients (93.8%), and 4 of them (25%) were also CXCR4 mutants (1 patient harbored WHIM-FS mutation and 3 harbored WHIM-NS mutation). CXCR4 mutations were associated with more aggressive disease (higher IPSS score, pancytopenia, higher levels of paraprotein and free light chains in serum, higher bone marrow infiltration; Table 1), and CXCR4$^{WHIM-WT}$ patients were often asymptomatic. CXCR4 mutations were also associated with worse treatment response (2 CXCR4$^{WHIM-MUT}$ patients were resistant and 2 patients had a partial response to first-line therapy).

Table 1. The medians of laboratory parameters of the WM patients with and without CXCR4 mutation.

	MYD88^{MUT} CXCR4$^{WHIM-WT}$	MYD88^{MUT} CXCR4$^{WHIM-MUT}$
Paraprotein level [g/l]	14.3 (3.7-32.2)	25.4 (10.6-30.9)
Bone marrow infiltration [%]	25 (10-72.4)	64 (10-96)
Involved free light chain level [mg/l]	103.5 (26-633)	341 (317-507)
IPSS	1 (n=6); 2 (n=5);3 (n=1)	2 (n=1); 3 (n=3)
Hemoglobin [g/l]	112.5 (89-149)	111.0 (90-117)
Thrombocytes [x10^9/l]	258 (37-585)	189 (15-281)

Summary/Conclusions: The mutational analysis of MYD88 and CXCR4 genes is essential for the diagnostics and prognostic stratification of patients with WM and it allows a deeper understanding of the molecular pathogenesis of the disease. In accordance with published data, we confirmed that CXCR4 mutations are associated with more aggressive disease presentation and thus affect treatment outcome.
This work was supported by grant IGA-LF-2016-001.

E1327

DESCRIPTION OF PATIENT CHARACTERISTICS, TREATMENT PATTERNS AND RESOURCE USE FOR PATIENTS WITH MULTIPLE MYELOMA TREATED IN THREE LOCAL HEALTH UNITS (LHUS) IN ITALY
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Background: Recent data on resource utilization and treatment patterns in multiple myeloma (MM) in Italy are limited.
Aims: The objective of this study was to examine the resource utilization and treatment patterns of newly-diagnosed patients with MM in Italy.
Methods: Retrospective claims data from the administrative databases of three LHUs in Lazio, Sicily, and Lombardy in Italy were used. Patients with a hospitalization discharge diagnosis of MM between 1 January 2008 and 1 September 2013 were included. To identify newly-diagnosed cases, patients with a prior MM diagnosis or a prior prescription for bortezomib (Bor), thalidomide (Thal), melphalan (Mel), lenalidomide (Len) or cyclophosphamide (Cyc) 12 months prior to entry into the study cohort were excluded. Index date was defined as the earliest of: date of first MM diagnosis or first prescription of Bor, Thal, Mel, Len, Cyc, prednisone or dexamethasone in patients with a diagnosis of MM within six months following prescription date; or the visit date to an oncologist or hematologist in patients with a diagnosis of MM within six months following the visit date. The baseline period was defined as 12 months prior to index date and the follow-up period ranged from the index date (inclusive) until date of last data collection or patient's death. Data on patients' demographics and clinical characteristics were collected at baseline. Healthcare resource use and treatment patterns were explored during the entire study period. The identification of treatment lines was based on assumptions given the sequencing of treatments based on key literature and treatment guidelines in Italy.
Results: Overall, 938 patients with MM entered the study cohort; average follow-up was 1.7 years. A total of 157 patients underwent a stem-cell transplantation (SCT) during the study period. The mean age at diagnosis was 69.7 years (standard deviation [SD]: 12.3). A slight majority of patients were female (52%). The most common comorbidities at baseline were renal disease (5.9%), other malignancies (5.8%) and congestive heart failure (4.4%). Among 814 patients with at least three months of follow-up, 76.4% of patients received at least one treatment line and 40.8% and 18.3% received at least two and three lines, respectively. Mel (27.7%) and Bor (21.5%) were the most frequently administered first- and second-line treatments, while Len showed an increased uptake in third line and beyond. Bor was the most commonly used first-line treatment in SCT patients (31.3%), while Mel was the most commonly used first-line treatment for non-SCT patients (36.5%). During the entire follow-up period, 80% of patients had at least one hospitalization for MM, 74% of patients had at least one laboratory test and 63% at least one diagnostic test, with the most common procedure being biopsy of bone marrow (53%). The mean annual cost of hospitalizations and medications per patient was €13,558 (SD: 17,559) and €2,401 (5,532), respectively. The average annual cost of medication per patient increased from €2,590 (5,566) for patients in first-line to €3,253 (8,767) and €4,330 (11,439) for patients in second- and third-line, respectively.
Summary/Conclusions: MM poses a significant economic burden on the national health system in Italy with hospitalizations being the main cost driver. Treatment patterns data indicate an unmet need for more effective therapies in Italy.

E1328

OUTCOMES FOR ASIAN PATIENTS WITH RELAPSED MULTIPLE MYELOMA TREATED WITH CARFILZOMIB AND DEXAMETHASONE *VS* BORTEZOMIB AND DEXAMETHASONE: A SUBGROUP ANALYSIS OF THE PHASE 3 ENDEAVOR STUDY (NCT01568866)
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Background: Carfilzomib is a selective proteasome inhibitor that is approved as a single agent and in combination with dexamethasone or lenalidomide/dexamethasone for relapsed or refractory multiple myeloma (RRMM). The ENDEAVOR study (NCT01568866) demonstrated statistically and clinically significant improvements in progression-free survival (PFS) for carfilzomib and dexamethasone therapy (Kd) compared with bortezomib and dexamethasone therapy (Vd; median 18.7 *vs* 9.4 months [mo]; hazard ratio [HR], 0.53; 95% confidence interval [CI]:0.44-0.65; 1-sided *P*<0.001) (Dimopoulos et al, *Lancet Oncol* 2016).
Aims: This preplanned subgroup analysis evaluated the efficacy and safety outcomes in Asian patients (pts) with relapsed MM from the ENDEAVOR study.
Methods: Adult Asian pts with RRMM (1-3 prior regimens) were included. Patients in the Kd arm received carfilzomib at 20/56 mg/m^2 IV of a 28-D cycle. The Vd arm received bortezomib 1.3 mg/m^2 IV or SC of a 21-D cycle. Cycles were repeated until disease progression or unacceptable toxicity. The primary end point was PFS; secondary end points included overall survival (immature at the time), overall response rate (ORR), incidence of grade ≥2 peripheral neuropathy, and safety.
Results: Of 929 pts randomized, 109 (11.7%) were of Asian ethnicity (Kd=54,Vd=55) from the JAPAC countries and received study treatment. The

majority were from Japan (n=44; 40.4%) followed by Taiwan (n=24; 22.0%), Singapore (n=20; 18.3%), Republic of Korea (n=16; 14.7%), and Thailand (n=5; 4.6%). Median PFS follow-up was 8.4 mo (Kd) and 7.6 mo (Vd). Median PFS was 14.9 mo (Kd; 95% CI:13.1–17.7) *vs* 8.8 mo (Vd; 95% CI:6.6–NE [not estimable]) (HR=0.57, [95% CI:0.29–1.14]; Figure 1A), representing a greater than 6 mo improvement. The ORR was 79.6% (Kd; 95% CI:66.5–89.4) *vs* 70.9% (Vd; 95% CI:57.1–82.4) (odds ratio=1.604 [95% CI:0.664–3.872]). The proportion of pts who achieved a best overall response (OR) of ≥complete response (CR) was higher in the Kd arm (9.3%) *vs* the Vd arm (1.8%). Also, the rate of ≥very good partial response (VGPR) in the Kd arm (63.0%) was more than twice of that in the Vd arm (23.6%). Grade ≥2 peripheral neuropathy occurred in 0% of pts in the Kd arm and 29% in the Vd arm. Rates of serious treatment-emergent adverse events (TEAE), significant AEs of interest, and AEs leading to treatment reduction or discontinuation (Kd or Vd) are shown in Figure 1B. Similar pt incidence rates of AEs, ≥Grade 3 AEs, and ≥Grade 3 treatment-related AEs were observed between the Kd and Vd arms except for higher cardiovascular events and hypertension being observed in Kd arm.

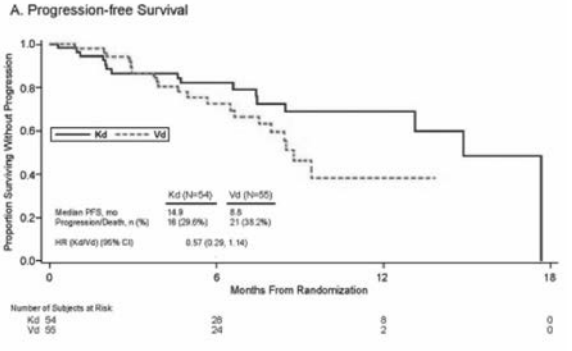

Figure 1.

Table 1.

B. Safety Results

	Asian Population (Safety Population)	
	Vd (bortezomib and dexamethasone) N=55, n (%)	Kd (carfilzomib and dexamethasone) N=53, n (%)
Number of Patients Reporting Serious TEAE	20 (36.4)	23 (43.4)
Significant AEs		
Cardiac failure	0	2 (3.8)
Dyspnea	2 (3.6)	9 (17.0)
Hypertension	4 (7.3)	14 (26.4)
Renal failure	0	0
Dose Reduction Due to an AE	34 (61.8)	13 (24.5)
Treatment Discontinuation due to an AE	7 (12.7)	8 (15.1)

Summary/Conclusions: In general, the efficacy and safety results from the Asian population analyses paralleled and are consistent with the results from the overall population of the ENDEAVOR study although the small sample size limits definitive conclusions regarding rates of AEs. For pts of Asian ethnicity, Kd therapy lead to clinically meaningful improvements in PFS, ORR, and CR/VGPR compared with the Vd arm.

E1329

THE PROGNOSTIC SIGNIFICANCE OF DEL1P IN MULTIPLE MYELOMA PATIENTS

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Background: The genomic characteristics of the malignant clone are an important aspect of multiple myeloma pathogenesis. It is well known that the disease is associated with certain cytogenetic abnormalities, some of which confer poor prognosis. The detection of these abnormalities with fluorescence in situ hybridization (FISH) can identify a group of patients with high risk who should be treated differently compared to those with standard risk.
Aims: Identification of genetic markers as independent prognostic factors for survival in multiple myeloma patients and assessment of their significance in the era of novel agents.
Methods: 92 newly-diagnosed multiple myeloma patients with performed FISH and/or conventional cytogenetics were evaluated. FISH analysis was performed in 76 patients, using specific probes for the most frequent high-risk genetic markers, including t(4;14), del17p, del13, amp1q and del1p. Prognostic factors for progression-free survival (PFS) and overall survival (OS) were identified by means of the Cox proportional hazard model for covariate analysis. As possible prognostic factors the following parameters were included in the regression model: age, β2-microglobulin levels, hemoglobin levels, platelet counts, creatinine, calcium, percentage of bone-marrow infiltration, extramedullary disease, plasmablast morphology and presence or absence of the above mentioned genomic aberrations. Median survival times were calculated and compared according to the presence or absence of a particular high-risk aberration and the type of induction therapy (bortezomib-based or conventional). Kaplan-Meier curves were plotted and compared using the log-rank test. Statistical analyses were performed with the program SPSS v21.
Results: The prevalence of high-risk genomic aberrations in our patient cohort was as follows: del13 in 39 (42.4%) patients; amp1q - in 22 (23.9%); del1p and del17p each in 15 (16.3%), and t(4;14) in 8 (8.7%) patients. In our analyses only high β2-microglobulin levels (p=0,035) and del1p (p=0,000) were independent prognostic factors for PFS, while high β2-microglobulin (p=0.002), thrombocytopenia (p=0.037) and del17p (p=0.006) were independent predictors of poor OS. Among the high-risk genetic abnormalities del1p and del17p had the greatest negative influence on patients' outcome, regardless of the induction therapy performed (conventional or bortezomib-based). Patients with del1p and 17p had a significantly shorter median survival, compared with patients without these aberrations, even after induction with a novel agent: 16 months *vs* 79 months for del1p (p=0.014) and 12 months *vs* 79 months for del17p (p=0.001).
Summary/Conclusions: Del17p is a well-known adverse prognostic factor in multiple myeloma which is included in the risk stratification models. Presence of this aberration predicts short survival and such patients are candidates for clinical trials. Only few data however are present on the prognostic significance of del1p in the era of novel agents. According to our results patients with del1p should be considered high-risk, similarly to patients with del17p, and may be candidates for more aggressive induction therapy.

E1330

PEGFILGRASTIM *VERSUS* FILGRASTIM IN THE MANAGEMENT OF POST-CHEMOTHERAPY NEUTROPENIA IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA IN TREATMENT WITH BVD (BENDAMUSTINE BORTEZOMIB-DEXAMETHASONE)

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Background: Patients receiving cancer chemotherapy are at increased risk of neutropenia, leading to increased risk of infections and delay in subsequent chemotherapy treatments. Recombinant granulocyte colony stimulating factors (G-CSFs) have been developed to stimulate proliferation and differentiation of neutrophils in patients receiving chemotherapy. Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF that extends the half-life and allows for once-per-cycle dosing, requiring less frequent dosing than nonpegylated G-CSF. Multiple Myeloma (MM) in advanced phases of disease may be managed by regimens combining agents not frequently employed in early phases of treatment (e.g. Anthracyclines, Alkylating agents, etc), but myelotoxicity is the main expected side effect. In this context, G-CSFs are often necessary to counteract the risks of febrile neutropenia: their use is bound to frequent evaluation of neutrophil counts that may not be easy for patients in home-care. Avoiding severe neutropenia by prophylactic pegfilgrastim seems particularly useful in these cases.
Aims: The objective of this study was to compare the efficacy and safety of pegfilgrastim in patients affected by Multiple Myeloma in advanced phase of disease. treated with BVD (Bendamustine, Bortezomib, Dexamethasone), in order to determine whether a single subcutaneous injection of pegfilgrastim is as effective as daily injections of standard filgrastim, in terms of haematological toxicity, febrile neutropenic episodes, antibiotic usage and hospedalization duration.
Methods: 47 patients (25 male and 22 female) with a median age of 58.4 years (range 36-82) affected by multiple myeloma, all relapsed and refractory to a median of 6 lines of therapy (range 2-11), all previously exposed to Bortezomib, Lenalidomide, Melphalan and all relapsed after at least one autologous bone marrow transplantation, had been enrolled.
Results: Since first course, received in our out-patient department, patients performed blood counts twice weekly and received, from day +8 to day +19 (considering "day+1" the day in which the chemotherapy protocol starts), prophylactic oral chinolonic antibiotics and anti-fungal drugs. During neutropenia after first cycle of chemotherapy, Filgrastim (5μgr/kg/day for 3 days) was given if neutrophils count was <1500x10^9cells/L. Median number of filgrastim administrations was 4.1 (r. 3-6); nadir neutropenia was registered after a median of 11.6 days (r. 8-14); median of nadir neutrophil count was 1.22x10^9cells/L (range 0.4-1.5x10^9cells/L), with maximum duration of 13 days. From the second course of chemotherapy, all patients switched to prophylactic therapy with pegfilgrastim (6 mg), injected subcutaneously with a single administration on

day +3 independently from the neutrophil count at that time. Primary endpoint of this study was the duration of neutropenia (neutrophil count <1.5x10^9 cells/L), comparing pegfilgrastim and filgrastim. During pegfilgrastim, neutropenia was never longer than 8 days, with a consequent reduction of neutropenia-related infections. Median nadir neutrophil count, evaluated for every patients for at least three courses of therapy (r. 3-6) registered at day +11, was 1.484 (range 1.04-2.33x10^9cells/L); only six patients (12.7%) needed, one week after pegfilgrastim administration, a supplement of 3 administrations of filgrastim. During pegfilgrastim prophylaxis, neutropenia was shorter than during Filgrastim treatment. Besides the mono-administration, pegfilgrastim was well tolerated in all patients: main side effects in our patients were mild fever and bone pain, (9/47 patients,19.1%).
Summary/Conclusions: In conclusions, in patients affected by MM exposed to myelosuppressive agents in advanced phases of myeloma disease, pegfilgrastim seems to reduce the incidence of neutropenia and may increase the possibility to maintain the scheduled time of treatment.

LB2262

PREDICTIVE VALUE OF AUTOANTIBODIES AGAINST M2-MUSCARINIC ACETYLCHOLINE RECEPTOR FOR THE ONSET OF CARDIAC AMYLOIDOSIS IN MULTIPLE MYELOMA
L Li*

Background: Multiple myeloma (MM) is a clonal late B-cell disorder in which malignant plasma cells (PCs) expand and accumulate in the bone marrow, leading to anemia, lytic or osteopenic bone disease and renal failure. Nowadays MM has accounted for the second most common hematological malignancy after non-Hodgkin lymphoma with its incidence of 1% in neoplastic diseases and 13% in hematological malignance[1,2]. According to the data[3], approximately 10% of multiple myeloma tends to be complicated by cardiac amyloidosis, mainly AL amyloidosis, in which the amyloidosis protein is consisted of monoclonal immunoglobulin light chains produced excessively by plasma cell malignancy. Moreover, up to 50% of patients with AL amyloidosis would progressively develop restrictive heart failure[4]. Unfortunately the prognosis is still poor. M_2 muscarinic receptor is attached to the family of cardiac G-protein-coupled receptors. Previously the auto-antibodies against the second extracellular loop of M_2-muscarinic acetylcholine receptor (anti-M_2-R) have been verified to involve in the development of various heart diseases characterized by heart failure, such as idiopathic dilated cardiomyopathy, Chagas's heart disease and peripartum cardiomyopathy[5-7]. Furthermore, the level of anti-M_2-R is positively associated with the increased risk of onset of peripartum cardiomyopathy[7]. Consequently, our study aims to explore whether the level of anti-M_2-R would rise in MM patients with or without cardiac amyloidosis and whether anti-M_2-R would increase the risk of cardiac amyloidosis in MM patients.
Aims: 1. Anti-M_2-R might participate in the phathophysiologic mechanism of cardiac impairment complicated by MM;
2. Anti-M_2-R can be an earlier predictor to estimate the risk of cardiac amyloidosis in MM patients than NT-proBNP.
Methods: Totally 78 subjects were recruited, including23 MM with cardiac amyloidosis, 25 MM without amyloidosis (MM) and 30 normal control (NC). The frequency and titer of anti-M_2-R were compared in the three groups. All MM were under detection of serum anti-M_2-R by ELISA before chemotherapy. The risk of cardiac amyloidosis was estimated using the univariate and multivariate logistic regression.
Results: The positive rates of anti-M_2-R in the three groups are 69.6% (16/23) in MM with cardiac amyloidosis, 40.0% (10/25) in MM and 10.0% (3/30) in NC respectively (p=0.040,69.6% *vs* 40% and p=0.003, 40.0% vs.10%).Moreover, multivariate logistic regression indicates that the presence of anti-M_2-R is associated with an increased risk of complicated cardiac amyloidosis in MM patients (OR, 5.5; 95% CI, 1.4-21.4; p=0.013).
Summary/Conclusions: This study demonstrates that the elevated level of anti-M_2-R is an earlier independent predictor for the risk of cardiac amyloidosis in MM patients.

Myeloproliferative neoplasms - Biology

E1331

MOLECULAR CHARACTERIZATION OF MYELOPROLIFERATIVE NEOPLASMS WITH CONCOMITANT BCR-ABL1 AND JAK2 V617F OR CALRETICULIN MUTATIONS
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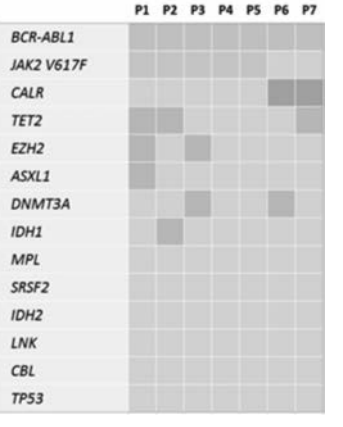
Background: Myeloproliferative neoplasms (MPN) are classified based on the presence of the *BCR-ABL1*, *JAK2* and *CALRETICULIN* mutations. These are considered to be mutually exclusive, however rare cases with concomitant occurrence of these driver mutations have been reported, representing an ideal model to chronicle the molecular alterations and to investigate the clonal architecture in these cases. We report a comprehensive clinical and genetic analysis of sequential samples obtained from 7 patients with concurrent *BCR-ABL1* and *JAK2* V617F or *CALR* mutations diagnosed at our centers between 1998 and 2015.
Aims: To perform a detailed clinical, morphological and quantitative genetic analysis of sequential samples obtained from 7 patients with MPN with concomitant *BCR-ABL1* and *JAK2* V617F (n=5) or *CALR* mutations (n=2) to gain further insight into clonal architecture and pathogenesis of these rare double-mutated cases.
Methods: DNAs and RNAs extracted from peripheral blood or bone marrow samples were used to perform quantitative analysis of the *BCR-ABL1* fusion transcript and *JAK2* V617F/*CALR* mutations as part of the routine molecular diagnostic workup. The mutation status of 13 genes (*JAK2, CALR, MPL, ASXL1, EZH2, SRSF2, IDH1, IDH2, LNK, CBL, TET2, DNMT3A, TP53*) frequently mutated in MPNs was analyzed using next generation sequencing (NGS). The NGS analysis for performed using the AmpliSeq technology with an IonTorrent instrument at an average depth of 1000x on 15 sequential samples from 7 patients.
Results: The quantitative analyses of the *BCR-ABL1* fusion transcript and *JAK2* V617F or *CALR* mutations suggested that these driver mutations occurred in different clones in 5 out the 7 cases analyzed. The next generation sequencing analysis of the 13 target genes identified additional mutations in 5 of the 7 cases with concurrent driver mutations (summarized in the heat map in *Figure 1*). The most frequently mutated gene was *TET2* with mutations identified in 3 cases (2 cases with *BCR-ABL1* and *JAK2* mutation and one case with *BCR-ABL1* and *CALR* mutation). Both *EZH2* and *DNMT3A* mutations were detected 2 cases. *ASXL1* and *IDH1* were found to carry mutations in single cases with BCR-ABL1 and *JAK2* mutation. The 5 patients harboring additional mutations carried 1-3 mutation per sample with one of the cases presenting with 3 additional mutations affecting the *TET2, EZH2* and *ASXL1* genes.

Table 1.



Summary/Conclusions: Our analysis of the 7 patients with concurrent MPN driver mutations suggested that the *BCR-ABL1* and *JAK2* or *CALR* mutations occur in different clones in majority of cases with frequent acquisition of additional phenotype modifying genes including *TET2, EZH2, ASXL1* and *DNMT3A*.

E1332

ABNORMAL EXPRESSION OF LCN2 (NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN) IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), are clonal hematopoietic stem cells' disorders. Clonal proliferation in MF is accompanied by a secondary inflammatory reaction characterized by bone marrow (BM) stromal changes and abnormal cytokine expression. We recently reported *LCN2/NGAL* upregulated mRNA expression in CD34+ cells of MF patients (Norfo et al. Blood 2014;124:e21-e32); increased BM expression of LCN2 might facilitate clonal predominance and contribute to a dysfunctional BM microenvironment (Lu M at al. Blood.2015;126:972-982).
Aims: We aimed to investigate the clinical significance of LCN2 levels and its correlation with clinical and molecular aspects in patients with MPNs.
Methods: One hundred well characterized MPN patients (20 PV, 20 ET, 30 PMF, 30 PPV-MF and PET-MF) were included in this study, and 20 health subjects. *LCN2* mRNA expression was performed in granulocytes by RTQ-PCR. Measurements of NGAL plasma protein (PP) were performed by ELISA (Bioporto Diagnostics). Mutational status of *JAK2, MPL, EZH2, ASXL1, IDH1/2, CBL, TP53, TET2, DNMT3A, SRSF2* was evaluated as previously reported. Statistical analyses were conducted with SPSS and Origin software.
Results: Significantly higher levels of *LCN2* mRNA and PP were found in PMF pts (mRNA: median RQ 9.70; PP: median 6.03ng/ml) in comparison with controls (mRNA:median RQ 1.09, P=.02; PP: median 1.19ng/ml; P=.005). mRNA LCN2 expression in PMF was also significantly higher compared with PV and ET (median RQ 1.89; P=.01 and median RQ 3.55; P=.02, respectively). Higher levels of PP were also observed in PPV-MF (median 4.82ng/ml) compared to controls (P=.003). LCN2 PP levels comparison among MPN sub-types is reported in Figure1. In particular LCN2 PP levels in PET-MF (median 2.86 ng/ml) were two-fold greater than in ET (median 1.395 ng/ml; P=.01), suggesting a link between increased LCN2 levels and progression to MF. mRNA expression inversely correlated with hemoglobin levels (P=.03, r=-0.29). LCN2 mRNA levels were positively correlated with IPSS scores (P=.01), and were higher in ASXL1+ pts (n=23) compared with WT (3.2 *vs* 10.4, P=.02). CALR+ pts showed lower levels compared with JAK2+ or Triple Negative (2.60 *vs* 4.85 *vs* 8.71 ng/ml respectively, P=.04), while PP levels were significantly higher in SRSF2+ pts (n=4) compared to WT (17.35 *vs* 4.04, P=.02). PP levels were linearly correlated with CD34+ count (P=.01, r=0.28). In 17 of 33 MF pts, reduction of PP LCN2 levels was observed after treatment with ruxolitinib; of these 6 (54.5%) achieved a spleen volume reduction and in 7 (63.6%) constitutional symptoms resolved.

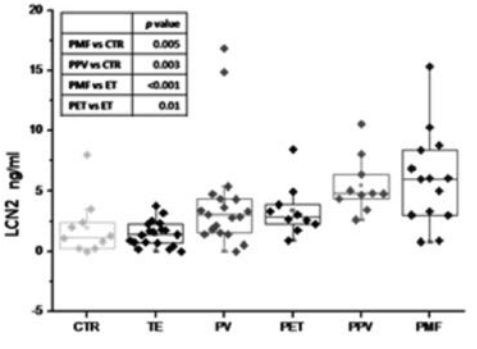

Figure 1.

Summary/Conclusions: Lipocalin might represent a novel diagnostic and/or prognostic marker and could also provide a new therapeutic target in MPNs, particularly myelofibrosis. Further studies are required to fully clarify the clinical significance of Lipocalin overexpression in myelofibrosis.

E1333

FACS SORTED PERIPHERAL BLOOD CELLS IDENTIFY CALR MUTATIONS IN B-AND T CELLS

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Background: Somatic mutations in exon 9 of the calreticulin gene (*CALR)* are found in the majority of the *JAK2V617F* negative Philadelphia-negative myeloproliferative neoplasms (MPNs) essential thrombocythemia (ET) and primary myelofibrosis (PMF). A hallmark of the mutated CALR protein is its preferential expression in megakaryocytes, and so far, mutations in CALR have only been found in cells of myeloid lineage. However, mutations in the *CALR* gene are considered an early event and thus expected to be present in cells of the lymphoid compartment as well. The mutant allele burden has been suggested to have an impact on the disease phenotype, but patients with high mutant allele burden appear to be rare and it is unknown if homozygous sub-clones is common in patients.
Aims: Here we wish to determine if the *CALR* mutations are present in B- and T- lymphocytes and investigate if homozygous clones are present in the patients.
Methods: In this study, we separated the myeloid and lymphoid cell fractions from 11 *CALR* mutated patients (4 ET, 1 pre-fibrotic primary myelofibrosis (pre-PMF), 5 PMF, and 1 post-ET MF) using fluorescent activated cell sorting (FACS). The mutations included the type 1 and type 2 subtypes, and two different 34 base-pair deletions. The allele burden was quantified for type 1 and 2 mutations using allele specific qPCR and the 34 base-pair deletions quantified by fragment analysis. Granulocytic and erythroid cell colonies from 15 patients (4 ET, 3 pre-PMF, 7 PMF, and 1 post-ET MF) were cultured in the presence of erythropoietin and after expansion individual colonies was isolated and analyzed for the *CALR* mutant allele burden.
Results: The mutant allele burden was determined in whole-blood (median: 41%, range: 3-47%), granulocytes (CD16+ median: 51%, range: 4.8-63%), and monocytes (CD14+; median: 47%, range: 5.6-61%). In 3 of 11 patients (27%), the type 1 mutation was found in T-lymphocytes (CD3+; median: 6.4%, range: 5-7.8%), and in 2 of 11 patients (11%) in B-lymphocytes (CD19+; median: 25%, range: 18-31%). One patient had the type 1 mutation in both cell types. The type 2 mutation was found in T-lymphocytes of a single patient (16%). Isolated granulocytes and erythroid cells from cultured cell colonies revealed the *CALR* positive clones from all patients to be heterozygous for the mutations.
Summary/Conclusions: The presence of the *CALR* mutations in both myeloid and lymphoid lineages indicates the *CALR* mutation to be an early event in a multipotent hematopoietic stem cell expanding as a heterozygous mutated clone of the myeloid lineage to become dominant in the majority of *CALR* mutated MPN patients.

E1334

FAILURE OF MEGAKARYOCYTE APOPTOSIS IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Megakaryocytic hyperplasia and morphological atypia are characteristic features of the myeloproliferative neoplasms (MPN) and its entities: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (MF). Data from murine and *ex vivo* models suggest that multiple molecular abnormalities (*e.g. JAK2*^{V617F}, *CALR* mutations) underpin megakaryocyte expansion in the MPN. Limited studies also show that megakaryocytes utilize apoptotic caspases to shed platelets and in the MPN, they may have apoptotic disturbances. Despite these claims, there is little data demonstrating the precise apoptotic defects and pathways affected in megakaryocytes of human MPN.
Aims: We investigated the apoptotic pathobiology of megakaryocytes in human MPN. We determined whether these disturbances targeted the intrinsic and/or extrinsic apoptotic pathways and examined their differential associations between MPN disease entities and the *JAK2*^{V617F} and *CALR* driver mutations.
Methods: Bone marrow trephines of MPN (PV=20, ET=72, MF=53) and controls (n=15) were studied. These included *JAK2*^{V617F}, *CALR*-mutated (*CALR*-^{Mut}) and double negative (JAK2^{V617F}-/*CALR*^{WT}) cases. Sections were stained with antibodies to pro-apoptotic (BNIP-3, caspase-8, caspase-9, Diablo, p53) and anti-apoptotic (Bcl-XL, survivin) effectors involved in the intrinsic and extrinsic apoptotic pathways using an immuno-alkaline phosphatase and Fast Red chromogen detection system. Megakaryocyte positivity was assessed by light microscopy and the percent positive determined per case. Mean differences (MD) in megakaryocyte positivity between MPN entities and *JAK2*^{V617F}/*CALR* mutation states were statistically evaluated. Megakaryocyte positivity was also correlated with platelet count.
Results: Megakaryocytes in MPN showed significantly greater positivity for anti-apoptotic Bcl-XL and survivin, and pro-apoptotic Diablo, caspase-8 and p53 than controls. However, MPN had significantly fewer pro-death BNIP-3 positive megakaryocytes (MD=42.8%, p<0.0001). Caspase-9 was only marginally increased in MPN (MD=4.3%, p=0.02). The apoptotic dysregulation was most marked in MF. Significantly fewer BNIP-3 positive megakaryocytes were seen in MF than ET (MD=14.7%, p=0.008). MF also had fewer caspase-9 positive megakaryocytes compared with both PV (MD=33.8%, p=0.003) and ET

(MD=19.8%, p=0.02). However, p53 was increased ~2.7-fold in MF *versus* PV (p=0.007). *CALR*^{Mut} megakaryocytes typically had pyknotic chromatin and lower pro-apoptotic BNIP-3 levels relative to *JAK2*^{V617F} (MD=24.2%, p=0.0008). Platelet counts correlated positively with caspase-9 megakaryocyte positivity in MPN (r=0.28, p=0.002), and strengthened among MF (r=0.34, p=0.03) and *CALR*^{Mut} cases (r=0.50, p=0.03).
Summary/Conclusions: Megakaryocytic hyperplasia in the MPN is assisted by disruptions of the intrinsic apoptotic pathway. Our data shows overexpression of anti-apoptotic Bcl-XL and survivin to be the key apoptotic inhibitors mediating megakaryocyte survival. In MF, apoptotic failure is exacerbated by reductions in pro-death BNIP-3 and caspase-9; this may relate to the marked formation of cohesive megakaryocyte sheets and abnormal nuclear morphology common in MF. *CALR* mutations also showed greater reductions in pro-apoptotic BNIP-3 compared with *JAK2*^{V617F}. Finally, the upregulation of caspase-9 seen in megakaryocytes of *CALR*-mutated cases may account for the thrombocytosis associated with this molecular subgroup.

E1335

INCREASED PLASMA NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) IS ASSOCIATED WITH A HYPERPROLIFERATIVE PHENOTYPE AND RESTRAINS DISEASE PROGRESSION IN MPN-ASSOCIATED MYELOFIBROSIS

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Background: Myeloproliferative neoplasm (MPN)-associated myelofibrosis is a clonal, neoplastic disorder of the hematopoietic stem cells, in which inflammation and immune dysregulation play an important role. Somatic mutations of JAK2 (JAK2V617F), CALR or MPL genes ar pathogenetically linked to the disorder. JAK-STAT pathway activation is the main mechanism of myeloproliferation; however, available evidence indicates that inflammatory/immune mechanisms play an important role in the phenotype of MPN-associated myelofibrosis by increased production of inflammatory cytokines and reduced number of regulatory T-cells. Nicotinamide phosphoribosyltransferase (NAMPT), intracellularly, is an enzyme that converts nicotinamide into nicotinamide mononucleotide, precursor of nicotinamide adenine dinucleotide (NAD^+), which is essential for cellular metabolism, energy production, DNA repair, and survival. NAMPT exists also as an extracellular protein defined in literature as Pre-B cell colony-enhancing factor (PBEF) or as an adipokine called visfatin. Different type of cells are able to release this protein, but its role as a cytokine is still unknown. Emerging data implicate eNAMPT in the pathogenesis of a number of different human diseases that share an inflammatory basis, such as rheumatoid arthritis, type 2 diabetes, acute life-threatening processes such as acute lung injury, sepsis, and tumorigenesis. In the tumour microenvironment, eNAMPT induces monocyte polarization to M2 macrophages secreting tumor-promoting cytokines and inhibiting T-cell responses rendering this pleiotropic molecule a novel player in tumor/host cross-talk. The function of NAMPT in MPN-associated myelofibrosis biology is completely unknown
Aims: Here we examined plasma levels of eNAMPT in patients with MPN-associated myelofibrosis and their effects on disease phenotype and outcomes. We also studied the concordance of eNAMPT levels with the marker of general inflammation high-sensitivity C-reactive protein (hs-CRP).
Methods: A total of 333 MPN-associated myelofibrosis patients (187 males and 146 females) and 31 age- and gender-matched normal-weight controls were enrolled in the study. The control group was selected among the general population excluding individuals with a body mass index greater than 25. At study entrance, all participants gave their informed consent, and study protocol was approved by the Ethics Committee of our Hospital before implementation. Since there is no accepted definition of disease severity, we evaluated a "severity score" by indexing leukocytosis, thrombocytosis, and splenomegaly (myeloproliferation index), and anemia, leukopenia, and thrombocytopenia (myelodepletion index) Levels of eNAMPT and hs-CRP were simultaneously assayed in 209 MPN-associated myelofibrosis patients. Twenty-four polycythemia vera or essential thrombocythemia patients were used as controls. eNAMPT levels were measured using a commercial immunoassay kit (BioPlex, BioRad Hercules, CA, USA) according to manufacturer instructions. All samples were assayed in duplicate.
Results: In the 333 MPN-associated myelofibrosis patients enrolled in the study main body, risk stratification according to the IPSS/DIPSS prognostic scoring system showed a predominance of low and intermediate I risk classes (74% of the patients). The severity score of the disease based on spleen size and hematological parameters measured at the time of eNAMPT analysis ranged from 0 to 6 with a median value of 3. The "myeloproliferation index" (range 0 to 4) had a median value of 3 and was equal to or lower than 1 in 39 patients (46.4%), while the "myelodepletion index" (range 0 to 4) had a median value of 2 and was equal to or lower than 1 in 34 patients (40.5%). eNAMPT was over expressed in MPN-associated myelofibrosis (n=333, median 1,487 ng/L, range 81-96,031) compared to healthy subjects (n=31, median 268 ng/L, range 81-4,981; P=0.001). There was no difference in the median eNAMPT level between patients with PMF and post-PV or post-ET myelofibrosis. eNAMPT expression was correlated with higher white blood cell count (R=0.13;P=0.029), higher hemoglobin (R=0.20;P<0.001) and higher platelet count (R=0.25;P=<0.001), suggesting that eNAMPT is an indispensable permissive agent for myeloproliferation of MPN-associated myelofibrosis. In 209 patients with MPN-associated myelofibrosis we simultaneously assayed eNAMPT and hs-CRP. Patients with MPN-associated myelofibrosis had higher levels of hs-CRP than healthy controls (median, 0.15 ng/µl, range, 0.02 to 11.4). However, the individual values of eNAMPT were not correlated with those of hs-CRP level, suggesting that eNAMPT in MPN-associated myelofibrosis does not behave as a canonical inflammatory cytokine. In addition, higher levels of eNAMPT predicted longer time to blast transformation, and protected against progression towards thrombocytopenia and large splenomegaly.
Summary/Conclusions: In conclusion, in MPN-associated myelofibrosis high levels of eNAMPT mark the myeloproliferative potential and, at variance with a high number of cancers, are protective against disease progression.

E1336

DOES THE MUTATIONAL STATUS AFFECT THE BONE MARROW MORPHOLOGICAL DATA IN PATIENTS WITH CLASSICAL PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS?

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Background: It is known that there may be a correlation between a genetic or molecular alteration and the presence of characteristic histomorphological changes in the bone marrow (for example, 5q- syndrome). In recent years, molecular alterations described in chronic Philadelphia-negative myeloproliferative neoplasms, mainly *JAK2* V617F, calreticulin (*CALR*) and *MPL*, have gained great diagnostic and prognostic interest.
Aims: Our objective was to analyze the possible influence of the mutational status of these diseases on the histomorphological appearance of the bone marrow.
Methods: We have retrospectively studied 140 patients atended at our center between 1998 and 2013, with a diagnosis of Philadelphia-negative myeloproliferative neoplasm. The series included 44 patients with polycythemia vera (PV, male 61%, mean age 61, range 28-82), 78 with essential thrombocythemia (ET, female 62%, mean age 54, range 16-81) and 18 with primary myelofibrosis (PMF, male 61%, mean age 63, range 46-95). We determined the presence or not of the mutations for *JAK2*, *CALR* and *MPL* by standard methods. All patients had performed a bone marrow trephine biopsy at diagnosis, and samples were examined by two hematologists and a pathologist with proven experience on these entities. A total of 18 histopathological characteristics were analyzed: overall and differential (erythroid, myeloid and megakaryocytic) cellularity, megakaryocytes features (size, disposition in dense or loose clusters, nuclei morphology, presence of "naked" nuclei, intrasinusoidal and paratrabecular location), anormal location of immature precursors, emperipolesis, presence of lymph nodes, microvasculature density, vascular ectasia, iron stain, osteoclerosis, collagen fibers, CD34+ precursors, and intrasinusoidal hematopoiesis. Arrangement was considered when the degree of consensus included at least two observers. Statistical analysis was performed using the SPSS 17.0 version.
Results: The frequency and distribution of mutations were as follows: 1) PV: *JAK2* V617F mutation in 88.4% of cases and *JAK2* exon 12 mutation in 4.7%; 2) ET: *JAK2* V617F mutation in 43.4% of analyzed patients, *CALR* mutation in 26.4% and none was carrier of *MPL* mutation; 3) PMF: *JAK2* V617F mutation in 37.5%, *CALR* mutation in 31.2% and none with *MPL* mutation. No morphological differences were found in the bone marrow of patients diagnosed with PV or ET according to their mutational status (carriers or not of mutations in *JAK2* in PV cases, and carriers or not of *JAK2* or *CALR* mutation in patients with ET). However, some significant changes were observed in patients with PMF. In the latter group of patients, the *JAK2* V617F mutation is associated with a significantly increased overall marrow cellularity when compared with *CALR* mutation (predominantly normocellular) (p=0.025). Meanwhile, carriers of *CALR* mutation, compared to those with *JAK2*, show significant changes in nuclear lobulation of megakaryocytes (mostly hyperlobulated *versus* normo- or hypolobulated in *JAK2* mutated) (p=0.019). The emperipolesis phenomenon was significantly more frequent in PMF patients carrying the *JAK2* V617F mutation when compared with JAK2 non-mutated (p=0.024), and a substantially higher frequency of "naked" nuclei of megakaryocytes was observed in PMF *CALR* mutated cases in comparison with *CALR* non-mutated (p=0.035).
Summary/Conclusions: In our experience, the mutational status appears to influence the histopathological changes observed in the bone marrow of patients with primary myelofibrosis, but not in polycythemia vera or essential thrombocythemia. Further studies including a larger volume of patients are necessary to confirm these findings.

E1337

HDAC INHIBITION SYNERGIZES WITH ANTIOXIDANT THERAPY TO TARGET MYELOPROLIFERATIVE NEOPLASMS

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Background: The BCR-ABL-negative myeloproliferative neoplasms (MPN) are a group of heterogeneous hematological diseases with constitutive JAK-STAT pathway activation. However, despite the recent advances in unravelling disease etiology (JAK2 activating mutations), there is still no curative treatment outside bone marrow transplantation. Epigenetic alterations, like histone acetylation, play pivotal roles in the pathogenesis of several hematological malignancies, including MPN. Preliminary data showed that histone deacetylase inhibitors (HDACis) promoted in cell death and growth arrest of myeloid neoplastic cells *in vitro*. Importantly, HDACis have proven efficacy in clinical practice, producing remissions and increasing the overall survival in hematological malignancies. HDAC inhibition has also produced results in MPN patients, an effect severely limited by toxicity.
Aims: In order to explore the mechanism of action of HDACis in MPN, we analyzed the impact of HDACi in the cellular biology of MPN cell lines and primary bone marrow cells.
Methods: Primary MPN cells were obtained from bone marrow samples at diagnosis following informed consent. SET2, HEL and UKE-1 MPN cell lines were used. Both primary cells and cell lines were incubated with the different pharmacological reagents and at different time points, the cells were stained for Apoptosis and Reactive Oxygen Species (ROS) for detection by Flow Cytometry.
Results: Vorinostat decreased cellular viability in primary MPN cells, particularly affecting the monocytic lineage, and this was associated with a concomitant decrease in ROS levels. In MPN cell lines we showed that a wide range of HDACi (Vorinostat, Trichostatin A, Butyric Acid and Depsipeptide) produced these effects. Interestingly, HDACi-induced apoptosis was dependent on decreased ROS levels suggesting that both events are connected and necessary for MPN cell death induced by HDACi to occur. By combining both HDACi and ROS reducing drugs (ROS production inhibitors; ROS scavengers and AntiOxidants) we were able to increase MPN cell death in a synergistic manner.
Summary/Conclusions: These results point to a promising synergistic effect of HDACi with ROS depleting drugs in the context of MPN, which must be confirmed *in vivo* and in the clinical setting. These combinations could improve efficacy a tolerability of these agents in MPN, opening the door to a new therapeutic alternative.

E1338

REDUCED FREQUENCY OF CIRCULATING CD4+CD25+CD127LOWFOXP3+ REGULATORY T CELLS IN PRIMARY MYELOFIBROSIS

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Background: A systemic dysregulation of immune system, represented by increased plasma levels of several inflammatory cytokines, and clinical and laboratory findings of autoimmunity, has been documented in primary myelofibrosis (PMF). Regulatory T cells (Tregs), currently identified as CD4+T cells with high expression of CD25 (interleukin-2 receptor), low expression of CD127 (IL-7 receptor), and high expression of FOXP3, are known to play a crucial role in the maintenance of T cell homeostasis and immunologic tolerance. Moreover, Treg cell defects have been associated with different autoimmune diseases, and a Treg cell deficit due to a mutation in the FoxP3 gene has been shown to cause aggressive autoimmunity and early death. Although a clinical study has indicated decreased number of Treg cells in the active status of PMF, conflicting results have been reported up to now.
Aims: In this study we investigated the frequency of Tregs, identified as CD4+CD25+CD127lowFoxP3+ cells, to clarify whether or not Treg cell number is reduced in PMF, and to investigate the associations between Tregs frequency and disease phenotype
Methods: Tregs frequency was assessed by flow cytometry in 202 patients with PMF, all out of therapy at time of sampling, and in 24 healthy subjects (HS), comparable for sex and age. Spleen-derived Tregs were evaluated in 17 different patients undergoing splenectomy for symptomatic splenomegaly and in 8 HS splenectomized for abdominal trauma. At study entrance, all participants gave their informed consent, and study protocol was approved by the Ethics Committee of our Hospital before implementation. A single cell suspension was obtained from splenic samples by means of mechanical dissociation followed by density gradient centrifugation. The frequency of $CD4+CD25+CD127^{low}FoxP3+$ cells was calculated as a percentage of positive cells in the total CD4 gate. For the Treg suppression assay circulating $CD4^{+}CD25^{+}$ T cells were immunoselected and cultured in triplicate with soluble anti-CD3/anti-CD28 and autologous irradiated cells. At day 4, ^{3}H thymidine (2Ci/well) was added to cultures for 16 hours and proliferation measured by the amount of incorporated ^{3}H.
Results: Compared with HS who had a median Treg frequency of 1.99% (range, 0.48% to 6.51%), patients with PMF had a median frequency of Tregs of 0.80% (range 0% to 10.2%; P<0.001). A similar trend was observed in the spleen were HS had a median Treg frequency of 1.20% (range 0.36% to 3.97%) and patients with PMF a median of 0.35% (range 0.04% to 1.26%; P<0.001). This result secures that the reduced frequency of circulating Tregs was not due to cell recruitment to sites of active malignancy, like spleen. To support that blood and spleen Tregs were independently regulated among total CD3+CD4+ cells, we measured the percentages of CD3+CD4+ in blood and in total spleen lymphocytes. They resulted non different from healthy individuals (data not reported). The percentage of suppression of Tregs on the proliferation of effector T-cells was comparable in patients with PMF (38.7%) and HS (36.5%) indicating that Tregs isolated from patients were functionally active. In patients bearing *JAK2*V617F mutation (N=126) we observed a higher frequency of circulating Tregs than those non mutated (N= 74; median, 1.00 *vs* 0.70; P= 0.002), and patients with high *JAK2*V617F allele burden (≥50%) had higher frequency of circulating Tregs than those with low allele burden (P= 0.07). In *CALR* mutants, the reduction of circulating Tregs was statistically associated with older age, longer disease duration, and higher IPSS/DIPSS prognostic score, and a strict direct correlation between Treg frequency and haemoglobin was documented.
Summary/Conclusions: Our findings showed that a large fraction of PMF patients had reduced frequency of Tregs, a non clonal component of bone marrow and spleen microenvironment. Blood Treg cell defect was particularly evident in patients lacking *JAK2*V617F mutation: in *CALR* mutated patients the defective Treg percentage was associated with an advanced and prognostically unfavourable disease, and strongly correlated with severity of anemia. These results open new research perspectives and support new therapeutic strategies aimed at increasing the number of Tregs in PMF.

Myeloproliferative neoplasms - Clinical

E1339

IMPACT OF SPLENOMEGALY ON MPN SYMPTOMS AND ASSOCIATION WITH CLINICAL FEATURES: AN ANALYSIS BY THE MPN QUALITY OF LIFE INTERNATIONAL STUDY GROUP

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Background: Splenomegaly (SM) is frequently observed in Myeloproliferative Neoplasms (MPNs). Many MPN symptoms have been attributed to splenomegaly but studies exploring these associations are lacking.
Aims: We sought to evaluate how the presence of severe splenomegaly (>10cm below costal margin [BCM]) impacts patient symptomatology and relates to other clinical features.
Methods: Data was collected among an international cohort of MPN patients including polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) without splenectomy as reported previously (Emanuel et al., JCO 2013). Subjects completed BFI and MPN-SAF instruments. Items were scored on a 0 (absent) to 10 (worst imaginable) scale. For individuals completing at least 6 of the 10 MPN-SAF TSS items, the survey was scored by multiplying the average score across items by 10 to achieve a 0 to 100 scaled score. Demographics, clinical features and symptom scores were compared between splenomegaly subgroups using ANOVA F-tests or chi-squared tests. Splenomegaly was assessed by clinicians based on physical examination and reported as an estimated value BCM. Patients were categorized into 'No-SM' (non-palpable), 'Mild-SM' (1-10cm) and 'Severe-SM' (>10cm).
Results: *Demographics*: A total of 2043 patients were included in the analysis (No-SM, n=1462; Mild-SM, n=404; Severe-SM, n=177) with a mean spleen size of 2.6 cm BCM (range 10.1-31.0 cm). Patients were of expected age (59.7 years) and similar gender distribution (54.6% female). MPN subtypes included ET (41.9%), PV (35.2%) and MF (22.9%). The most prevalent prognostic risk group for each subtype was as follows: ET, intermediate (46.1%); MF, Int.-1 (54.9%); PV, High (49.2%). Compared to a history of prior thrombosis (20.3%), anemia (8.7%), leukopenia (9.9%), thrombocytopenia (11.0%), red blood cell transfusions (5.8%) and prior hemorrhage (5.0%) were relatively uncommon. *Clinical Correlations*: When compared to No-SM populations, patients with Severe-SM were most likely to be >60 years (54.5% *vs* 45.5%, p=0.015) and have MF (62.7% *vs* 10.8%, [p<0.001]). Severe-SM patients were also most likely to suffer from anemia (29.3% *vs* 4.0%), leukopenia (16.4% *vs* 7.5%) and thrombocytopenia (34.2% *vs* 7.1%, all p<0.001), along with prior hemorrhages (8% *vs* 4.2%, p=0.029) and have the highest transfusion requirements (15.3% *vs* 2.8%, p<0.001). In contrast, No-SM patients were most likely to have a history of prior thrombosis (20.1% *vs* 14.3%, p=0.046). SM did not correlate with gender or individual prognostic risk categories. *Symptoms*: Severe-SM was associated with the highest Total Symptom Scores (TSS, 28.5 *vs* 16.3 in No-SM, p<0.001) and individual symptom scores for all items assessed, with the exception of fever and bone pain (both p>0.05; Figure 1). Highest scores were noted for fatigue (4.9 *vs* 4.1, p=0.006), early satiety (3.6 *vs* 2.3, p<0.001), inactivity (3.4 *vs* 2.2, p<0.001), and impact on overall quality of life (3.8 *vs* 2.7, p<0.001). Abdominal discomfort (2.9 *vs* 1.7, p<0.001) and weight loss (1.9 *vs* 1.1, p<0.001) scores were of relatively low severity in comparison to other complaints.

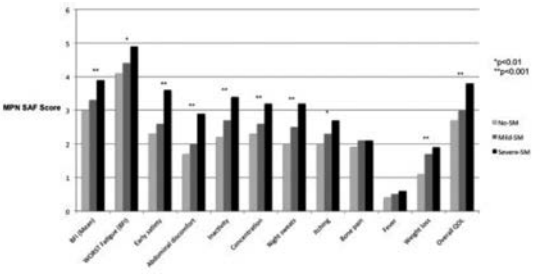

Figure 1.

Summary/Conclusions: Spleen size directly correlates with symptoms unrelated to the abdominal cavity and overall MPN disease burden. The lack of association between spleen size and prognostic risk scores suggests low risk patients may still suffer from significant, undermanaged symptomatology.

E1340

ANALYSIS OF CLINICAL PHENOTYPE AND OUTCOME IN CALR *VERSUS* JAK2 MUTATED OR TRIPLE-NEGATIVE ESSENTIAL THROMBOCYTEMIA

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Background: About 85% of patients (pts) with Essential Thrombocythemia (ET) harbors one of three driver mutations: JAK2, calreticulin (CALR) and MPL; the remaining are defined "triple-negative". Recent studies have analyzed the clinical and hematological features of ET or primary myelofibrosis (MF) according to mutational genotype. However the clinical impact of these mutations seems to differ between cases of ET and MF. In particular controversial data were reported regarding the risk of MF progression in triple-negative and CALR mutated ET.
Aims: We retrospectively analyzed a cohort of 350 ET pts, diagnosed between 1990 and 2015 according to WHO classification, and we compared the clinical phenotypes and outcome of CALR mutant cases with the group of JAK2V617F positive and triple-negative pts.
Methods: Hematological parameters, cardiovascular factors, IPSET score, microvascular symptoms and thrombotic complications were reported for each cohorts of ET pts, as well as cytoreductive treatment. During follow-up, progression to MF and leukemic evolution were also recorded. Mann-Whitney test was used for numerical comparisons while Chi-square test was used for nominal ones. Overall survival (OS) and myelofibrosis-free-survival (MFS) and Leukemia-free-survival (LFS) were estimated by Kaplan–Meier analysis and compared with the Log-rank test.

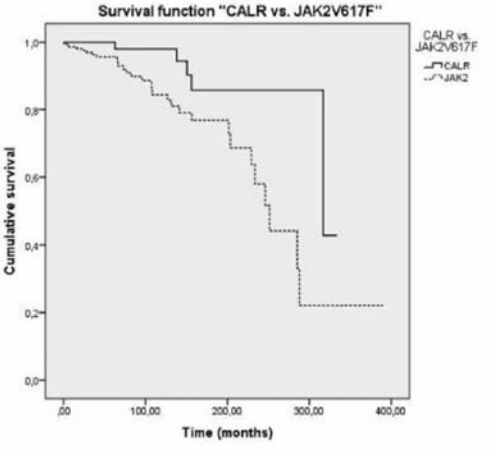

Figure 1.

Results: Among 350 ET pts, CALR mutations were detected in 69 (19.7%), JAK2V617F mutations in 216 (61,7%) and MPL mutations in 13 (3.7%). 52 (15%) pts were triple-negative. The median follow-up was 77,5 months, with a longer time of observation for CALR mutants (median 125 months). JAK2-mutated ET had a higher risk of thrombosis at onset, which was about three times higher than CALR mutated pts or triple-negative ET (20,4% *vs* 7,2% *vs* 17,3% respectively, p=0,05). No difference between JAK2, CALR mutated and triple-negative ET was identified in terms of incidence of recurrent thrombosis during follow-up. Compared to those with JAK2 mutation, CALR-mutated ET

pts were characterized by younger age (p=0,012), lower leukocyte count (p<0,0001) and hematocrit (p<0,002) and higher platelet count (p<0,0001) and presented a lower IPSET score (p<0,0001). Triple-negative pts showed a low IPSET score, similar to CALR-mutated pts; they had a lower incidence of microvascular symptoms (13.5%) in respect to JAK2 (39,8%) and CALR-mutated (34,8%) pts (p<0,0006 and p=0,014, respectively), with a low intensity of cytoreductive treatment. Interestingly, CALR-mutated ET presented an increased risk of progression to MF (24,6%) in comparison with JAK2V617F positive (5,6%) and triple-negative (7,7%) ET (p<0,0001). CALR type 1 deletion seemed to be strongly associated with MF evolution (15/17 pts). Median MFS seemed shorter in JAK2 positive pts (79,77 months) compared to CALR-mutated (106,44 months) and triple-negative ET pts (96,04 months). As shown in Figure 1, we finally documented a better OS in CALR pts than in JAK2 positive group (median OS 317 *vs* 251 months respectively; p=0,007). No difference in LFS according to different genotypes was demonstrated.

Summary/Conclusions: This study in a large cohort of ET supports and extends the growing body of evidence showing that different mutational genotypes in ET present significant differences with regards to clinical and hematologic presentation as well as risk of MF progression. Even though OS was higher, *CALR* mutation-positive patients appear to have a higher risk of evolution to MF compared to the *JAK2* V617F-positive and triple-negative pts.

E1341

A MULTICENTER EVALUATION OF THE REVISED 2015 WHO MINOR CRITERIA FOR PREFIBROTIC PRIMARY MYELOFIBROSIS

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Background: The gold standard for the diagnosis of prefibrotic primary myelofibrosis (prePMF) remains the histomorphologic assessment of bone marrow (BM) biopsy samples. Clinically, especially early cases are difficult to distinguish from other myeloproliferative neoplasm (MPN), in particular essential thrombocythemia (ET). Most prominently, thrombocyte counts are not different in those two entities. However, several clinical features are frequently encountered in prePMF, that are not routinely found in ET. The revised 2015 WHO diagnostic criteria recognize four of these features as minor criteria for prePMF, at least one of which must be present to establish the diagnosis: anemia, leukocyte counts ≥11 G/L, elevated lactate dehydrogenase levels (LDH) as well as palpable splenomegaly.

Aims: The aim was to evaluate the new WHO minor criteria for prefibrotic primary myelofibrosis on a cohort of histomorphologically characterized patients.

Methods: From a multicenter database, 161 cases of prePMF in which the diagnosis had been established through BM biopsy, were selected. The presence of the minor criteria was then assessed at diagnosis. The results were compared to 186 patients diagnosed with ET from the same database. A fiber grade of 1 was present In 54 of prePMF patients, while no ET patients showed increased fibrosis in the BM.

Results: One or more minor criteria could be found in 90.7% (146/161) patients with prePMF, while this was true for only 52.7% (98/186) of ET cases. In both entities elevated LDH levels were the most prevalent feature (77.0% *vs* 29.0%, p<0.001), followed by high white blood cell counts. There were no significant differences between patients with fiber grade 0 or 1, except in splenomegaly (37.4% *vs* 42.9%, p=0.030). The number of minor criteria increased with age. While prePMF patients with one criterion were aged 60 years, patients that displayed all four symptoms were aged 74 years. Accordingly, overall survival was shorter in patients with a higher disease burden.

Table 1.

Parameter	prePMF		ET		p-value
	%	n	%	n	
Anemia (Hemoglobin m/f ≤ 13/12 g/dl)	26.7	43	8.6	16	<0.001
Splenomegaly (palpable or increased length diameter by imaging analysis ≥13 cm)	43.5	70	11.3	21	<0.001
LDH (above upper institutional limit)	77.0	124	29.0	54	<0.001
WBC ≥ 11 x 10^9/L	47.2	76	25.8	48	<0.001

Summary/Conclusions: The revised minor criteria for prePMF could be well reproduced in this population. In combination with the histomorphologic assessment, they are suitable to correctly establish the diagnosis of prePMF. However, there is a small subgroup of prePMF patients that show no minor criteria at time of diagnosis. These patients should be monitored closely and their diagnosis should be re-evaluated if they should present with one of the features. The proportion of ET patients that also display one or more minor clinical symptoms underlines the central role of histomorphologic differentiation of MPN patients.

E1342

THE MEGAKARYOCYTIC INDEX: A NEW TOOL FOR THE DIAGNOSIS OF ESSENTIAL THROMBOCYTHEMIA

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Background: The 2008 WHO diagnostic criteria of Essential Thrombocythemia (ET) require the integration of clinical, molecular and histological parameters. Megakaryocytes (Mk) are the mainstay of bone marrow (BM) histological assessment, as they are present at increased numbers and display peculiar cytological features. While the morphology of ET-associated Mk has been widely studied, their objective quantification may prove challenging (e.g. biased count due to variable BM cellularity in patients of different age).

Aims: This study aimed to: (i) identify a quantitative parameter to define the number of Mk, irrespective of BM cellularity; (ii) define a Mk threshold to objectively discriminate between ET from non-neoplastic BM samples.

Methods: The present study considered BM biopsies from: (i) 89 patients with WHO-defined ET; (ii) 6 patients with secondary/reactive thrombocytosis (ST); and (iii) 22 controls with normal platelet counts and no evidence of BM disease. For the aims of this study, each biopsy was assessed for the following histological parameters: (i) BM cellularity; (ii) total number of Mk; (iii) BM biopsy area (as measured by digital imaging techniques). These parameters were used to calculate Mk density (ratio between total number of Mk and BM biopsy area) and its cellularity-normalized value (here referred to as "megakaryocytic index"), calculated as: Total number of Mk/(BM biopsy area) x (BM cellularity). Statistical analysis was performed using one-way ANOVA and ROC curve analysis.

Results: Differences in BM cellularity among ET and control cases were not statistically significant. ET patients had higher values of Mk density and Mk index compared to both ST and normal controls (Table 1) (p<0.01). At ROC curve analysis, Mk density had limited efficacy in distinguishing ET from non-neoplastic BM samples (threshold value: 10.5/mm^2; sensitivity: 89.9%; specificity: 90.9%). Better results were obtained with the Mk index (threshold value: 25.5/mm^2; sensitivity: 94.4%; specificity: 95.5%).

Table 1. Histological parameters (mean±SD) of ET, ST and control marrow biopsy samples.

	ET	ST	Controls
Cellularity (%)	42.6 ± 17.3	61.7 ± 13.3	34.3 ± 18.8
Mk density (n°/mm^2)	20.7 ± 9.4	9.6 ± 3.3	6.1 ± 2.9
Mk index	52.3 ± 24	15.3 ± 2.9	18.9 ± 5

Summary/Conclusions: BM cellularity can greatly vary among patients, mainly as a result of the age-related decrease of hematopoietic elements. This may impair the evaluation of the Mk number (a key feature for the diagnosis of ET and other myeloproliferative neoplasms). Our model allows the quantification of Mk, irrespective of BM cellularity. In particular, a megakaryocytic index ≥25.5/mm^2 can sensitively and specifically discriminate between ET and non-neoplastic controls. Poorer results have been obtained by Mk density. In conclusion, the megakaryocytic index may represent a valuable tool for the histological diagnosis of ET.

E1343

MOLECULAR CHARACTERIZATION OF ESSENTIAL THROMBOCYTHEMIA PATIENTS EVOLVED TO POLYCYTHEMIA VERA

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Background: *JAK2*V617F-mutated essential thrombocythemia (ET) and polycythemia vera (PV) represent a biological spectrum of the same *JAK2*V617F disease modulated by several acquired and constitutional factors. In this sense, the phenotypic transformation of ET to PV occurs in 3-10% of patients. There is scarce information comparing the clinical and molecular profile of this group of patients, at baseline and at the time of transformation.

Aims: To characterize the clinical and molecular profile of a group of 10 ET patients who evolved to post-ET PV, before and after transformation.

Methods: From a whole cohort of 262 ET patients, 10 cases that evolved to post-ET PV (3.8%) were included in the study. In all patients, PV diagnosis was established according to the demonstration of an increased red cell mass (>125%). *JAK2*V617F was analyzed by quantitative allele specific PCR in granulocytes at ET and post-ET PV diagnosis. To determine acquisition of 9p loss of heterozygosity (LOH), breakpoints for chromosome 9p were mapped using fluorescence microsatellite PCR. Targeted next generation sequencing (NGS) was used to detect additional non-driver somatic mutations. Clonality based on X-chromosome inactivation pattern by *HUMARA* and *JAK2* 46/1 haplotype were also analyzed. The study was approved by the local Ethics Committee and informed consent was obtained according to the Declaration of Helsinki.

Results: All 10 patients (male/female: 2/8) initially diagnosed with ET according to WHO criteria fulfilled the criteria for PV after a median of 75 months (range:

13-305). One patient started cytoreductive treatment prior to the transformation. The main clinical and hematological data at the time of ET and post-ET PV are summarized in Table 1. *JAK2*V617F evolutionary pattern was available in 8 patients. In all instances, a progressive increase in the *JAK2*V617F allele burden was documented. Mitotic recombination of 9p was observed in 4/10 patients (40%) and 3 of them corresponded to the patients with higher *JAK2*V617F increase. Mutations in *ASXL1* (n=1), *DNMT3A* (n=1) and *TP53* (n=1) were detected at the time of transformation. Clonal hematopoiesis could not be demonstrated in any case. The *JAK2* 46/1 haplotype was present in 7 out of 8 available patients (87.5%), 4 in heterozygosis and 3 in homozygosis.

Table 1. Main clinical and hematological features at the ET and Post-ET PV diagnosis.

	ET	Post-ET PV	*p*
Age (years)	42 (32-63)	56 (40-75)	0.003
Hemoglobin (g/l)*	147 (123-166)	171 (140-192)	0.001
Hematocrit (%)*	45.1 (38-49)	52.7 (45.6-58.9)	<0.001
Platelet count (x10^9/l)*	615 (513-1064)	916 (533-1347)	0.097
Leukocyte count (x10^9/l)*	9.86 (6-15)	12.9 (8.7-18.2)	0.042
***JAK2*V617F allele burden, (%)**	30.5 (16.9-42.3)#	47.4 (25.5-84.9)	0.006

**Median (range). # Available in 8 patients. M: male. F: female. ET: Essential thrombocythemia. Post-ET PV: polycythemia vera post essential thrombocythemia.*

Summary/Conclusions: An increase in *JAK2*V617F allele burden is observed in all ET patients transforming to PV and may be accompanied by other mechanisms such as acquisition of 9p LOH or additional somatic mutations.
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E1344

PALPITATIONS AND ARRHYTHMIA IN 3649 HIGH-RISK PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: RESULTS FROM THE PROSPECTIVE LONG-TERM OBSERVATIONAL EXELS STUDY

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Background: The Evaluation of Xagrid Efficacy and Long-term Safety (EXELS) study (NCT00567502) is the largest prospective observational cohort of high-risk patients with essential thrombocythemia (ET) reported to date. Although anagrelide (ANA) is typically well tolerated, as patients with ET often have cardiac risk factors, it is important to analyze ANA's cardiac safety in these patients.
Aims: The primary objective of EXELS was safety and pregnancy outcomes of ANA compared with other cytoreductive therapies (CRT). Here we assess palpitations and arrhythmia events as reported in EXELS.
Methods: High-risk patients (≥1 of age >60 years, previous thrombotic event, platelet count >1000x10^9/L) with ET were enrolled across 13 countries in Europe from 2005-2009. Patients were required to be receiving CRT. Data, including events predefined in the protocol (PDEs), were collected every 6 months for 5 years for all patients.
Results: 3649 patients were categorized according to treatment at registration as follows: ANA (n=804), ANA+other CRT (n=141), other CRT (n=2666). Over 80% of patients received either hydroxycarbamide (HC) or ANA, and 69.8% of patients received anti-aggregatory therapy. At registration, median age was lower in the ANA (55.5 years) and ANA+other CRT (59.0 years) groups *vs* the other CRT group (70.0 years). Due to the non-interventional nature of the study, patients were able to switch treatments and did not necessarily remain in the same group over the entire observation period. As such, the analysis of palpitations and arrhythmia events were performed according to the treatment group patients were on for at least one day during the study. Higher rates of palpitations were observed in patients receiving ANA alone (1.69%, n=19) *vs* other CRT alone (0.21%, n=6; Table). Similar differences were observed for tachycardia# events; 2.04% of patients (n=23) receiving ANA experienced tachycardia compared to 0.24% (n=7) of those receiving other CRT. There did not appear to be a clear difference in rates of supraventricular arrhythmia* events observed between patients receiving ANA (1.06%, n=12) or other CRT (1.34%, n=39). This was also observed with ventricular arrhythmias** with no apparent difference between the ANA only group (0.09%, n=1) *vs* other CRT (0.10%, n=3). Very low rates of other arrhythmias*** were observed across all groups. Similar rates of palpitations, tachycardia and arrhythmias (supraventricular, ventricular and other) were seen in patients receiving ANA+other CRT compared with ANA alone.

Table 1.

	ANA only (n=1127)		Other (n=2909)		ANA + other (n=451)		No treatment (n=645)	
	Patients n (%)	Events (n)	Patients n (%)	Events (n)	Patients n (%)	Events (n)	Patients n (%)	Events (n)
Palpitations	19 (1.69)	21	6 (0.21)	6	8 (1.77)	8	1 (0.16)	1
Tachycardia#	23 (2.04)	26	7 (0.24)	8	6 (1.33)	6	2 (0.31)	2
Supraventricular arrhythmia*	12 (1.06)	13	39 (1.34)	51	4 (0.89)	4	5 (0.78)	6
Ventricular arrhythmia**	1 (0.09)	1	3 (0.10)	3	0	0	0	0
Other arrhythmia***	3 (0.27)	3	3 (0.10)	4	1 (0.22)	1	1 (0.16)	1

#Tachycardia comprises tachycardia, tachycardia paroxysmal and tachyarrhythmia (not otherwise defined).
**Supraventricular arrhythmia comprises atrial fibrillation/atrial flutter, arrhythmia supraventricular, supraventricular extrasystoles, supraventricular tachycardia and sinus tachycardia.*
***Ventricular arrhythmia comprises ventricular fibrillation/ventricular tachycardia/ventricular extrasystoles.*
****Other arrhythmia comprises arrhythmia (not otherwise defined), bradycardia, extrasystoles, sinus bradycardia.*

Summary/Conclusions: Of the most commonly reported cardiac PDEs, palpitations and tachycardia were more frequently observed with ANA *vs* other CRT. Arrhythmia events (supraventricular, ventricular and other) were observed at similar rates in the ANA and other CRT groups. Palpitations, tachycardia and arrhythmia events were reported at a similar rate in the ANA+other CRT group compared with the ANA group. While there may be an increase in specific cardiac events with ANA, these are mostly mild and reversible with appropriate treatment.

E1345

SYMPTOMS, RISK CLASSIFICATION, AND SPLEEN SIZE IN JAK2 INHIBITOR-NAÏVE MYELOFIBROSIS: IMPLICATIONS FOR JAK2 INHIBITOR TREATMENT

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Background: Symptom burden in myelofibrosis (MF) is severe and a risk factor for mortality (Blood 2010;115(9):1703-8). In trials comparing JAK2 treatment to best available therapy, patients receiving JAK2 therapy had significant alleviation in disease related symptom burden and decreased spleen size (N Engl J Med 2012;366:787-798).
Aims: To date no studies have evaluated which cutoffs are most associated with Dynamic International Prognostic Scoring System (DIPSS) risk score and thus may identify patients for further symptom-directed treatment.
Methods: Patient demographics, symptom burden, DIPSS risk classification, and spleen size were collected from MF patients at an office visit. Symptoms were assessed via the MPN-10 total symptom score (TSS, JCO 2012;30(33)4098-103). The TSS was calculated as the summated 10-item score, reported in a range of 0-100 for patients completing at least 6/10 symp-

tom items. Associations of symptoms (worst single symptom score and TSS utilizing various cutoffs) with DIPSS risk and spleen size (categorized as >10cm and separately as >15cm) were investigated using ANOVA F-tests and ordinal logistic regression. Lowest Akaike Information Criteria (AIC) was used to select an optimal model among all a priori models.

Results: Demographics and symptom burden: 851 MF patients with known DIPSS risk score or spleen size were selected, of which 695 were JAK2 inhibitor-naïve (420 with DIPSS risk, 425 with known spleen size). Among participants, 58% had a TSS >20. The worst single symptom (or multiple worst if tied) was most often fatigue (50%) followed by night sweats (19%) and satiety (19%). The worst single symptom score was >5 for 57% patients, and 50% had both a TSS >20 and worst single symptom score >5. Associations of symptoms with DIPSS risk and spleen size: Similar to prior published data, increased TSS was significantly associated with higher DIPSS risk score (p<0.001) with mean TSS of 15.8, 24.5, 30.1, and 37.7 for low, int-1, int-2, and high risk groups, respectively. Increased TSS was also significantly associated with spleen size (>10cm: mean TSS 25.2 *vs* 30.0, p=0.02; >15cm: 25.5 *vs* 32.9, p=0.004). Increased worst single symptom severity was associated with higher DIPSS risk score (mean worst single symptom score 5.0 *vs* 6.3 *vs* 7.0 *vs* 7.5, p<0.001) as well as the largest spleen size (>10cm: 6.3 *vs* 6.8, p=0.11; >15cm: 6.3 *vs* 7.3, p=0.02). Ordinal logistic regression modeling of DIPSS risk: When comparing models, worst single symptom score >5 had the lowest AIC (Table 1), suggesting this as an optimal model predicting increased risk. In this model, worst single symptom score >5 was associated with 14.1%, 49.3%, 32.3%, and 4.3% probabilities of being low, int-1, int-2, and high risk, compared to 20.8%, 52.8%, 23.7%, and 2.7% for single score ≤5. The next best model was based on a TSS score >20 alone (AIC 936.657). Among combined TSS and single item models, a combined TSS >20 and single item >5 had the relative lowest AIC (938.51).

Table 1. Ordinal logistic regression models of DIPSS risk score (N=420) by symptoms in JAK2-naïve myelofibrosis patients.

Model	DIPSS Risk AIC
TSS >20	936.657*
Worst single symptom score >5	935.281*
Worst single symptom score >6	942.198
Worst single symptom score >7	942.684
TSS >20 & single score >5	938.510
TSS >20 & single score >6	943.335
TSS >20 & single score >7	944.867

*Optimal models based on lowest AIC.

Summary/Conclusions: Alleviation of MPN symptoms, now measurable through standardized instruments such as the TSS, are an integral part of assessing therapeutic impact in both clinical practice and trials. In our modeling, a cutoff criteria of the worst single symptom being >5/10 is optimal for predicting increased DIPSS risk score suggesting that this criterion may differentiate between which patients will most benefit from symptom-based treatment. We propose that JAK2 inhibitor treatment be strongly considered in any JAK2-inhibitor naïve MF patient with an individual symptom score >5.

E1346

OSTEOPROTEGERIN (OPG) AND INTERLEUKIN-8 (IL-8) IMMUNOSTAINING IN BONE MARROW (BM) BIOPSIES OF PATIENTS WITH PH-NEGATIVE MYELOPROLIFERATIVE DISORDERS (MPD) AT DIAGNOSIS: CLINICAL CORRELATIONS

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Background: Both IL-8 and OPG have been implicated in the pathogenesis and clinical manifestations of myelofibrosis (MF), the most severe Ph(-) MPD. IL-8 and its receptors were reported to promote altered megakaryocyte (MGK) growth (Emadi et al), a typical MF finding, while OPG was found upregulated in endothelial cells (Bock et al).

Aims: To evaluate the expression and intensity of IL-8 and OPG immunostaining in BM biopsy of MPD patients at diagnosis and to study any eventual correlations between them and clinical characteristics or outcome.

Methods: 78 patients were studied, diagnosed with MPN from 1984 to 2012 with a median follow up period of 55 months. Median age was 65 years, 49 were males. 22% were diagnosed with polycythemia vera (PV), 42% with essential thrombocytosis (ET) and 36% with MF. Of those, 58% had splenomegaly, 8% had bone pain, 81% presented with fatigue, 9% had sweating, 11.5% experienced a thrombotic event, 5% lost weight and 77% were JAK2 positive. Karyotype was abnormal in 32% while 7% presented unfavourable karyotype findings. Immunostaining for IL-8 and OPG was performed on paraffin-embedded 4μm sections of formalin fixed BM biopsies, carried out at the time of diagnosis, using the two-step peroxidase conjugated polymer technique. Grade of positivity and intensity of IL-8 and OPG expression was scored according to a 0 to 3 scale. Statistical analysis was performed conventionally, using the SPSS version 22.0 package, and p values<0.05 were considered significant.

Results: There was no significant difference observed concerning the grade of expression of both stainings among the different MPN-groups. High intensity of IL-8 expression in MGK correlated, in the whole cohort, with a high neutrophil count (p=0.029), low lymphocyte count (p=0.026), and increased haemoglobin (p=0.02). OPG was also expressed in cells of myeloid lineage (MLC) and its high intensity in MLC was observed in patients with high platelet counts (p=0.015). High intensity of OPG expression in MGK correlated with high neutrophil counts (p=0.045), high hemoglobin (p=0.045), high platelet counts (p=0.02), and a favourable karyotype (p=0.006). Most importantly, OPG low intensity in MGK correlated with poor survival (p=0.012). There was no correlation found between OPG and bone pain.

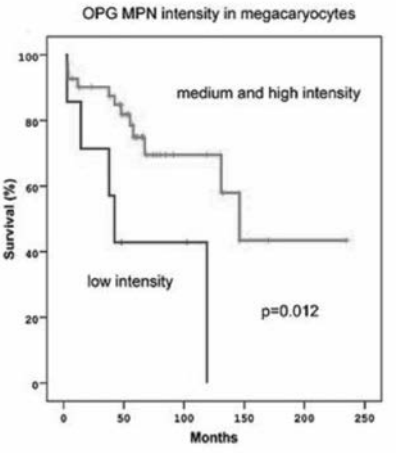

Figure 1.

Summary/Conclusions: Our most important finding is that high intensity of OPG expression in MGK correlated with increased survival and a favourable karyotype in patients with MPDs (PV, ET and MF). To the best of our knowledge, this finding has not been reported yet. It should indeed be further validated.

References

1. Emadi et al. IL-8 and its CXCR1 and CXCR2 receptors participate in the control of megakaryocytic proliferation, differentiation, and ploidy in myeloid metaplasia with myelofibrosis. Blood. 2005 Jan 15;105(2):464-73;
2. Bock et al. Osteosclerosis in advanced chronic idiopathic myelofibrosis is associated with endothelial overexpression of osteoprotegerin. Br J Haematol. 2005 Jul;130(1):76-82.

E1347

MYELOPROLIFERATIVE NEOPLASMS AND THROMBOSIS: A DANISH COHORT STUDY

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Background: Arterial (AT) as well as venous thromboembolism (VTE) are causes of morbidity and mortality in patients with myeloproliferative neoplasms (MPN). Despite cytoreductive therapy and antiplatelet drugs MPN patients seem more prone to develop both AT and VTE than the background population. The influence of known cardiovascular risk factors on the risk of thrombosis in MPN patients is debated.

Aims: To investigate the absolute and relative risks of AT and VTE in MPN patients compared to matched controls in a prospective population based cohort study, adjusting for cardiovascular risk factors.

Methods: The Diet, Cancer and Health (DCH) Study is a Danish prospective population based cohort study enrolling 57.053 subjects between 1993-1997. Follow-up for this study was until 2008. Subjects were excluded if they had pre-baseline history of AT, VTE or cancer. Diagnosis of MPN was identified by linking to the Danish National Patient Registry, which provides information on date of diagnosis coded in the ICD-8 or ICD-10 system. All identified cases of

VTE and AT, which in this study was either myocardial infarction (MI) or stroke, have been objectively confirmed by trained personnel looking through medical records or discharge letters, biochemical investigations and diagnostic images. Information on cardiovascular risk factors was obtained by self-administered questionnaires at baseline. Biometric measures were obtained by trained technicians at enrolment. For each MPN patients five participants matched on age and sex were identified in the DCH cohort. We used Cox regression models to estimate the relative risk of AT and VTE in MPN patients.
Results: During follow-up 72 women and 71 men were diagnosed with MPN, mean age was 56.8 years. Among MPN exposed subjects 10 MI's, 6 cases of stroke and 3 VTE events were observed after MPN diagnosis, corresponding to incidence rates of 15.4 events per 1000 person years (10^{-3} p-y), $9.3*10^{-3}$ p-y and $4.4*10^{-3}$ p-y. In the Cox regression analysis MPN was associated with a significantly higher risk of MI, Hazard Ratio (HR) 5.7, 95% confidence interval (95%CI) 2.4-13.7. The HR for stroke among MPN exposed was 2.2, 95%CI 0.8-5.7. The observed VTE events resulted in a HR of 5.6, 95%CI 1.1-27.8 in the regression analysis. Our study was underpowered regarding the adjusted regression analysis.

Table 1. Absolute and relative risks of thrombosis.

	MI	Stroke	VTE
MPN exposed (N= 143)			
n	10	6	3
Incidence ($*10^{-3}$ p-y), (95% CI)	15.4 (8.3-28.6)	9.3 (4.2-20.7)	4.4 (1.4-13.7)
Matched controls (N= 715)			
n	10	15	3
Incidence ($*10^{-3}$ p-y), (95% CI)	2.6 (1.4-4.8)	4.0 (2.4-6.7)	0.8 (0.3-2.4)
Relative risk of thrombosis			
HR, (95% CI)	5.7 (2.4-13.7)	2.2 (0.8-5.7)	5.6 (1.1-27.8)
p value	< 0.001	0.11	0.035

Summary/Conclusions: In our prospective cohort study a 6-fold increased risk of MI was observed among MPN patients. The risk of stroke was increased by 2-fold, however not statistically significant. The risk of VTE was increased by 6-fold in MPN patients, however the number of events in this study was too few for adjusted analysis.

E1348

PROCOAGULANT MICROPARTICLE IS A NEW SURROGATE BIOMARKER FOR THROMBOTIC EVENTS IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background: Previous studies have identified several clinical and genetic markers for thrombosis in myeloproliferative neoplasm (MPN) patients, such as age >60 years, thrombosis history, cardiovascular risk factors, and the presence of JAK2 mutation. Microparticles (MPs) are circulating small vesicles released from the membranes of activated or apoptotic cells, which contribute to thrombosis with their procoagulant activities. However, to date, there have been a very few reports evaluating the relationship between the levels of MPs and procoagulable state in MPN patients.
Aims: In this study, we characterized MPs in MPN patients and assess their relationship with the thrombotic events in MPN patients in comparison with previously reported risk factors in the practical setting.
Methods: We analyzed 52 patients with MPNs: 17 with PV, 31 with ET, 1 with Primary myelofibrosis (PMF), and 3 with secondary MF, according to the revised WHO criteria and 12 healthy controls. The patient group consisted of 35 men and 17 women with a median age of 68 years ranging from 30 to 87 (at analyses). Thrombotic events were observed in 16 cases (30.8%). To assess the levels of MPs, we extracted platelet free plasma (PFP), in which MPs were enriched from sodium citrate-anticoagulated blood by centrifugations. Quantification and phenotypic characterization of MPs were performed by Flow cytometry. This study was approved by the ethics committee at Kindai University, and patients provided written informed consent.
Results: There was no difference in the total amount of MPs between MPN patients and controls (MPNs *vs* controls: median 2240 *vs* 3110/μL PFP; p=0.058), whereas MPN patients had more AnnexinV$^+$ or tissue factor (TF)$^+$procoagulant MPs (PCMPs) than controls (median±SD 15.1±6.2% *vs* 11.5±7.4%, p<0.05; 2.5±1.6% *vs* 0.89±0.52%, p<0.05, respectively). The majority of PCMPs derived from platelets and endothelial cells in MPN patients, which was confirmed by positivity for CD41a and CD146, respectively. The levels of AnnexinV$^+$ and TF$^+$ MPs tended to decrease in response to cytoreductive treatment in MPN patients but with no significant difference (n=35: p=0.143, p=0.084, respectively). Also, there was no statistically significant association between PCMP levels and common hematopoietic parameters (e.g. Hb, WBC, and PLT counts), suggesting that PCMPs were elevated independently of excessive hematopoiesis in MPN patients. In addition, none of age, cardiovascular risk factors, and JAK2 mutation showed a statistically significant relationship with thrombosis history in untreated MPN patients (n=17) by univariate analysis. In this setting, only high levels of TF$^+$ MPs showed a strong association with thrombosis history (p<0.01). The ROC analysis revealed that the best cut-off value of TF$^+$ MPs in accordance with thrombosis history was >52.0/μL PFP with 100% sensitivity and 75.0% specificity (AUC: 0.88, 95% CI: 0.71-1.0; p=0.102). These data indicate that elevated levels of TF$^+$ MPs will be a new surrogate biomarker to predict thrombotic events in MPN patients.
Summary/Conclusions: The current study shows the presence of elevated PCMPs in the peripheral circulation of MPN patients, which was supposed to contribute to the thrombotic events in these patients. Moreover, measuring PCMPs, especially TF$^+$ MP levels, seems to be a promising strategy to predict thrombosis in MPN patients. Further studies to analyze their pathologic roles in thrombosis may provide us with a new approach to prevent thrombotic events in MPN patients.

E1349

SPLENOMEGALY AND ELEVATED ALKALINE PHOSPHATASE ARE STRONG AND INDEPENDENT ADVERSE PROGNOSTIC MARKERS IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS

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Background: Systemic mastocytosis (SM) comprises a group of rare myeloid neoplasms characterized by accumulation of mast cells (MC) in various tissues, predominantly skin, bone marrow (BM), and visceral organs. Depending on the affected organ(s), SM-related organ damage, and involvement of other hematopoietic lineages, SM can be divided into indolent SM (ISM) and advanced SM (advSM). The *KIT* D816V mutation is a primary driver of disease manifestations while the presence and number of additional mutations, e.g. in *SRSF2*, *ASXL*, or *RUNX1* (S/A/R gene panel), have a strong adverse impact on disease phenotype and prognosis. However, only little is yet known about the prognostic impact of aberrant clinical, laboratory or hematological parameters.
Aims: We sought to evaluate the influence of organomegaly, as measured by magnetic resonance imaging (MRI)-based volumetry of liver and spleen as well as other known clinical and hematological characteristics, on disease evolution and overall survival (OS) in SM.
Methods: We retrospectively analyzed 108 patients (median age 64 years, range 27-82; male 57%) with ISM (n=41) or advSM (n=67) (aggressive SM/mast cell leukemia [ASM/MCL], n=12 or SM/ASM/MCL with associated hematologic non-mast cell lineage disease [AHNMD], n=55). Median observation time from the date of MRI was 26 months (range 1-79); 33/108 (31%) patients have died in the observation period. The 2-year OS probability of all patients was 77%, with a median OS of 5.4 years.
Results: The median volumes of liver and spleen were 2035 mL (range 1034-4265) and 540 mL (range 92-3193), respectively. Overall, MRI-documented hepatomegaly (>2400 mL) was observed in 31/108 (29%) patients, and splenomegaly in 67/108 (62%) patients (ISM 12%, advSM 88%). Splenomegaly was mild (≥450 mL) in 43/67 (64%) patients and marked (≥1200 mL) in 24/67 (36%) patients. High disease burden, presence of an AHNMD and organ damage (C-findings) were strongly associated with mildly or markedly increased spleen volume (P<0.05). In multivariate analysis, however, only an increased spleen volume ≥450 mL (hazard ratio [HR], 9.4; 95% confidence interval [CI], [2.2-39.9]; P=0.0002) and an elevated alkaline phosphatase (AP; HR 8.4 [3.2-22.1]; P<0.0001) remained independent predictive adverse prognostic markers for OS. The 2-year OS was 100%, 76%, and 56%, respectively (P<0.001), for patients with 0 (low-risk, n=37), 1 (intermediate-risk, n=32) or 2 (high-risk, n=39) parameters. If available (n=74), the relative frequency of mutations in the S/A/R gene panel was significantly different in the various risk groups (low: 0/15, intermediate: 12/24 [50%], high: 28/35 [80%], P<0.01).
Summary/Conclusions: Splenomegaly and elevated AP provide relevant prognostic information in SM patients that is independent of other clinical, laboratory or hematological parameters. In addition to the recently identified adverse prognostic impact of mutations in the S/A/R gene panel, this data will have major implications for the implementation and validation of novel prognostic scoring systems on the basis of larger patient cohorts, such as the dataset of the ECNM registry, with the long-term objective to develop an IPSS for patients with SM.

E1350

DEFERASIROX IN THE TREATMENT OF IRON OVERLOAD DURING MYELOPROLIFERATIVE NEOPLASMS (MPN)

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Background: Deferasirox (DFX) is an oral iron chelator widely employed in the treatment of iron overload during thalassemic syndromes and myelodysplastic syndromes.
Aims: At present, very few data are available on the treatment with DFX in patients with Ph- Myeloproliferative Neoplasms(MPN) and transfusional requirement.
Methods: To address this issue, we report here on 37 patients (M 29; F 8) with MPN and iron overload secondary to transfusional requirement enrolled in the database of our regional cooperative group who received a treatment with DFX. Of them, 33 had a primary Myelofibrosis, 3 a post Essential Thrombocythemia myelofibrotic phase and 1 a post Polycythemia Vera myelofibrotic phase.
Results: According to IPSS classification, 7 patients (18.9%) resulted low/intermediate-1 risk, 13 (35.1%) intermediate-2 risk and 17 (46.0%) high-risk. The main features of the patients at diagnosis and at baseline of DFX treatment are reported in the Table 1.Treatment with DFX was started after a median interval from diagnosis of 12.3 months [interquartile range (IR) 7.6-37.6] and from start of transfusion dependence of 11.0 months (IR 6.0-17.4), with a median of 27 packed red cells units received (IR 17-39). The starting DFX dose was 20 mg/Kg in 21 patients (56.7%), 15 mg/Kg in 11 patients (29.7%) and 10 mg/Kg in 5 patient (13.5%). All patients were evaluable for toxicity: extra-hematological toxicity of all WHO grades was reported in 20/37 patients (54.1%) and consisted of gastro-intestinal symptoms in 7 patients, transient renal impairment in 10 patients and skin reactions in 3 patients: however, only 3 patients (8.1%) needed a permanent discontinuation for toxicity. As to chelation efficacy, after a median treatment period of 15.4 months (IR 8.1-22.3), 4 patients achieved ferritin levels<500 ng/ml, 9 patients ferritin levels<1,000 ng/ml and 3 patients presented a reduction >50% of basal ferritin but with levels >1,000 ng/ml, with a global response rate of 16 out 37 patients (43.2%): among the remaining 21 patients, 2 discontinued for early toxicity, 18 did not have any ferritin reduction and 1 had an early unrelated death (<6 months of treatment). As to hematological improvement, 7/37 patients (18.9%) showed an unexpected and persistent rise of Hb levels >1.5 g/dl, with disappearance of transfusional requirement in 5 cases. The median overall survival of the whole cohort from DFX initiation was 20.7 months (95% CI 16.1-25.1): the median overall survival from DFX initiation in patients with chelation response was 46.9 months (95% CI 23.6-70.1) compared to 15.8 months (95% CI 2.9-28.7) in patients without chelation efficacy (p=0.007).

Table 1.

	DIAGNOSIS	BASELINE
Median age, years [interquartile range (IR)]	69.8 (63.8 – 74.7)	71.1 (67.2 – 76.2)
Median Hb, g/dl (IR)	8.3 (7.2 – 9.7)	7.9 (7.3 – 8.4)
Median ferritin, ng/ml (IR)	439 (150 – 1086)	1486 (1177 – 2209)
Median creatinine level, mg/ml (IR)	NR	1.0 (0.8 – 1.1)

Summary/Conclusions: Treatment with DFX is feasible and effective in MPN with iron overload. Moreover, also in this setting an hematological improvement can occur in a sizeable rate of patients.

E1351

CLINICAL FEATURES OF DIFFERENT GENOTYPES IN A COHORT OF 172 PATIENTS WITH CALRETICULIN-MUTATED ESSENTIAL THROMBOCYTHEMIA

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Background: Calreticulin (CALR) mutations occur in 15-24% of patients with essential thrombocythemia. The 52-bp deletion (mutation type 1) and the 5-bp insertion (mutation type 2) are the most frequent alterations, being 45-53% and 32-39% of all mutations; the remaining types are described in very few patients each. CARL-mutated ET has a higher platelet count, a lower leukocyte count, a lower haemoglobin level, and a lower thrombotic risk in respect to the JAK2 V617F-ET. However, the differences between types 1 and 2 have been rarely investigated (Tefferi A et al, Am J Hematol 2014; 89: E121).
Aims: To assess in a retrospective multicenter cohort of CALR-mutated ET patients the clinical and laboratory features of type 1, type 2, and types non-1/non-2.
Methods: We analysed a retrospective cohort of 172 patients with CALR-mutated ET diagnosed according to the updated WHO 2014 criteria. CALR mutation was type 1 in 91 patients (52.9%) (M/F 46/45, median age at diagnosis 59 yrs, range 18-84), type 2 in 55 patients (32.0%) (M/F 17/38, median age at diagnosis 43 yrs, range 17-85), and type non-1/non-2 in 26 patients (15.1%) (M/F 11/15, median age at diagnosis 46 yrs, range 20-83). Laboratory and clinical features were compared among the groups. The relative risk of thrombosis between groups was estimated by a Cox proportional hazards model.
Results: The total observation time was 1586 pt-years (median 7.5, range 0.1-34 years). As previously reported, male sex and younger age were more present in type 1 mutation than in type 2 (p=0.02 and p=0.002, respectively). On the other hand, no significant difference in sex and age distribution was found between patients with type non-1/non-2 mutation and those with type 1 and type 2. Patients with type 1, type 2, and type non-1/non-2 mutations did not significantly differ in the rate of splenomegaly (10.9% vs.14.5% *vs* 0%), Hb gr/dL (median 13.4, range 9.4-16.5 *vs* median 13.6, range 11-16 *vs* median 13.6, range 10.9-13.4), Hct (median 0.40, range 0.28-0.49 *vs* median 0.41, range 0.33-0.43 *vs* median 0.40, range 0.32-0.48), leukocyte count $x10^9$/L (median 8.43, range 4.63-17.15 *vs* median 8.05, range 3.56-13.40 *vs* median 7.82, range 4.55-15.30), and platelet count $x10^9$/L (median 787, range 454-2000 *vs* median 920, range 465-1743 *vs* median 887, range 453-2042). As for clinical phenotype, patients with type 1, type 2, and type non-1/non-2 mutations did not significantly differ in the rate of microvascular disturbances (17.5% *vs* 16.3% *vs* 15.3%), need of cytoreduction (71.4% *vs* 72.7% *vs* 73.0%), major bleeding (19.7% *vs* 27.2% *vs* 23.0%), transformation to myelofibrosis (9.8% *vs* 12.7% *vs* 11.5%), occurrence of solid neoplasia (14.2% *vs* 9.0% *vs* 7.6%). No patient evolved to acute leukemia. Thrombosis occurred in type 1 in 26.3% of cases (arterial thrombosis in 14 cases, venous in 8, both sites in 2), in type 2 in 21.8% of cases (arterial thrombosis in 6 cases, venous in 6), and in type non-1/non-2 in 7.6% of cases (arterial thrombosis in 2 cases). In patients with type 1 the risk of thrombosis was increased over time with a borderline statistical significance in comparison with type 2 (hazard ratio, HR, 1.77, 95%CI 0.91-3.64) and type non-1/non-2 (HR 3.31, 95%CI 0.91-6.29).
Summary/Conclusions: Male sex and younger age are confirmed to be more present in type 1 than in type 2. In this cohort of ET patients the type of CALR mutation did not influence significantly other laboratory and clinical features. However, a trend towards a higher thrombotic risk was displayed in type 1, deserving further studies.

E1352

GERMLINE VARIATIONS AT JAK2 AND TERT ARE ASSOCIATED WITH MYELOPROLIFERATIVE NEOPLASMS IN TAIWANESE POPULATION

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Background: In addition to the *JAK2* 46/1 haplotype, a recent study reported that germline variations at *JAK2* rs12339666, *TERT* rs2736100, *HBS1L-MYB* rs9376092 and *MECOM* rs2201862 are associated with myeloproliferative neoplasms (MPNs) in European populations.
Aims: In this study, we sought to evaluate the association of these five germline variations (*JAK2* 46/1 haplotype is tagged by rs12343867) with MPNs in Taiwanese population.
Methods: A total of 178 MPN patients (111 ET, 53 PV and 14 PMF) seen at Mackay Memorial Hospital were enrolled into this study. Genetic testing in MPN patients has been approved by the institutional review board. All patients were screened for $JAK2^{V617F}$, *CALR* and *MPL* mutations. All the above 5 germline variations were genotyped by high-resolution melting analysis and/or Sanger sequencing. Data of healthy controls were obtained from 118 local controls, published papers, and Taiwan Biobank database (https://taiwanview.twbiobank.org.tw). Chi-square or Fisher's exact test were used to analyze difference between patients and healthy controls, and to calculate odds ratios and 95% confidence intervals. Population attributable risks (PAR) were calculated as previously described. The SPSS software (version 22.0) was used for statistical analysis, and a two-side *p* value<0.05 was defined as significant.
Results: The *JAK2* 46/1 haplotype, *JAK2* rs12339666 and *TERT* rs2736100 were significantly associated with MPN cases (*p* value, $7.5x10^{-8}$, $3.2x10^{-8}$ and $3.0x10^{-6}$, respectively), and $JAK2^{V617F}$-positive cases (n=121) (*p*=$7.0x10^{-10}$, $2.7x10^{-9}$ and $5.9x10^{-7}$, respectively). For the whole cohort, the PAR for *JAK2* 46/1 haplotype, *JAK2* rs12339666 and *TERT* rs2736100 were 43.6, 42.1 and 28.5, respectively. In $JAK2^{V617F}$-negative cases (n=55), only the *JAK2* 46/1 haplotype remained statistically significant (*p*=0.044). When stratified by disease subtypes, the *JAK2* 46/1 haplotype, *JAK2* rs12339666 and *TERT* rs2736100 were significantly associated with both polycythemia vera (*p*=$7.9x10^{-10}$, $6.8x10^{-10}$ and $6.1x10^{-4}$, respectively) and essential thrombocythemia (*p*=$1.5x10^{-4}$, $1.8x10^{-3}$ and $4.9x10^{-4}$, respectively). The *JAK2* 46/1 haplotype, *JAK2* rs12339666 and *MECOM* rs2201862 were significantly associated with primary myelofibrosis (*p*=0.021, 0.01 and 0.007, respectively).
Summary/Conclusions: In this cohort, germline variations at *JAK2* (both the 46/1 haplotype and rs12339666) and *TERT* rs2736100 were significantly associated with MPNs in Taiwanese population.

E1353

MORPHOLOGIC DYSPLASIA IS ASSOCIATED WITH A MORE AGGRESSIVE DISEASE PHENOTYPE IN MYELOFIBROSIS

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Background: Primary myelofibrosis (PMF), post-essential thrombocytemia-MF (PET-MF) and post-polycythemia vera-MF (PPV-MF) are Philadelphia-chromosome negative myeloproliferative neoplasms (MPNs). These diseases are frequently associated with driver mutations within the JAK-STAT signaling cascade. However, additional mutations may be acquired during disease progression. MDS/MPNs are distinct myeloid diseases with similar genetic and morphologic characteristics of myelodysplasia and MPNs. To better understand the significance of these mutations, we correlated DNA mutation profiles with bone marrow (BM) dysplasia and clinical outcomes.
Aims: Evaluate the impact of non-driver mutations and BM dysplasia on clinical outcomes in patients with PET-MF, PPV-MF, and PMF.
Methods: We identified 19 patients diagnosed with PMF and 18 patients with PET- or PPV-MF, reviewed their BM biopsies and aspirates, and performed NGS with a 37 gene myeloid panel. At least 2 hematopathologists, blinded to original diagnosis and genetic results, classified the BM biopsies according to WHO guidelines and morphologic dysplasia. A committee of hematologists and hematopathologists reviewed all data and rendered a consensus diagnosis. Statistical comparisons between groups relied on the two-sided student's t-test; the log-rank test was utilized for survival assessments. This study was approved by our IRB
Results: The mean age at diagnosis was 57.1 years and NGS occurred at a median of 2.2 months from diagnosis; the mean length of follow-up is 8.2 years. Fourteen patients (39%) had an abnormal karyotype. *JAK2* mutation V617F was identified in 25 (68%) patients, *CALR or MPL* mutations were present in 8 (22%), and 4 had no identifiable driver mutation. Patients had a mean of 2 mutations; *TET2*, *EZH2*, *IDH1*, *IDH2*, *DNMT3A*, and *ASXL1* represent the most common mutations. For analysis, patients were divided according to the dynamic international prognostic scoring system plus risk: high (7), int-2 (17), and low/int-1 risk (13) disease. Those with high-risk disease were significantly more likely to have BM dysplasia relative to those with low/int-1 disease (77% *vs* 33%, *P:*<0.05) and twice as likely to be re-categorized as MDS/MPN overlap syndrome (67% *vs* 35% and 23%) during consensus review. These patients had a trend toward a higher mutational burden (3* *vs* 2.2 and 1.7* mutations, **P:* 0.09), and were twice as likely to have an unfavorable karyotype (*67% *vs* 41% and *8%, **P:*<0.05). Clinically, these patients experienced a more aggressive disease phenotype with a shortened overall survival (OS) compared to patients with non-dysplastic MF. Five patients in this analysis had *TET2* mutations. *TET2* mutated patients were more likely to have BM dysplasia compared to wild type (WT) (80% *vs* 50%). These patients were younger at diagnosis (mean: 49.3 *vs* 60.4 years, *P*:<0.05), had a higher number of non-driver mutations (3.8 *vs* 1.2, *P*: 0.001), were more likely to have an unfavorable karyotype (80% *vs* 42%) and circulating blasts (40% *vs* 14%). Six year OS was significantly shorter in the *TET2* mutated group *vs* WT (*P:*<0.05).

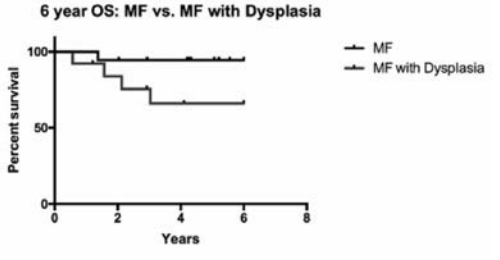

Figure 1.

Summary/Conclusions: Distinguishing between 'MF with dysplasia' and MDS/MPN is a diagnostic challenge because the significance and natural history of MPN dysplasia is poorly understood. Our data demonstrate that this dysplasia is associated with established clinical and genetic markers of aggressive disease, including mutations of *TET2.* Taken together, this suggests that MPN with dysplasia represents a MPN with aggressive disease biology and shortened survival.

E1354

PROPOSAL FOR A REVISED PROGNOSTIC MODEL IN PRIMARY MYELOFIBROSIS THAT ACCOUNTS FOR JAK2, MPL AND CALR MUTATIONS: AN EXPERIENCE OF A SINGLE CENTER IN CHINA

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Background: Primary myelofibrosis (PMF) is a Philadelphia-chromosome-negative myeloproliferative neoplasm (MPN) in which JAK2V617F, CALR and MPLW515 mutations are the most frequent driver mutations. PMF appears to have different clinical features in different population. For example, Chinese patients have different clinical and laboratory features compared with PMF in patients of predominately European descent. These differences may affect prognostic scoring systems which might require revision based on the population being evaluated.
Aims: In current study, we focus on the contribution of driver mutations (JAK2, MPL and CALR) to survival and propose a clinical-molecular prognostic model based on the data from a single center in China.
Methods: 402 consecutive subjects with PMF had a bone marrow sample collected at diagnosis or referral, February 1990 to February, 2015 at the Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences were enrolled. Follow-up data were available for 361 subjects. Median follow-up of survivors was 36 months (range, 1-385) months. Patients were divided randomly into a study group (n=279) and a test group (n=123). The impact of JAK2, MPL or CALR mutations on prognosis was analyzed by univariate- and multivariate survival analyses.
Results: JAK2V617F mutations were detected in 189 subjects (47%), MPLW515 mutations in 13 (3.2%) and CALR mutations in 81 (20.1%). 119 subjects (29.6%) had no detectable mutation in JAK2, MPL or CALR. There were 30 (37%) type-1 and 48 (59.3%) type-2 CALR mutations. In the study group, patients with CALR type-2 mutations or no detectable mutation had briefer survival compared to those with JAK2, MPL or CALR type-1 or other less common CALR mutations (HR 2.99, 95% CI 1.935-4.619, P<0.001). Therefore, patients were categorized into the high-risk with CALR type-2 mutations or no detectable driver mutation and the low-risk without aforementioned mutations status. A multivariate analysis of prognostic factors in the study group identified the following independent factors (P<0.05): Dynamic International Prognostic Scoring System (DIPSS) risk group, palpable spleen and the status of JAK2, MPL or CALR mutations. We assigned each factor a weight: (1) 3 for DIPSS high-risk; (2) 2 for DIPSS intermediate-2 risk; (3) 1 for DIPSS intermediate-1 risk; (4) 0.5 for no splenomegaly, (5) 1 for CALR type-2 mutation or non-mutated JAK2, MPL and CALR. Subjects were categorized into 4 risk cohorts: (1) low (score 0-1; 39.5% of patients); (2) intermediate-1 (score 1.5-2; 34% of patients); intermediate-2 (score 2.5-3; 19% of patients); and high (score ≥3.5; 7.5% of patients; Fig. 2A).The revised clinical-molecular prognostic model divided patients into 4 prognostic groups with significantly different outcomes (Figure 1) and had a significantly higher predictive power for survival than the DIPSS. The model was validated in the test group.

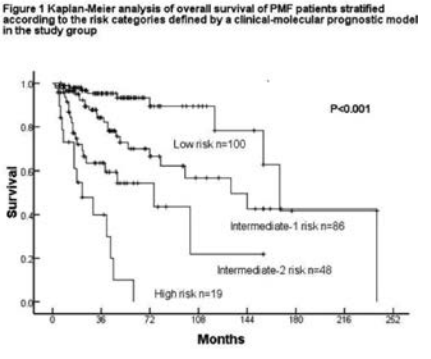

Figure 1.

Summary/Conclusions: This study indicates that the prognostic system including driver mutations (JAK2, MPL and CALR) status could more accurately evaluate survival for patients with PMF.

E1355

INCREASED RISK OF AGE-RELATED MACULAR DEGENERATION IN PATIENTS WITH PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background: Patients with Philadelphia (Ph) -negative chronic myeloprolifer-

ative neoplasms (MPNs) can experience visual symptoms which are caused by microcirculatory disturbances, but the risk of other ophthalmic manifestations such as age-related macular degeneration (AMD) is unknown. AMD causes progressive loss of the central vision and it is the most common cause of blindness in elderly in western countries. Chronic inflammation is involved in the development and progression of both AMD and MPNs, and similar inflammatory pathways are dysregulated. Our previous findings suggest that patients with Ph-negative MPNs have increased prevalence of AMD at time of diagnosis. The potential link between the diseases is possibly explained by the degree of systemic inflammation in patients with MPNs, which may predispose some individuals to develop AMD.
Aims: To determine the risk of AMD in patients with essential thrombocythemia (ET), polycythemia vera (PV), myelofibrosis (MF) and unclassifiable MPN (MPN-U) compared to the general population.
Methods: We undertook an observational matched cohort study and included patients diagnosed with Ph-negative MPNs in Denmark between 1994 and 2013. We included nationwide cohorts of patients with ET, PV, MF and MPN-U by using population-based registries. To compare the risk of AMD, we identified 10 individually sex-and-age matched comparisons from the general population for each patient. We excluded patients and comparisons who had a prior AMD diagnosis or a follow-up time of less than 30 days after the MPN was diagnosed. Everyone was followed until death, December 31, 2013, or until they were diagnosed with any AMD diagnosis at a hospital. Cox proportional hazards models were used to estimate the hazard ratio (HR) of AMD in patients with Ph-negative MPNs (ET, PV, MF, MPN-U) as well as separately for each MPN subgroup. We adjusted for smoking (proxy measure used by including smoking related diagnoses from the Danish National Patient Registry) as smoking is a risk factor for developing AMD. Additionally, we also adjusted for risk-time (1994-2001, 2002-2006, 2007-2013), since new AMD treatments were introduced in Danish hospitals in 2002 and 2007. This could imply that individuals were more likely to be diagnosed with AMD at hospitals after these treatments had been introduced.
Results: We included 7 958 patients with Ph-negative MPNs and 77 445 sex-and-age matched comparisons in the study, after 365 patients and 2 619 comparisons had been excluded. Of the excluded individuals, 222 patients (1 907 comparisons) had a prior AMD diagnosis and 143 patients (712 comparisons) had a follow-up time of less than 30 days. In total, we included: ET=2 628; PV=3 063; MF=547 and MPN-U=1 720 patients. Significant more patients with ET, PV and MPN-U had smoking-related diagnoses compared to comparisons ($p<0.05$), but no difference was seen for patients with MF. Smoking was associated with an increased risk of AMD in both patients and comparisons. We found an increased risk of AMD in patients with Ph-negative MPNs (ET, PV, MF, MPN-U), adjusted hazard ratio (HR) [95% confidence interval] =1.28 [1.10-1.48]. Subset analyses of the HR in each MPN subgroup revealed an increased risk of AMD in each group, but the risk was only significantly increased in patients with PV. The smoking and risk-time adjusted HR (95% CI) was 1.15 [0.88-1.49] for ET; 1.42 [1.15-1.76] for PV; 1.54 [0.67-3.53] for MF and 1.33 [0.93-1.88] for MPN-U.
Summary/Conclusions: Our study indicates that patients with Ph-negative MPNs have increased risk of AMD compared to the general population. We speculate that the increased risk is caused by chronic inflammation, but further studies are needed to investigate the potential causal relationship.

E1356

IMPACT OF JAK2 AND CALR MUTATIONS ON THE DISEASE FEATURES AND OUTCOME AMONG PATIENTS WITH PRIMARY MYELOFIBROSIS OR ESSENTIAL THROMBOCYTHEMIA TREATED WITH STANDARD OF CARE (INCLUDING RUXOLITINIB)

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Background: Primary myelofibrosis (PMF) and essential thrombocythemia (ET) share the same mutations in *JAK2* and *CALR* but differ in their clinical features and outcomes.
Aims: Here, we assessed the impact of *JAK2* and *CALR* mutations on the clinical features at diagnosis and on the outcome of patients with PMF or ET treated with standard of care including the JAK2 inhibitor ruxolitinib.
Methods: We studied the clinical and genetic features of 212 patients (median age at diagnosis, 60 years [y]; range, 22-93 y) with PMF (n=93), ET (n=84) or PMF/ET (n=35). Diagnosis was made applying the WHO 2008 criteria, with no clear assignment to PMF or ET possible for patients with PMF/ET. *CALR* mutations were primarily assessed in patients without *JAK2* V617F. Median follow-up time was 5.9 y. Associations were assessed using the Mann-Whitney U-Test for continuous and Fisher's exact test for categorical variables. Overall survival (OS) and leukemia-free-survival (LFS) were estimated using the Kaplan-Meier-method and compared using the log-rank-test.
Results: In the entire cohort of 212 patients, 58.6% carried a *JAK2* V617F and, of the *JAK2* V617F negative patients tested for *CALR* mutations, 38.3% had a *CALR* type 1/type 1-like and 25.5% a type 2/type 2-like mutation. Patients with *CALR* mutations were of younger age at diagnosis (p=0.003) and of predominantly male gender in comparison to *JAK2* V617F positive patients (58.8% *vs* 48.8%). Patients with *CALR* mutations tended to present more often with splenomegaly at diagnosis than patients with *JAK2* V617F (p=0.19). Considering ET patients only, patients with *JAK2* V617F presented with higher white blood counts compared to those with *CALR* mutation (p=0.0006). Among the PMF patients, those with *CALR* mutations had higher platelet counts at diagnosis than those with *JAK2* V617F (p=0.043). Of the 212 patients, 77.4% received treatment specific for PMF or ET (excluding aspirin); 58% had hydroxyurea, 12.8% received an allogeneic blood stem cell transplantation. Regarding the outcomes, patients with *JAK2* V617F had slightly higher rates of thromboembolic events compared to those with *CALR* mutations (35.8% *vs* 23.5%, p=0.21). There were significant differences in OS and LFS in the PMF cohort with patients with *CALR* mutations having better OS (p=0.047; 10y rates 88.9% *vs* 65.1%) and LFS (p=0.049) than patients with *JAK2* V617F; the difference in the OS remained significant among patients aged >60y (p=0.02). Among the ET patients, we did not observe differences in OS or LFS according to the *JAK2* V617F or *CALR* mutation status. Importantly, PMF patients with *CALR* mutations had a similar risk profile in terms of OS as ET patients (p=0.71), implicating that *CALR* mutations attenuate the generally accepted adverse prognosis associated with the diagnosis of PMF as opposed to that of ET. Among the 24 PMF patients treated with ruxolitinib, there were no significant differences in the reduction of spleen size, changes in blood counts or OS between patients with *JAK2* V617F or *CALR* mutations, suggesting that response to ruxolitinib is independent of the *JAK2* and *CALR* mutation status.
Summary/Conclusions: *JAK2* V617F and *CALR* mutations are associated with distinct clinical features in ET and PMF. Patients with PMF and *CALR* mutation have an OS better than that of PMF patients with *JAK2* V617F and similar to that of patients with ET. The treatment response to ruxolitinib is similar among PMF patients with *JAK2* V617F or *CALR* mutations, which refers to the role of JAK-STAT signaling in both mutation types.

E1357

JAK2 AND CALR MUTATION STATUS DEFINES SUBTYPES OF ESSENCIAL THROMBOCYTHEMIA WITH SUBSTANTIALLY DIFFERENT CLINICAL COURSE

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Background: The JAK2V617F mutation is found in about 50 to 60% of cases of essential thrombocythemia (ET) while 5% to 10% of JAK2V16F negative ET patients carry MPL mutations at codon 515. Recently, mutations at exon 9 of CALR, were identified in 50% to 70% of ET patients with nonmutated JAK2 and MPL (double-negative).
Aims: To analyze the prevalence of JAK2V617, CALR and MPL mutations in a population of patients with WHO-defined ET and their association with clinical and laboratory features.
Methods: We retrospectively analyzed a cohort of ET patients in a single center, between 2010 and 2014. The JAK2V616F mutation was detected by allele-specific polymerase chain reaction (PCR) testing. For the detection of MPL exon 10 and CALR exon 9 mutations, we applied PCR amplification and Sanger sequencing.
Results: Of the 172 patients studied, 111 (64.5%) carried JAK2V617F mutation (JAK2+), 35 (20.3%) CALR mutation (CALR+), 6 (3.5%) MPLW515K/L mutation (MPL+) and 20 (11.6%) patients had nonmutated JAK2, MPL and CALR (triple-negative). These mutations were mutually exclusive and therefore, CALR-positive cases represented 63.5% of double-negative ET cases. Within the 35 CALR+ patients, 21 (60%) carried the type 1 mutation, 10 (28.6%) the type 2 mutation and 4 (11.4%) carried other less frequent indels. Compared to JAK2+ patients, CALR+ patients had a younger age at diagnosis (<60 years) (57.1% *vs* 31.5%; p=0.006) and a higher platelet count (≥900x10^9/L) (51.4% *vs* 26.1%; p=0.005). On the other hand, the JAK2+ patients presented a higher hemoglobin level (≥15g/dL) compared to CALR+ patients (45.9% *vs* 20%; p= 0.006). No differences were found between type 1 and type 2 CALR+ subgroups. The distribution according to thrombotic risk classification was statistically different (p=0.003) among the two groups: the percentage of low/intermediate risk patients was 48.6% (n=17/35) in the CALR+ group *vs* 22.5% (n=25/111) in the JAK2+ group. The distribution of International Prognostic Score for Essential Thrombocythemia (IPSET) categories was also statistically different (p=0.002) among the three groups analyzed: the percentage of high risk patients was 38.7% (n=43/111) in the JAK2+ group, 20% (n=7/35) in the CALR+ group and 10% (n=2/20) in the triple-negative group. The IPSET-thrombosis model also stratified patients with significant difference (p<0.001) among the three groups: the percentage of high risk patients was 82.9% (n=92/111) in the JAK2+ group, 17.1% (n=6/35) in the CALR+ group and 5% (n=1/20) in the triple-negative group. When comparing the two groups, CALR+ patients belonged more frequently to the low-risk group than those with JAK2+ (48.5% *vs* 5.4%; p<0.001). The incidence of thrombotic events was 25% (n=43/172) and most occurred prior to the diagnosis of ET (74.4%; n=32/43). The group

of triple-negative patients had the lower incidence of thrombotic events (5%) *vs* the CALR+ group (17.1%) and *vs* the JAK2+ group with the highest incidence of thrombotic events (32.4%) (p=0.015). Transformation to post-ET myelofibrosis occurred in 7 patients (3 CALR+, 2 triple-negative, 1 MPL+ and 1 JAK2+; p=0.093). The median overall survival (OS) was not reached in any group and no differences were identified in OS by univariate analysis according to mutational status (CALR+ *vs* JAK2+ *vs* MPL+ *vs* triple-negative; p=0.3).
Summary/Conclusions: Our study confirms that CALR-mutated ET is phenotypically and biological distinct from JAK2-mutated ET with regard to clinical presentation and thrombotic risk.

E1358

THROMBOSIS IN MYELOPROLIFERATIVE NEOPLASIA ASSOCIATED WITH DIFFERENT COAGULATION FACTOR POLYMORPHISMS

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Background: The group of myeloproliferative neoplasms (MPNs) are known for their different phenotypes but similar vascular events complications. Blood cells, interaction between them, and the activation of coagulation factors play a role in the pathogenesis of thrombosis in MPNs. Platelet-specific polymorphisms, FVII as well as β-fibrinogen single nucleotide polymorphisms (SNP's) have been never analyzed together in patients with MPNs.
Aims: To evaluate the effects of coagulation factor VII, β-fibrinogen and TT genotype of GP c.807C>T single nucleotide polymorphisms, and the risk of thrombosis in patients with PV, ET, and PMF at the Department of Oncology and Hematology of the Institute of Oncology, the Lithuanian University of Health Sciences.
Methods: We included 108 patients in this survey. Findings of clinical and hematological analyses were collected. Genotyping was done using PCR and PCR-RFLP analysis
Results: TT genotype of GP c.807C>T polymorphism was more frequently found in the group of MPN patients with arterial thrombosis compared to the MPN patients who were thrombosis-free (26.5% *vs* 11.5%, p=0.049). CT genotype of β-fibrinogen c.-148C>T polymorphism occurred significantly more frequently in MPN patients with arterial and total thrombosis compared to the wild type or homozygous genotype (respectively, 57.7% *vs* 40.0 *vs* 12.5%; p=0.027), (64.7% *vs* 44.4% *vs* 25% p=0.032). The carrier state for 323P10 variant of FVII SNP (summation of P10/10 and P0/10) was significantly more frequent in MPN patients with total thrombosis compared to the wild-type genotype carriers (71.4% *vs* 43.4%, p=0.049). The coexistence of both genotypes - heterozygous β-fibrinogen c.-148C>T and FVII -323P0/10 SNP - statistically significantly increased the odds of arterial thrombosis in MNP patients (21.1% *vs* 3.7%, p=0.008).
Summary/Conclusions: We found that 323P10 variant of FVII, TT genotype of GP c.807C>T and CT genotype of β-fibrinogen c.-148C>T SNP polymorphism may be associated with risk of thrombosis in patients with MPNs.

E1359

A PHASE 1 OPEN LABEL STUDY TO DETERMINE THE PHARMACOKINETICS OF PACRITINIB IN PATIENTS WITH MILD TO SEVERE RENAL IMPAIRMENT AND END STAGE RENAL DISEASE (ESRD) COMPARED WITH HEALTHY SUBJECTS

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Background: Treatment and management of patients with hematologic malignancies and co-morbid renal impairment (RI) is challenging as the kidneys are a major route of excretion for many therapeutic agents. The conduct of a RI study is an important regulatory requirement to support appropriate dosage recommendation in patients with RI. Patients with myelofibrosis (MF) and moderate to severe RI treated with the JAK1/JAK2 inhibitor ruxolitinib require dosage reductions. Pacritinib is an oral kinase inhibitor with specificity for JAK2, FLT3, IRAK1, and CSF1R being evaluated for patients with primary or secondary MF.
Aims: Evaluate the pharmacokinetics (PK) and safety of pacritinib and its major metabolite in patients with RI (mild, moderate, severe, and ESRD requiring hemodialysis) compared with healthy age-, sex-, and weight-matched control participants.
Methods: Study participants (N=39) who provided written informed consent included healthy volunteers and subjects with RI (mild, moderate, severe based on estimated glomerular filtration rate and ESRD on hemodialysis). All participants except those with ESRD received a single dose of pacritinib 400 mg, with PK and urine assessments conducted for up to 168 h post-dose. Subjects with ESRD received 1 dose of pacritinib 400 mg in each of 2 periods (dialysis and inter-dialysis) separated by a 14-day washout. Pacritinib was administered immediately before and after hemodialysis in the dialysis and inter-dialysis periods, respectively, followed by PK assessments for 72 h post-dose.
Results: Time to maximum plasma concentration (t_{max}) was reached at 8, 6, and 5.5 h post-dose in patients with mild, moderate, and severe RI, respectively; t_{max} was 7 h in patients with ESRD. In healthy controls, t_{max} was 8 h post-dose. Pacritinib plasma concentrations demonstrated comparable elimination phases across RI groups. Excretion of pacritinib in urine was minimal (1.0-1.4% of dose over 72 h interval) and RI did not decrease urinary excretion. PK analysis revealed slightly higher maximum plasma concentration (C_{max}) and total exposure (area under the plasma concentration-time curve [AUC]) in patients with RI *vs* healthy controls, which are unlikely to be of clinical relevance (Table). C_{max} and AUC were similar for patients with ESRD during dialysis and inter-dialysis periods. Apparent total clearance (CL/F) was comparable across RI groups. All adverse events (AEs) were grade 1-2; diarrhea was the most frequent AE.

Table 1.

PK Parameter (unit)	Group 1: Mild RI	Group 2: Moderate RI	Group 3: Severe RI	Group 4: Subjects With ESRD N=8		Group 5: Matched Healthy Subjects
	N=8	N=8	N=8	Dialysis	Inter-Dialysis	N=7
C_{max} (µg/mL)	5.00 ±2.22	4.75 ±0.896	5.83 ±2.86	6.01 ±2.45	5.97 ±2.27	4.26 ± 0.596
t_{max} (h)	8.00 (4.00-12.00)	6.00 (5.00-24.00)	5.50 (3.00-8.00)	6.96 (4.08-12.00)	7.00 (5.00-8.00)	8.00 (3.00-24.00)
AUC_{0-t} (µg.h/mL)	274 ±106	334 ±103	430 ±287	243 ±149	267 ±136	268 ±75.5
$t_{1/2}$ (h)	39.4 ±9.11	58.0 ±13.3	55.6 ±17.2	44.0 ±23.3	54.1 ±37.6	44.7 ±11.4
CL/F (L/h)	1.57 ±0.622	1.32 ±0.622[a]	1.41±1.11[b]	2.96 ±1.15[c]	NC[d]	1.58 ±0.862
Vz/F (L)	86.1 ±28.8	98.1 ±49.2[a]	90.9 ±43.8[b]	87.8 ±6.20[c]	NC[d]	99.3 ±48.9

Values are arithmetic mean ±SD, except median (range) for tmax.
[a] n=6; [b] n=7; [c] n=3; [d] n=2
AUC_{0-t}, area under the plasma concentration time curve from time of dosing to last measurable concentration; NC, not calculated; $t_{1/2}$, half-life; Vz/F, apparent total volume of distribution.

Summary/Conclusions: PK parameters and renal excretion of pacritinib were not significantly affected by RI. Administration of 400 mg single doses of pacritinib to patients with RI was well tolerated. Dosage adjustment for pacritinib is not warranted in patients with RI.

E1360

RUXOLITINIB TREATMENT FOLLOWING INTERFERON IN PATIENTS WITH POLYCYTHEMIA VERA: AN ANALYSIS FROM THE RESPONSE TRIAL

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Background: Polycythemia vera (PV) is characterized by excessive proliferation of erythroid, myeloid, and megakaryocytic components in the bone resulting in an increased risk of thromboembolic events, cardiovascular complications, and disease progression. Both hydroxyurea (HU) and interferon-alpha (IFN) are used as first-line treatment for high-risk pts with PV but pts may become resistant/intolerant (Barbui. *JCO* 2011; 29(6):761-770). The recent phase 3 RESPONSE study showed that ruxolitinib (RUX) was superior to best available therapy (BAT) following HU resistance/intolerance; however, little information has been presented on the sub-group of pts treated with IFN in BAT arm.
Aims: This subgroup analysis of RESPONSE study evaluates the safety and efficacy of pts treated with IFN in the randomized BAT arm before and after crossover to RUX.
Methods: RESPONSE, an open-label, phase 3 study enrolled pts with PV, who were resistant to or intolerant of HU per modified European LeukemiaNet criteria, had splenomegaly and required phlebotomy (PBT) to control hematocrit (HCT). Pts were randomized 1:1 to RUX (110 pts) 10 mg bid or BAT (112 pts), of which 13 pts received at least one dose of IFN during the randomized treatment phase. Pts were evaluated for HCT control (<45%) without PBT; spleen response (≥35% reduction in spleen volume from baseline by magnetic resonance imaging); and complete hematologic response [CHR: HCT<45%, white blood cell (WBC) ≤10×10^9/L, platelet (PLT) count ≤400×10^9/L]. JAK2V617F allele burden, adverse events (AEs) and hematologic abnormalities were assessed for randomized and crossover treatment.
Results: Baseline demographics were comparable among RUX (N=110) and IFN-treated pts of the BAT arm (N=13). Three pts (23%) received IFN at some time prior to inclusion in the RESPONSE trial. On trial, 9 pts were treated with PEG-IFN and 4 pts were treated with non-PEG-IFN at varying doses. All IFN pts discontinued and crossed over to RUX due to lack of efficacy (11 pts crossed

over prior to wk 48 and 2 pts after wk 48). No pt treated with IFN achieved spleen response and only 23% of pts achieved HCT control without PBT from wk 8 to 32 (40% and 60% while on RUX therapy, respectively). Thirty-eight percent, 31%, and 31% of pts required no PBT, 1 PBT, and ≥2 PBT, respectively, from wk 8 to 32 of randomized treatment and only 15% of pts achieved CHR at wk 32. Following crossover to RUX, 62% of pts achieved spleen response and PBT requirements decreased (64% of pts did not require any PBT and no pt required ≥2 PBT from wk 32 to 80 after switching to RUX). A majority of pts randomized to IFN had modest reductions in WBC, PLTs, and JAK2V617F allele burden over the course of treatment (figure), with most pts showing further reduction after crossover to RUX. The rates and incidence of hematologic abnormalities (WBC, PLT, and neutrophils) reduced after crossover to RUX with the exception of hemoglobin (prior to crossover: grade 1 [N=2] and grade 2 [N=1]; after crossover: grade 1 [N=5] and grade 2 [N=2]). Similarly, rates and incidence of nonhematologic AEs (systemic, metabolic, gout, pruritus, and fatigue) reduced after crossover to RUX with the exception of infections (primarily grade 1 or 2). Four pts discontinued crossover treatment due to AE (N=3) or pt decision (N=1). One death due to CNS hemorrhage occurred after crossover to RUX (not related to study drug).

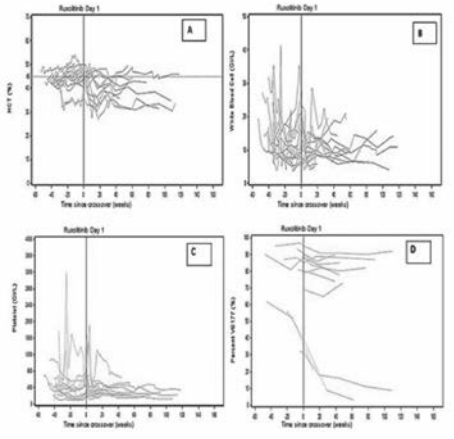

Figure 1. Spaghetti plot of hematocrit (A), WBC (B), platelet counts (C) and JAK2V617F allele burden (D) over time in interferon patients.

Summary/Conclusions: In the HU-resistant/intolerant PV population of the RESPONSE study, no pt treated with IFN achieved spleen response and a limited number of pts achieved HCT control. However, following crossover to RUX, pts showed improvement in hematologic and spleen response with an overall reduction in PBT procedures and a majority of pts being able to achieve spleen response.

E1361

DEVELOPMENT OF AN MF PATIENT REPORTED OUTCOME (PRO) TOOL FOR FDA QUALIFICATION: COMPREHENSIVE LITERATURE SEARCH AND PHYSICIAN COGNITIVE DEBRIEFING RESULTS

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Background: Myelofibrosis (MF) is recognized for its debilitating constitutional symptoms, splenomegaly, profound cytopenias and inflammatory state. Current MPN PROs (MF-SAF, MPN-SAF and MPN-10) have proven sensitive for symptom detection. Formal FDA qualification requires that these tools integrate a comprehensive literature search of previously reported symptoms as well as complete physician cognitive debriefing.
Aims: In this investigation, we aimed to complete these required steps to ascertain the most prevalent and pertinent MPN symptoms for PRO inclusion.
Methods: *Literature Search:* An initial review of the published literature was performed using OVID Medline®. The Medical Subject Headings (MeSH) terms included 'primary myelofibrosis' or 'MF', 'Myeloid metaplasia' and 'agnogenic myeloid metaplasia' which were meshed with 'symptom' or 'signs and symptoms' and limited from 1980 to 2011. Articles that were not original research or in the English language were excluded. Articles were then individually reviewed for content with further exclusion of case reports where search terms occurred concurrently with other medical conditions, articles presenting in-vitro data and articles with a primary focus on pharmacotherapy that utilized the MPN-SAF, MPN-SAF TSS or MF-SAF. Articles were then evaluated for all symptoms and recorded by the number of publications they were reported in. *Cognitive Debriefing:* International MPN specialists were contacted via email to complete the Physician Cognitive Debriefing Evaluation via a REDCAP® survey. Implied consent was provided through survey completion and demographic data was recorded. Responders were asked a series of questions on each symptom acquired through the literature search, along with three free-standing questions on MF PRO development. Thirteen responders were also contacted to complete an in-person interview and respond to eight questions on MF-PRO development. Descriptive statistics were used for data analysis.
Results: The OVID Medline search yielded 166 articles for MF which were then individually reviewed. A total of 30 symptoms were extracted from the literature and recorded for incorporation into the REDCAP survey. The survey was distributed to 48 MPN specialists and 17 (35.4%) agreed to participate. Most responders were Caucasian (94.1%) and male (76.5%) with >5 years experience (94.1%) as physicians. Providers saw between 1-5 (58.8%), 6-10 (23.5%) or >10 (11.8) MF patients per week and most held primary practices in Europe (70.6%). Survey responses to symptom questions are provided in Figure 1. During in-person interviews, some providers felt that a different list of symptoms should be provided to patients with: early *vs* late MF (61.5%), primary *vs* secondary MF (7.7%) and clinical trial settings *vs* office environments (23.1%). Most providers felt that 'inactivity' (100%), 'fever' (92.3%) and 'weight loss' (92.3%) were signs as opposed to symptoms of the disease, though should still be included in the PRO (92.3% for each). Most providers believed that the ideal survey length should be between 10-20 questions. Two providers believed that 'health economics' and 'activities of daily living' should be integrated into a final PRO.

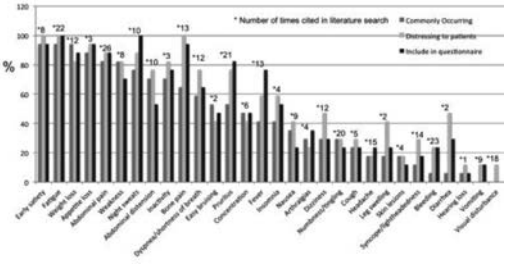

Figure 1.

Summary/Conclusions: Results of the literature search and Physician Cognitive Debriefing support that the MPN-SAF currently addresses the most frequent and symptomatic MF symptoms, with the exception of four items (dyspnea, easy bruising, arthralgias, nausea). Addition Patient Cognitive Debriefing is required to ensure that the final PRO is comprehensive of the most important symptoms to patients.

E1362

STUDY OF ADHERENCE RATES AND SIDE EFFECTS OF ORAL TREATMENTS IN POLYCYTHEMIA VERA OR ESSENTIAL THROMBOCYTEMIA (OUEST STUDY)

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Background: Polycythemia vera (PV) and essential thrombocythemia (ET) are two chronic hematological malignancies with no curative project. Interna-

tional consensus recommends combining long-term low dose antiplatelet drug and a cytoreductive therapy to reduce hyperviscosity (thrombosis) and phenotypic evolutions (myelofibrosis and acute leukemia). There is actually no study in the literature which evaluates the level of adherence in this population.
Aims: The aim of our study was to evaluate patient adherence rate to physicians prescriptions, explore the reasons and identify the occurrence of side effects.
Methods: A total of 134 patients treated for PV or ET with a median follow-up of 6.8 years were asked for about their adherence using a one-shot questionnaire designed by investigators. Data on complications (thrombosis and industrial) were collected from diagnosis to the time of consultation. All the patients signed inform consent before completing the form.
Results: Twenty-eight percent (37 patients) of the patients reported non-adherence to their cytoreductive drug. Patients who forgot their anti-platelet or anticoagulant drugs (22 patients=X%) are mostly those who belong to the first non-adherent group (15/22; *p<0.0001*). In total, 44 patients (32.8%) were declared as non-adherent. A typical profile of these patients could be described: patient who lives alone (*p=0.007*), rather young (p=0.06) and male (p=0.09). The main cause of non-compliance was oblivion (23/37, 69.7%). Polymedicated patients were significantly more adherent (*p=0.001*). Thrombotic history were recorded in 21.7% of patients (21/97) members against 24.3% of non-adherent patients (9/37) (*p=0.74*). On the other hand, myelofibrotic evolutions were more seen in adherent group (14.4% *vs* 5.4%, *p=0.233*). Side effects related to anti-proliferative treatments were reported by 101 patients (75.4%), and were most frequently cutaneous (53/101, 52.5%) or mucosal toxicity (16/101, 15.8%) likely due to the high prevalence of hydroxycarbamide (94/134, 70%).
Summary/Conclusions: For patients treated for PV or ET, non-adherence to anti-proliferative treatment and anti-agregant therapy seems to be frequent. We did not found any correlation with history of thrombosis and myelofibrotic evolution. The patients will be followed for five years to assume the occurrence of new events instead to correlate them with adherence in a prospective way. An analysis of data on a total population of 286 patients will be presented at the conference.

E1363

ERYTHROPOIESIS-STIMULATING AGENTS (ESA) FOR ANEMIA IN MYELOFIBROSIS (MF): A REAL LIFE EXPERIENCE ON 31 UNSELECTED PATIENTS

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Background: Anemia in MF is common but few therapeutic options are available. ESA are safe and widely used but the experience in MF is limited and only a few reports are available.
Aims: We evaluated the safety and efficacy of ESA in MF in a real life context.
Methods: We retrospectively analyzed all the MF patients treated with ESA in our institutions. ESA was started in transfusion-dependent patients or if hemoglobin (Hb) was <10g/dL. According to IWG-MRT and ELN 2013 revised response criteria, anemia response (AR) was defined as a 2 g/dL increase in Hb level for transfusion-independent patients or becoming transfusion-independent for transfusion-dependent patients. The possibility of pretreatment variables to predict a AR to ESA was analyzed using Chi-squared test and Student's t-test. The duration of response was estimated using the Kaplan-Meier method.
Results: Thirty-one MF patients were treated for anemia with ESA (median age at diagnosis 68 years, range 38-85). MF was primary in 42% of the patients while 26% and 32% of the cases were secondary to polycythemia vera or essential thrombocythemia, respectively. JAK2 mutation was detected in 62% of the patients. Seven patients were transfusion-dependent while in the other group median Hb value was 9.1 g/dL (range 7.9-9.9) at the beginning of ESA treatment. Median time from MF diagnosis to ESA start was 3 months (range 0-89). Fourteen patients received epoetin-alpha (originator product) while 11 cases were treated with epoetin-zeta and 5 with other biosimilar ESA. Median ESA starting dose was 40000 U/weekly (range 8000-40000). One patient received low-dose darbopetin-alpha. Median follow up of living patients after ESA start was 27 months (range 3-80). Globally the AR rate was 52%. Most response occurred in the first 3 months, with a median time to AR of 2 months (range 1-7). Nine patients not responsive to standard-dose ESA increased dose to 80000 U/weekly and 2 of them responded. Two of the 5 patients treated with low-dose ESA (i.e.<20000 U/weekly) responded. Median duration of response was 29 months and the probability of maintaining AR at 12 months was 75%. The elapsed time from MF diagnosis to ESA treatment start was predictive of response to ESA: AR rate among patients who started ESA within 3 months from MF diagnosis was 87% *vs* 14% in the others, p<0.01. Transfusion-dependent patients showed a trend towards a reduced probability of AR (29% *vs* 67%, p=0.08). No other predictive factors of response could be found. Among patients with an available baseline serum erythropoietin level (13/31), only 3 showed a value above 125U/L and 1 of them achieved AR after ESA dose increase. Considering the most frequently used types of ESA in our patients, both originator epoetin-alpha and epoetin-zeta obtained favorable results (43% *vs* 70%, p>0.1).The treatment was well tolerated and no significant adverse events related to ESA were reported. In 5 patients an increase in splenomegaly was recorded during ESA therapy.
Summary/Conclusions: Our report confirms the safety and efficacy of ESA for anemia in MF with a fair response duration and it suggests that an early initiation of ESA treatment could yield better results. Besides, we confirm the negative predictive value of transfusion dependence. Epoetin-zeta achieved result at least equal to originator epoetin-alpha, an observation that need to be confirmed but could be important when treatment costs are taken into account.

E1364

A RETROSPECTIVE COHORT STUDY REPORTING ON THE DISEASE CHARACTERISTICS AND OUTCOMES OF PATIENTS DIAGNOSED UNDER THE AGE OF 50 YEARS WITH PRIMARY OR SECONDARY MYELOFIBROSIS

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Background: Myelofibrosis (MF), a rare clonal haematopoietic disorder is commonly diagnosed in the 6th or 7th decade of life. The clinical and molecular profile of younger patients remains poorly defined.
Aims: In this retrospective study we evaluated patients who were ≤50 years of age at diagnosis with WHO-defined primary MF (PMF), post-essential thrombocythaemia MF (PET-MF) or post-polycythaemia vera MF (PPV-MF) managed at our centre.
Methods: Forty three patients with a median age of 43 years (range 19-50 years) at diagnosis were included; 24/43 (55.8%) were male. Median follow-up was 44 months (range 0-208 months). Twenty (47%) had a diagnosis of PMF, 14/43 (33%) PET-MF and 9/43 (21%) PPV-MF.
Results: Concerning mutations 22/43 (51%) had *JAK2*V617F, 18/43 (42%) *CALR*, 0/43 (0%) *MPL* mutations and 3/43 (7%) were 'triple negative' (TN). The IPSS and DIPSS scores were low risk in 26/39 (67%) and 26/39 (67%), intermediate-1 in 8/39 (21%) and 11/39 (28%), intermediate-2 in 3/39 (8%) and 2/39 (5%) and high risk in 2/39 (5%) and 7/39 (18%), respectively. Univariate analysis did not demonstrate any significant impact of mutational status upon haemoglobin, leukocytes, platelet count, circulating blasts, red blood cell transfusion need, splenomegaly or constitutional symptoms at diagnosis. Additionally, we did not observe any influence of driver mutation on age at diagnosis, gender, IPSS or DIPSS scores. Comparing *JAK2*V617F and *CALR* mutated subjects, there was a significant increase in the presence of palpable splenomegaly in the *JAK2*V617F mutated patients (p<0.05). TN patients were more like to have a platelet count<100x109/L (p=0.073), adverse karyotype (p=0.06), a high DIPSS-plus score (p=0.076), and the only transformation to acute myeloid leukaemia (AML) occurred in a TN patient. Two thrombotic events occurred: a deep vein thrombosis in a *JAK2*V617F mutated patient, and a myocardial infarction in *CALR* mutated patient, giving an overall calculated incidence rate of 1% patient-year. No major bleeding events were identified. Only one patient progressed to AML with an overall calculated incidence rate of 0.5% patient-year. No deaths were recorded during the follow-up period.
Summary/Conclusions: The distribution of driver mutations in our cohort of patients with MF diagnosed age less than 50 years differs from that of the overall MF patient population; we report that 51% of patients had *JAK2*V617F mutation *vs* 58-64.7% for the broader patient population, 42% *CALR* mutated *vs* 22.7-25%, 0% *MPL* mutated *vs* 4-8.3% (1,2). The frequency of TN patients mirrors that of the wider MF population, 7% in our cohort *vs* 8.6-8.7% in the literature (1,2). Although we were not able to demonstrate any significant impact of driver mutation status on age, gender or laboratory parameters, we recorded a higher incidence of splenomegaly in *JAK2*V617F mutated patients compared to *CALR* mutated patients, (P=0.047). We found that TN status appeared to be associated with higher risk profile and transformation to AML.
Our findings suggest that younger MF subjects may have a higher frequency of *CALR* mutations and a more indolent disease course *i.e.* low IPSS and DIPPS score, lower incidences of thromboembolic events and with less evidence of negative prognostic variables and disease progression compared to the general MF population.

References

1. Tefferi et al. *CALR vs JAK2 vs MPL*-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia. 2014;28(7):1472-7;
2. Rumi et al. Clinical effect of driver mutations of *JAK2*, *CALR*, or *MPL* in primary myelofibrosis. Blood. 2014;124(7):1062-9.

E1365

USEFULNESS OF PERFORMING JAK2 MUTATIONAL ANALYSIS IN PATIENTS WITH MODERATE ABSOLUTE ERYTHROCYTOSIS

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Background: The diagnosis of polycythemia vera (PV) is based on clinical and biological criteria defined by the World Health Organization (WHO 2008).

The proposed revised version (WHO 2016) has lowered the threshold hemoglobin (Hb) level for the diagnosis of PV from 18.5 g/dl in men and 16.5 g/dl in women to an hemoglobin level of 16.5 g/dl in men and 16 g/dl for women. The importance to lower hemoglobin threshold in the evaluation of patients with absolute erythrocytosis has not been validated yet.

Aims: To evaluate the importance of the new hemoglobin threshold we retrospectively assessed the frequency of polycythemia vera *versus* secondary erythrocytosis in patients with absolute erythrocytosis categorized according to hemoglobin value at diagnosis.

Methods: We included in the current study all patients recorded in our database with a diagnosis of polycythemia vera or secondary erythrocytosis who showed isolated absolute erythrocytosis at diagnosis according to the new proposed WHO criteria (Hb >16.5g/dl in men and >16g/dl in women, leukocyte count <10x10^9/L, platelet count <450x10^9/L). We stratified these patients according to their hemoglobin level at diagnosis (<17g/dl, 17-19g/dl, 19-21g/dl, 21-23g/dl, >/=23g/dl) and calculated the percentage of PV cases in each subgroup. Only patients assessed for both V617F and exon 12 *JAK2* mutations were included in the study.

Results: Patients who satisfied the above criteria were 236 patients, including 82 PV and 154 secondary erythrocytosis. Diagnosis of PV was made according to WHO 2008 criteria (Hb >16,5g/dl (female) or 18,5g/dl (male) or >15g/dl (female) or 17g/dl with a sustained increase of >2g/dl from baseline, *JAK2* V617F or exon 12 mutation, low erythropoietin level and/or panmyelosis at bone marrow analysis). As shown in table 1, around one third of patients stratified in the subgroup with the lowest hemoglobin (Hb below 17 g/dl) had a final diagnosis of PV, thus reinforcing the importance to perform *JAK2* analysis also in patients with moderate erythrocytosis. This was particularly important in female patients. Namely, when we repeated the same stratification taking into account sex distribution, we observed that half (52.6%) of female patients with Hb level <17g/dl received a final diagnosis of PV. According to our data set, instead, no male patient with Hb lower than 17 g/dl was diagnosed with polycythemia vera. Patients who satisfied the above criteria were 236 patients, including 82 PV and 154 secondary erythrocytosis. Diagnosis of PV was made according to WHO 2008 criteria (Hb >16,5g/dl (female) or 18,5g/dl (male) or >15g/dl (female) or 17g/dl with a sustained increase of >2g/dl from baseline, *JAK2* V617F or exon 12 mutation, low erythropoietin level and/or panmyelosis at bone marrow analysis). As shown in table 1, around one third of patients stratified in the subgroup with the lowest hemoglobin (Hb below 17g/dl) had a final diagnosis of PV, thus reinforcing the importance to perform *JAK2* analysis also in patients with moderate erythrocytosis. This was particularly important in female patients. Namely, when we repeated the same stratification taking into account sex distribution, we observed that half (52.6%) of female patients with Hb level <17g/dl received a final diagnosis of PV. According to our data set, instead, no male patient with Hb lower than 17g/dl was diagnosed with polycythemia vera.

Table 1.

Hb range (g/dl)	Whole cohort		Female patients		Male patients	
	PV n°(%)	SE n°(%)	PV n°(%)	SE n°(%)	PV n°(%)	SE n°(%)
<17	10 (29.4)	24 (70.6)	10 (52,6)	9 (47,3)	0 (0)	15 (100)
17-19	36 (24.7)	110 (75.3)	16 (66,7)	8 (33,3)	20 (16,4)	102 (83,6)
19-21	28 (59.6)	19 (40.4)	10 (83,3)	2 (16,7)	18 (51,4)	17 (48,6)
21-23	6 (85.7)	1 (14.3)	3 (100)	0 (0)	3 (75)	1 (25)
≥23	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)

Table 1. Frequency of polycythemia vera (PV) versus secondary erythrocytosis (SE) according to hemoglobin value at diagnosis in the whole cohort and divided by gender

Summary/Conclusions: In patients with isolate erythrocytosis it is important to perform screening for *JAK2* mutations also when hemoglobin level is slightly increased (>16,5 in men and >16 in women) as a significative proportion of patients with polycythemia vera show at diagnosis only mild erythrocytosis. This result reinforces the importance to lower the hemoglobin threshold required for PV diagnosis as proposed by the new WHO criteria, especially as far as female patients are concerned.

E1366

Abstract withdrawn.

E1367

PROGNOSIS FACTORS CONDITIONING THE RISK OF MIELOFIBROTIC TRANSFORMATION IN ESSENTIAL THROMBOCYTHEMIA: SIGNIFICANCE OF ALLELIC BURDEN AND THE NUMBER OF LEUCOCYTES AT DIAGNOSIS IN YOUNG PATIENTS

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Background: Even though there are predictive prognostic scores of thrombotic risk in patients with Essential Thrombocythemia (ET), there is no agreement established on the factors influencing the evolution of myelofibrosis in these patients, this is a factor that adversely affects their life expectancy, which is initially similar to that of the healthy population of the same age.

Aims: To analyse in a group of patients with ET the impact of parameters such as age, platelet count at diagnosis, number of leucocytes, time until cytoreductive therapy was needed, time under observation, presence of JAK2 V617F mutation and allelic burden on the risk of myelofibrotic transformation.

Methods: We analysed a total of 100 patients diagnosed in our centre between 1996 and 2013 based on bone marrow study. All patients received anti- platelet agregation and began cytoreductive therapy with hydroxyurea and / or anagrelide according to IPSET criteria. JAK2 V617F mutation was detected by allele - specific real- time quantitative in peripheral blood samples. Homozygous patients for the mutation occurred when the load allelic was >50%, being the rest considered heterozygous. Statistical analysis was performed using SPSS version 15.0 software, considering a statistical significant difference if p<0.05.

Results: Average age of the study population was 63 years. 73% of patients had JAK2 V617F mutation, being homozygous for the same 7% of patients. 11% of patients progressed to myelofibrosis, with a median follow-up of 5 years from diagnosis to transformation. The transformed and untransformed groups were similar in platelet count at diagnosis, time under observation, time to need for cytoreductive treatment and presence of the mutation JAK2 V617F, although there is a tendency to older (74 *vs* 63) and more number of leukocytes (15,1x10e9 /L *vs* 9,6x10e9 /L) in the transformed *versus* non-transformed group. Patients with JAK2 V617F mutation and allelic burden above 40% had a higher risk of myelofibrotic transformation in the multivariate analysis, with an accumulated incidence at 5 years of 57.1% (Figure 1), p=0.04.

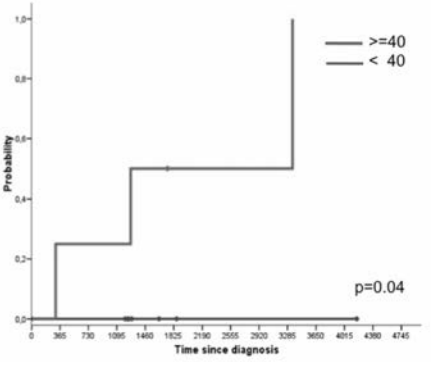

Figure 1. Probability of evolution to positive JAK2 myelofibrosis.

Summary/Conclusions: Patients with ET and JAK2 V617F mutation have an increased risk of myelofibrotic transformation when allelic burden is above 40%, which is why specific therapeutic strategies will be needed aimed at reducing allelic burden to maintain an adequate life expectancy in young patients with this condition. Leucocytes cell count at diagnosis would be a variant to be considered in the prognostic stratification, although specific studies are required to examine this in more detail.

Non-Hodgkin & Hodgkin lymphoma - Biology

E1368

Abstract withdrawn.

E1369

ACTIVATED STAT5 DRIVES PERIPHERAL T CELL LYMPHOMAS

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Background: STAT5 transcription factors are essential regulators of differentiation, survival and proliferation during hematopoiesis. Hyperactive STAT5 signaling requires enhanced tyrosine phosphorylation (pYSTAT5), which is frequently found in hematopoietic cancers and is associated with negative prognosis. Importantly, recurrent gain-of-function STAT5 variants have been reported in Peripheral T Cell Lymphoma (PTCL) patients, who suffer from aggressive diseases with no targeted therapy options.
Aims: We aim to analyze STAT5-dosage dependent effects on hematopoiesis. Here, we investigated whether constitutive activation of STAT5 suffices to drive PTCL and whether inhibition of the JAK/STAT pathway offers a novel therapeutic opportunity in this disease.
Methods: We used $cS5^{F}$, a hyperactive point mutant of STAT5A (S710F), to generate mouse models expressing the transgene under the vav-promoter at low ($vcS5^{lo}$) or high ($vcS5^{hi}$) levels from hematopoietic stem cells (HSC) on. The phenotypes were characterized by regular blood sampling, flow cytometric analysis of hematopoietic cells and HE, anti-CD3e and -Ki67 stainings on organ sections. RNA-seq and gene-set enrichment analysis was done to confirm the PTCL-like disease of $vcS5^{hi}$ mice. We quantified STAT5 expression levels in PTCL patient tissues by immunohistochemistry and qPCR. Effects on viability and pYSTAT5 levels of murine and human PTCL cell lines after JAK-STAT5 signalling inhibitor treatment were determined.
Results: High pYSTAT5 levels in $vcS5^{hi}$ mice led to an aggressive expansion of $CD8^{+}$ T cells being lethal between 25 and 45 weeks of age. In contrast, $vcS5^{lo}$ mice developed only a mild expansion of $CD8^{+}$ T cells with no effect on life expectancy. The $vcS5^{hi}$ PTCL-like disease was associated with lymphadenopathy, splenomegaly and T cell infiltrations into various organs. The $CD8^{+}$ T cells were transplantable and expressed T cell activation markers. Furthermore, the number of active cycling HSCs increased. The expression profile determined by RNA-seq correlated closely with human PTCL forms. STAT5 expression and activation levels were found to be elevated in PTCL patients. Treatment with Ruxolitinib, Tofacitinib and a novel STAT5 SH_2-domain inhibitor decreased murine and human PTCL cell line viability in response to pYSTAT5 decline.
Summary/Conclusions: Our results obtained from mouse models and patients support the concept that enhanced STAT5 signaling drives PTCL and that STAT5 represents a target in these life-threatening malignancies.

E1370

MECHANISM, CONSEQUENCES AND THERAPEUTIC TARGETING OF ABNORMAL IL-15 SIGNALING IN CUTANEOUS T-CELL LYMPHOMA

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Background: Cutaneous T-cell lymphoma (CTCL) is a type of non-Hodgkin's lymphoma of skin-homing T-cells. It represents about 70-80% of all cutaneous lymphoma with unknown pathogenic mechanisms and no cures. There is good evidence to support a divisive role of interleukin-15 (IL-15) in controlling oncogenic processes in CTCL, since it serves to increase T-cell survival, proliferation, migration and invasion in CTCL cell lines.
Aims: While the malignant CD4+ T-cells from CTCL patients appear to produce and be activated by IL-15, the role of this cytokine in the pathogenesis of CTCL remains unresolved. With the intent of gaining insight into mechanisms of IL-15 overexpression and its role in CTCL pathogenesis, we first confirmed the relevance of IL-15 by showing stage-dependent overexpression in lesional skin and peripheral blood CD4+ T-cells from CTCL patients. We elucidated at least one mechanism by which IL-15 can be overexpressed *in vivo,* and then investigated the biological and clinical aspects of a spontaneous epidermotropic CTCL that develops in mice that constitutively overexpressing IL-15.
Methods: We explored shared molecular aberrations between the CTCL in IL-15 transgenic mice and human CTCL, which could be replicated in normal human T-cells exposed to IL-15. We also interrogated the IL-15 animal model with the purpose of identifying mechanisms and pathways amenable to selective pharmacological targeting for preclinical validation using isotype specific HDAC inhibitor.
Results: Here we report that CTCL patients show increased IL-15 in a clinical stage-dependent manner. Mechanistically, we show that Zeb1 is a transcriptional repressor of IL-15 in T-cells and that hypermethylation of the Zeb1 binding region within the IL-15 promoter, as seen in CTCL patients, prevents Zeb1 binding and causes increased transcription of IL-15. Using a transgenic mouse model of IL-15, we provide evidence that overexpression of IL-15 induces a spontaneous CTCL that mimics the human neoplasm. Excessive autocrine production of IL-15 in T-cells inhibits an HDAC1-mediated negative autoregulatory loop, resulting in the upregulation of HDAC1 and HDAC6, and transcriptional induction of the onco-miR-21. Interruption of IL-15 downstream signaling with isotype-specific HDAC inhibitors halts (HDAC1) or significantly delays (HDAC6) the progression of CTCL *in vivo* and provides pre-clinical evidence supporting a hierarchical model of oncogenic signaling in CTCL.
Summary/Conclusions: In summary, we provide *in vivo* evidence that IL-15 likely can have a causal role in the pathogenesis of at least some cases of CTCL, in part via the epigenetic inhibition of the transcriptional repressor, Zeb1, that in turn leads to overexpression of IL-15 and activation of specific HDACs. IL-15 transgenic CTCL mice provide a novel model for studying the development of CTCL and evaluating potential therapies. Selective inhibition of HDAC1/2 produces a comparable halt in the progression of experimental CTCL as that seen with the pan-HDAC inhibitors, and thus may provide an equally potent yet less toxic alternative in the clinic. Our findings not only demonstrate a critical role for IL-15-mediated inflammation in cutaneous T-cell lymphomagenesis, but also uncover a new oncogenic regulatory loop in CTCL involving IL-15, HDAC1, HDAC6 and miR-21 that show differential sensitivity to isotype-specific HDAC inhibitors.

E1371

EXOME SEQUENCING REVEALS RECURRENT GERMLINE VARIANTS IN PATIENTS WITH FAMILIAL LYMPHOPLASMACYTIC LYMPHOMA

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Background: Waldenström's Macroglobulinemia (WM) represents a B-cell lymphoproliferative disorder, classified as a lymphoplasmacytic lymphoma, according to the WHO classification. Previous studies have identified familial aggregation of WM cases, and the clustering of B-cell lymphoproliferative disorders among first degree relatives of patients with WM, including chronic lymphocytic leukemia, non-Hodgkin lymphoma, multiple myeloma, IgM-MGUS and IgG/IgA-MGUS, thus suggesting a possible contribution of inherited susceptibility to familial WM. Nevertheless, whether relatively common germline variants may contribute specifically to familial WM cases, remains unexplored.
Aims: To identify the potential contribution for inherited susceptibility to familial WM. To define the functional relevance of the most recurrent observed variant.
Methods: Whole exome sequencing was performed on germline DNA obtained from four members of a single family with documented coinheritance of WM (three affected; 1 unaffected) and applied bioinformatic tools to identify candidate germline variants likely to have a biological role in WM. Additional 246 independent, unrelated WM cases (50 probands from familial cases; 196 from non-familial cases) were screened for the identified variants. The DNA was size-selected to exonic hybrid capture using SureSelect v2 Exome bait (Agilent). Samples were multiplexed and sequenced on Illumina HiSeq flow cells with the goal of an average depth of coverage of 100x. Allele frequencies (AF) in 1000G, as well as PolyPhen-2 prediction, were used to quantify the deleteriousness of SNVs. Variants with AF >0.05 in 1000G were filtered out, and the remaining SNVs were defined as potential familial WM-associated variants. Gene prioritization was obtained by using GRAIL and global coexpression network COXPRESdb. Functional validation was performed by engineering WM cells for the most recurrent variant, by using site-directed mutagenesis and LentiORF.
Results: LAPTM5(c403t) and HCLS1(g496a) represented the most recurrent variants, present in 3/3 affected members of the index family. Each of these variants was present in 8% of the unrelated familial cases, in 0.5% of the non-familial cases and in <0.05 of a control population. To interrogate a possible involvement of LAPTM5 and HCLS1 in WM biology, we used the HEFalMp

database to construct a combined LAPTM5/HCLS1 module and interrogated whether this module is disrupted in WM patients compared to healthy donors (HDs), using an independent gene expression profile dataset (GSE6691). We demonstrated a statistically significant high gene-to-gene connectivity in HDs, while the sub-network was disrupted in WM. Importantly, the change in mean connectivity was significantly different in the WM- *versus* the HD-modules ($P<0.0001$). Together, these findings suggest a conserved high interactivity between LAPTM5 and HCLS1 in normal B cells, while WM cells present a disrupted pattern of connectivity, which likely impacts disease biology. Previous studies have reported on LAPTM5 over-expression in patients with B-cell lymphomas. LAPTM5c403t was the most significantly predicted variant to be functionally related to the WM mRNA signature, and therefore dissected its functional relevance, by using site-directed mutagenesis in WM cells. LAPTM5c403t mutated cells presented with enhanced cell growth, NF-kBp65 activation and phospho- NF-kBp65 nuclear translocation, compared to the non-mutated cells, thus suggesting the role of the LAPTM5(c403t) variant in enhancing WM cell proliferation through modulation of NF-kB activation.
Summary/Conclusions: LAPTM5(c403t) and HCLS1(g496a) may represent predisposition alleles in patients with familial WM. Future studies will be needed to clarify the penetrance of specific alleles as well as possible combinatorial effects.

E1372

COMBINATION OF THE GLYCOENGINEERED TYPE II CD20 ANTIBODY OBINUTUZUMAB WITH THE NOVEL BCL-2 SELECTIVE INHIBITOR VENETOCLAX INDUCES ROBUST CELL DEATH IN NHL MODELS AND CLL PATIENT SAMPLES

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Background: Obinutuzumab (GA101) is a novel glycoengineered type II anti-CD20 monoclonal antibody that induces a high level of non-apoptotic direct cell death and, due to increased affinity for FcγRIII on effector cells, results in enhanced antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP). Venetoclax (ABT-199/GDC-0199) is a novel, orally bioavailable, selective BCL-2 inhibitor that induces intrinsic apoptosis in preclinical models of hematological malignancies (NHL, AML, MM) and primary CLL patient samples.
Aims: Based on their complementary mechanisms of action involving increased apoptosis (venetoclax) or direct cell death (obinutuzumab) we aimed to determine if the combination of both agents has the potential for greater efficacy in treating B lymphoid malignancies such as NHL and CLL when compared to monotherapy and co-treatment with other anti-CD20 antibodies such as rituximab.
Methods: In vitro studies primarily utilized fluorescence activated cell sorting (FACS) to measure direct cell death induction/apoptosis on NHL cell lines (WSU-DLCL2, SU-DHL4 and Z138) and primary CLL patient samples (n=27) as well as the impact of BCL-2 inhibition on ADCC induction. ADCC assays utilized peripheral blood mononuclear cells (PBMCs) isolated from healthy donors as effector (E) cells and SU-DHL4 as target (T) cells and measured by Lactate dehydrogenase (LDH) release using the LDH Cytotoxicity Detection Kit (Roche Applied Science). *in vivo* efficacy of venetoclax monotherapy or in combination with anti-CD20 antibodies was evaluated in WSU-DLCL2, SU-DHL4 and Z138 sub-cutaneous xenograft models. Venetoclax (100 mg/kg) was administered daily by oral gavage for 21 days and anti-CD20 antibodies (1 mg/kg) were delivered intrapertioneally once during the treatment period.
Results: Venetoclax enhanced induction of cell death when combined with obinutuzumab or rituximab in SU-DHL4, WSU-DLCL2 and Z138 cell lines. However, a greater degree of cell death was observed when venetoclax was combined with obinutuzumab *versus* rituxumab at lower drug concentrations. Importantly, venetoclax did not antagonize natural killer (NK)-cell mediated ADCC or B cell depletion induced by obinutuzumab or rituximab treatment. Treatment of SU-DHL4, WSU-DLCL2 and Z138 xenografts with venetoclax plus obinutuzumab resulted in complete regressions, increased overall response rates or greater tumor growth inhibition *in vivo*. Moreover, monotherapy with venetoclax following combination treatment with obinutuzumab resulted in sustained *in vivo* efficacy and increased duration of response in SU-DHL4 and WSU-DLCL2 xenograft models suggesting a potential benefit of utilizing venetoclax as maintenance therapy. In primary CLL samples (from untreated or relapsed patients), venetoclax plus anti-CD20 antibody drug combination treatment significantly increased B cell depletion (based on direct cell death/ADCC) after 7 days in culture ex vivo. Under these conditions, venetoclax in combination with obinutuzumab was more effective than the combination with rituximab or venetoclax alone. Moreover, co-culture with nurse-like cells that increases B-cell survival did not protect primary CLL cells from venetoclax demonstrating that drug efficacy is maintained in a protective tumor microenvironment such as lymph node.
Summary/Conclusions: Collectively, our data demonstrate that combination of obinutuzumab with venetoclax results in greater cell death and robust anti-tumor efficacy in xenograft models representing NHL sub-types and primary CLL patient samples that is superior when compared to co-treatment with rituximab. The preclinical data presented supports clinical investigation of obinutuzumab and venetoclax combination therapy, which is currently in phase Ib clinical trials for relapsed/refractory CLL (clinical trial.gov identifier NCT01685892), and warrants further investigation in other B-cell malignancies such as NHL.

E1373

THE CD47-BLOCKING CANCER IMMUNOTHERAPEUTIC TTI-621 HAS ANTI-TUMOR EFFECTS ACROSS A BROAD RANGE OF HEMATOLOGICAL MALIGNANCIES

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Background: CD47 binds to signal-regulatory protein α (SIRPα) on the surface of macrophages and delivers a "do not eat" signal that suppresses phagocytosis. There is strong evidence that many hematological and solid tumors upregulate cell surface expression of CD47 as a means to exploit the CD47-SIRPα pathway and escape macrophage-mediated immune surveillance. Blockade of CD47 using TTI-621, a soluble SIRPα-Fc fusion protein (SIRPαFc), is a promising therapeutic strategy to neutralize the suppressive effects of CD47 and promote the eradication of tumor cells by host macrophages.
Aims: Here we have examined the effect of TTI-621 on human hematological tumor cells in vitro and *in vivo*.
Methods: Human macrophages were derived from peripheral monocytes of healthy donors. For the in vitro phagocytosis assay, tumor cells were co-cultured with macrophages for two hours in the presence of TTI-621 or control Fc protein. Phagocytosis was assessed by confocal microscopy or flow cytometry. Acute myeloid leukemia (AML) xenografts were performed in NOD.SCID mice. Cells from AML patients were transplanted intra-femorally, and following a three week engraftment period, mice were dosed with 5 mg/kg mouse SIRPαFc or 3.3 mg/kg control Fc protein three times/week for four weeks. AML engraftment was determined by%$CD45^+CD33^+$ human cells. Lymphoma xenografts were performed in SHrN mice. Namalwa cells were injected subcutaneously into each flank. Toledo cells were injected in 50% Matrigel. Three days (Nawalma) or 10 days (Toledo) after tumor cell implantation, animals received 10 mg/kg mouse SIRPαFc or 6.75 mg/kg control Fc protein, daily five times/week, for three weeks. Tumor volumes were monitored until they reached the maximum volume of $1500mm^3$.
Results: We first assessed the ability of TTI-621 to trigger macrophage-mediated phagocytosis of cancer cells. In cultures left untreated or treated with a control Fc fragment, macrophages exhibited a low level of phagocytosis, consistent with CD47-mediated suppression. Blockade of CD47 by TTI-621 led to a dramatic increase in the phagocytosis of human tumor cell lines of both myeloid and lymphoid origin, including non-Hodgkin lymphoma lines. Furthermore, TTI-621 enhanced macrophage-mediated phagocytosis of primary tumor samples from patients with AML, myelodysplastic syndrome, multiple myeloma and B- and T-cell acute lymphoblastic leukemia. In total, 77% (23/30) of cell lines and 97% (32/33) of primary patient samples were readily phagocytosed following treatment with TTI-621. We next evaluated the *in vivo* efficacy of a mouse surrogate of TTI-621 in an AML xenograft model. Treatment of mice with mouse SIRPαFc for four weeks significantly reduced the tumor burden in the injected femur of 7/10 patient samples compared to a control Fc protein, while dramatically reducing the tumor burden in non-injected bone marrow in all 10 AML patient samples. Finally, we assessed the *in vivo* activity of mouse SIRPαFc in xenograft models of Burkitt lymphoma (Namalwa) and diffuse large B cell lymphoma (Toledo). Treatment with SIRPαFc ablated the growth of Namalwa and Toledo tumors and was superior to rituximab therapy in both models.
Summary/Conclusions: Collectively, these data indicate that TTI-621 is active across a broad range of human hematological tumors. A Phase I clinical trial of TTI-621 in patients with advanced hematological malignancies is currently underway (ClinicalTrials.gov #NCT02663518).

E1374

ACTIVATION OF MTORC2 COMPLEX CONTRIBUTES TO CELL CYCLE PROGRESSION AND SURVIVAL IN ALK+ ANAPLASTIC LARGE CELL LYMPHOMA

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Background: ALK+ anaplastic large cell lymphoma (ALK+ ALCL) is characterized by chromosomal translocations of ALK gene locus, the most frequent being the t(2;5)(p23;q35) resulting in aberrrant expression and activation of the NPM-ALK oncoprotein. The NPM-ALK activates multiple oncogenic pathways including the mTOR-Raptor (mTORC1) pathway, however, the potential role of mTOR-Rictor (mTORC2) complex in ALCL pathogenesis is yet unknown.
Aims: To investigate the potential role of Sin1, a critical component of the mTORC2 complex required for its integrity and activation, in ALK+ ALCL pathogenesis.
Methods: Expression, phosphorylation, and localization of Sin1 protein were assessed by Western blot after subcellular fractionation, immunofluorescence and confocal microscopy in 3 ALK+ and 2 ALK- ALCL cell lines. Physical interaction between various proteins was assessed by co-immunoprecipitation assays. Real-time RT PCR was used to assess Sin1 gene products at the RNA level. Transfection experiments were performed in BaF3 cells and in ALK+ and ALK- ALCL cells using NPM-ALK and Sin1 expressing plasmids and Sin1-shRNA constructs (gene silencing). Standard cell viability, proliferation and colony formation assays as well as flow cytometry were utilized to assess cell growth and survival following forced expression or gene silencing of Sin1 gene, as well as after treatment with ALK and STAT3 inhibitors. In a cohort of 32 previously untreated patients with ALK+ ALCL, Sin1 protein expression was assessed by immunohistochemistry performed on a tissue microarray.
Results: Sin1.1 and Sin1.5 were the main isoforms detected in immunoblots and they were differentially expressed among ALK+ and ALK- ALCL cell lines. Sin1 protein was co-localized with activated (Ser473-phosphorylated) AKT kinase in ALCL cells. Treatment of ALK+ ALCL cells with Crizotinib led to reduced expression and de-phosphorylation of Sin1 protein. Forced expression of Sin1.1 and Sin1.5 isoforms resulted in increased cell proliferation in ALK+ ALCL cells with downregulation of the CDK inhibitor p21. Inversely, knocking down Sin1 gene by two specific Sin1-shRNA constructs resulted in dramatic decrease of cell viability and colony formation (by 80%) of ALK+ ALCL cells, which was associated with AKT de-activation and downregulation of the anti-apoptotic BCL-XL and MCL-1 proteins. Using our in vitro system and ex vivo (xenografts) mouse model, Sin1 gene expression was, at least in part, regulated by STAT3 transcription factor. In the cohort of ALK+ ALCL patients, Sin1 protein was expressed in all 32 tumors studied with cytoplasmic and nuclear pattern.
Summary/Conclusions: Taken together, these novel findings suggest that mTORC2 complex, through Sin1-dependent activation of AKT and possibly other AKT-independent functions, may contribute to cell cycle and apoptosis deregulation and oncogenesis in ALCL. Thus, modulation of mTORC2 activity might represent a novel target for investigational therapy in patients with ALCL.

E1375

SEARCHING FOR NOVEL PROGNOSTIC BIOMARKERS IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) BY CIBERSORT-BASED ANALYSIS OF TUMOR MICROENVIRONMENT

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Background: Diffuse large B cell lymphoma (DLBCL) comprises a large group of disease entities with high molecular heterogeneity and variable treatment responsiveness. Results from gene expression profiling (GEP) studies highlighted the role of cell of origin, namely activated B cell-like cells (ABC) and germinal center B cells (GCB), and stromal gene signatures for predicting clinical outcome and stratifying patient risk. However, translation of GEP information in easily applicable prognostic biomarkers remains a challenge.
Aims: We applied an innovative GEP-based computational method, namely CIBERSORT, to determine the cellular composition of DLBCL microenvironment and investigate associations between abundance of certain tumor-infiltrating non-malignant cell types and molecular/clinical traits of the disease.
Methods: We generated a gene signature matrix including a total number of 1028 genes that distinguish 17 different immune and non-immune cell types, the latter including adipocytes, endothelial cells (EC), pericytes and myofibroblasts (MF). This matrix was used to perform CIBERSORT analysis (on-line access to the webserver http://cibersort.stanford.edu/) of GEP data from 3 publicly datasets (GSE10846, GSE19246 and GSE34171) of overall 604 DLBCL cases. The relative percentage of each tumor-infiltrating cell fraction was estimated and a global heatmap of tumor microenvironment composition was generated. Results were also stratified according to either cell of origin or clinical outcome data and significant differences in each subgroup calculated by a two-sided Mann-Whitney test.
Results: By applying CIBERSORT, we found that, among immune cells, memory B cells, plasma cells, NK cells, dendritic cells, CD4 T cells and macrophages mainly infiltrate DLBCL tissues. In particular, plasma cells, NK and dendritic cells showed significant higher proportion in ABC than GCB tumors, whereas CD4 T-cells and dendritic cells were prevalent in patients with better outcome. Notably, among stromal cells, MF was the most represented population and exhibited a significant predominance in patients with more favorable outcome and a strong positive correlation with overall survival (Figure 1). Consistently, the fraction of MF was significantly larger in GCB tumors compared with ABC subtypes. These latter resulted more enriched in EC.

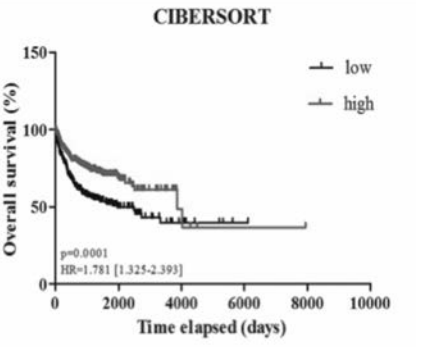

Figure 1 - Publicly available DLBCL GEP datasets (GSE10846 and GSE34171) were analyzed by CIBERSORT (cases=488) to estimate the percentages of myofibroblast infiltration. Kaplan-Meier curve shows patients stratified into the two groups 'high' or 'low', according to the median value of myofibroblast fraction (p=0.0001,log-rank test; n=238 low and 241 high patients).

Figure 1.

Summary/Conclusions: By estimating relative fractions of DLBCL-infiltrating cells using CIBERSORT, we studied unknown aspects of tumor microenvironment with potential prognostic implications. Our data suggest that the composition of DLBCL with diverse molecular and clinical features also differ in term of immune and, particularly, stromal cell infiltration. These findings are in line with previous reports highlighting the prognostic value of specific stromal gene signatures and support further studies aimed at uncover novel prognostic/predictive biomarkers correlated with non-malignant tumor-associated cell subsets.

E1376

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF ALK+ AND ALK- ANAPLASTIC LARGE CELL LYMPHOMA (ALCL)-DERIVED EXOSOMES AND THEIR INTERACTIONS WITH TUMOR MICROENVIRONMENT

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Background: Anaplastic large cell lymphoma (ALCL) is an aggressive type of T-cell non-Hodgkin lymphoma with high frequency in childhood. The current WHO classification recognizes two distinct types of ALCL based on the expression of the anaplastic lymphoma kinase (ALK), the ALK+ and the ALK- ALCL. The ALK+ ALCL is characterized by chromosomal translocations involving the *alk* gene locus, the most frequent being the t(2;5) leading to aberrant expression and activation of NPM.ALK oncoprotein. The latter is known to activate multiple oncogenic pathways including the Ras/ERK, JAK/STAT3, PI3K/AKT/MTOR, JNK/Jun, Sonic Hedgehog and others, resulting in cell cycle and apoptosis deregulation. However, the potential role of bi-directional crosstalk between the microenvironment (TME) and ALCL cells is not yet known. Exosomes are endosome-derived vesicles that contain DNA, RNA, proteins, lipids and other factors that have been reported to play significant role in intercellular signaling. Their role in the ALCL-TME interactions has not been investigated to date.
Aims: To characterize the ALCL-derived exosomes and investigate their possible functional interactions with microenvironment using an in vitro system as well as an ex vivo mouse model of ALK+ and ALK- ALCL.
Methods: Exosomes derived from ALK+ and ALK- ALCL cell lines were isolated using well established ultracentrifugation protocols. The exosomes were subsequently characterized by nanoparticle tracking analysis (NanoSight) and transmission electron microscopy for their size and shape. The molecular composition of exosomes at the RNA and protein level was assessed by real time RT-PCR and Western blotting, respectively. Stromal cells including bone marrow-derived fibroblasts and mesenchymal stem cells were co-cultured with ALK+ and ALK- ALCL cells or they were educated with exosomes and the biologic effects were investigated. Uptake levels of exosomes by recipient lymphoma or stromal cells were assessed by DIR labeling and flow cytometry. Expression of proteins associated with the Cancer-Associated Fibroblasts (CAF) was evaluated by immunofluorescence and confocal microscopy. The biologic effects of co-cultured lymphoma and stromal cells after ALK (Crizotinib) and STAT3 (Stattic, XIII) inhibition were analyzed with standard cell viability and proliferation assays and flow cytometry in our in vitro ALCL system. In addition, an ex vivo mouse model for ALK+ and ALK- ALCL (xenografts) was used in this study.

Results: We characterized for first time the ALK+ and ALK- ALCL-derived exosomes. Transmission electron microscopy and nanoparticle tracking analysis showed significant differences in exosome size among various ALCL cell lines. Western blot analysis performed on both, whole cellular and exosomal lysates, confirmed the expression of various exosomal markers including Rab5, Alix, and CD82. Lack of AIF in the exosomal lysates served as a quality control. More importantly, Western blot analysis revealed the presence of critical components of the ALK signaling in NPM-ALK positive ALCL-derived exosomes including pALK, pSTAT3, AP-1 transcription factors, pAKT and mTOR pathway kinases. ALCL-derived exosomes were uptaken by ALK+ and ALK- ALCL cells at a high level ranging from 30% to 80% at 6 hours, and similarly, they were uptaken by bone marrow-derived fibroblasts and mesenchymal stem cells at a high level as well. Co-culture of the ALCL cells with stromal cells resulted in activation of stromal fibroblasts that acquired a CAF phenotype (aSMA+) as shown by immunofluorescence and confocal microscopy. Furthermore, co-culturing of ALK+ ALCL and stromal cells altered response (sensitivity) of ALK+ ALCL cells to Crizotinib treatment at a moderate level.
Summary/Conclusions: This is the first study to characterize the ALK+ and ALK- ALCL-derived exosomes and demonstrate potential exosome-associated interactions between the ALCL and stromal cells. Ongoing functional studies will enrich our understanding for the involvement of the microenvironment in the mechanisms of resistance to targeted therapy that would lead to more efficient therapeutic approaches for the ALCL patients.

E1377

A 6-GENE EXPRESSION SIGNATURE IN MANTLE CELL LYMPHOMA: RESULTS FROM THE FONDAZIONE ITALIANA LINFOMI (FIL)-MCL-0208 TRIAL

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Background: The aggressive clinical behavior of mantle cell lymphoma (MCL) is mainly attributed to the t(11;14)(q13;q32) traslocation and cyclin D1 (CCND1) overexpression. Nevertheless, a certain degree of clinical/biological heterogeneity has been reported.
Aims: To identify MCL subsets with peculiar clinical/biological features in the context of a cohort of homogeneously treated MCL patients.
Methods: The study used gene expression profiling (GEP) and quantitative real-time PCR (qRT-PCR) validations in peripheral blood (PB, n=46) and formalin fixed paraffin embedded (FFPE, n=42) samples from 82 MCL cases enrolled in the Fondazione Italiana Linfomi (FIL)-MCL-0208 randomized clinical trial (high-dose therapy followed by autologous transplantation).
Results: i) Unsupervised and supervised analyses. GEP from 27 PB samples were analyzed by principal component analysis (PCA) and divided in two subgroups named PCA1 (14 cases) and PCA2 (13 cases). Supervised analysis, according to PCA classification, identified a gene expression signature of 902 probes (700 up-regulated). Gene Set Enrichment Analysis (GSEA) demonstrated a significant enrichment of five B Cell Receptor (BCR)-related gene sets, these genes being constitutively over-expressed in PCA2 samples. ii) Identification of a "PCA2-type" gene signature. By merging the lists of differentially expressed genes and the BCR signaling related genes according to GSEA, a group of 14 genes, all overexpressed in PCA2 cases, was obtained. Among these genes, 6 genes, *AKT3*, *BCL2*, *BTK*, *CD79B*, *PIK3CD*, and *SYK*, were selected for further validations. iii) Generation of a 6-gene prediction model. These 6 genes were analyzed by qRT-PCR and utilized to generate a prediction model by using 17 cases as training cohort and 10 cases as validation cohort (all from PB samples). By this approach, 10/10 cases of the validation cohort were correctly assigned according to the PCA2/PCA1 classification. qRT-PCR was then utilized to classify 19 additional cases (10 PCA2 cases) not employed in GEP. Overall, in the 46 cases, 23 cases were classified as PCA2 by the GEP/qRT-PCR approach. iv) Clinical/biological correlations. No association was found between the 6-gene signature and *IGHV* status (33/41 unmutated *IGHV* cases) and the overexpression of *SOX11* (15/27 cases over the median value). Moreover, no association was found with the presence of the main recurrent mutations of the *ATM*, *BIRC3*, *CCND1*, *KMTD2*, *NOTCH1*, *TP53*, *TRAF2*, *WHSC1* genes. Finally, an "ad-interim" analysis of progression free survivals (PFS) suggested a shorter PFS (2-years PFS 76% *vs* 44%, p=0.04) for PCA2 cases. v) Application of the 6-gene signature to FFPE samples. By testing the 6-gene signature, by qRT-PCR in FFPE samples, 22 and 20 cases were classified PCA1 or PCA2, respectively. Again, in this independent series, PCA2 group demonstrated a trend for shorter PFS (2-years PFS 95% *vs* 80%, p=0.15). Merging together cases analyzed using PB and FFPE material, PCA2 patients had a shorter PFS (2-years PFS 85% *vs* 61%, p=0.02). vi) 6-gene signature and sensitivity to the BCR inhibitor ibrutinib. We investigated the proliferation rate of the MCL cell lines Rec-1, Jeko-1, Mino, JVM-2, JVM-13, and Z-138 in presence or in absence of ibrutinib 10 nM for 7 days. By qRT-PCR sensitive cell lines showed higher expression levels of the selected six genes than the resistant counterpart, and were classified as PCA2.
Summary/Conclusions: A novel 6-gene expression signature related to the BCR pathway has been found to characterize MCL cells with peculiar clinical/biological features and sensitivity to BCR inhibitors.

E1378

BONE MARROW MESENCHYMAL STEM/STROMAL CELLS FROM SPLENIC MARGINAL ZONE LYMPHOMA PATIENTS SHOW DISTINCT INTRINSIC CHARACTERISTICS AND EFFECTS ON B-LYMPHOCYTE CHEMOTAXIS AND APOPTOSIS

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Background: Splenic marginal zone lymphoma (SMZL) originates from the neoplastic transformation of mature B-lymphocytes. In most cases, B-lymphocytes infiltrate bone marrow (BM), suggesting a potential involvement of BM microenvironment in disease pathology.
Aims: To examine BM-derived mesenchymal stem/stromal cells (MSCs), since they comprise key components of the BM hematopoietic stroma, in order to investigate if they exhibit altered intrinsic properties *in vitro* or if they stimulate altered B- and T-cell immunomodulatory properties in SMZL patients compared to healthy controls.
Methods: BM MSCs were isolated and expanded *in vitro* from 12 SMZL patients and 11 healthy controls. MSCs were cultured for a total of 5 passages (P) and were phenotypically characterized by flow cytometry (FC). The colony forming unit-fibroblast (CFU-F) assay was used to estimate MSC frequency within the BM mononuclear cell (BMMC) fraction in P0 and their clonogenic capacity in P2. MSCs differentiation to adipocytes and osteoblasts was assessed by cytochemical stains. Their proliferative potential was evaluated by Methyl Triazolyl Tetrazolium assay. To assess the effect of SMZL MSCs on lymphocyte proliferation, B- and T-cells were immunomagnetically isolated (Miltenyi Biotec) from peripheral blood (PB) of normal individuals, labeled with carboxyfluorescein succinimidyl ester (Gibco) and subsequently cultured in the absence or presence of confluent layers of SMZL or normal MSCs, in the presence of activating factors. B cell apoptosis was evaluated via FC using 7-AAD staining, after co-culturing with SMZL or normal MSCs. Finally, B-cell chemotaxis was evaluated by monitoring the migration of isolated B-cells through a 5μm pore membrane in a transwell plate towards the confluent layers of either SMZL or normal MSCs.
Results: In vitro expanded MSCs from both groups were adherent cells with a spindle-shape morphology, which expressed CD29, CD73, CD90 and CD105 and were negative for the CD14, CD34 and CD45 cell surface markers. SMZL and Normal MSCs were similar in their differentiation to adipocytes and osteocytes as evidenced by Oil Red O and Alizarin Red staining, respectively. The frequency of MSCs within the BMMC compartment was significantly lower in patients as compared to healthy individuals (2.51 *vs* 7.95 colonies per 10^5 BMMCs respectively; p=0.04) apparently due to the predominance of the lymphoma cells within patient BMMCs. SMZL MSCs in comparison to Normal MSCs at P2, displayed defective clonogenic potential (3.36 *vs* 6.23 colonies respectively, p=0.03) and reduced proliferative potential (p<0.03). In co-culture experiments, SMZL and Normal MSCs showed no difference in their effect on normal B-cell or T-cell proliferation. However, SMZL MSCs produced a statistically significant advantage on B-cell survival as measured in B-cell apoptosis experiments. More specifically, 50±1.46% of B cells cultured in medium alone were apoptotic, while only 23±2.63% and 17±0.77% of B cells co-cultured with either Normal MSCs or SMZL MSCs were apoptotic (p=0.009 when comparing SMZL *vs* Normal MSCs). Finally the transwell migration assays showed that SMZL MSCs displayed a stronger chemotactic activity on isolated normal B-cells, in comparison to Normal MSCs, with the percentages of migrated B-cells being 34% and 22% respectively, (p=0.009).

Summary/Conclusions: Our data suggest that SMZL MSCs are intrinsically defective in terms of proliferative and clonogenic potential and moreover they exert distint anti-apoptotic and chemotactic effects on healthy-donor derived B cells. The impact of SMZL MSCs on the properties of patient derived peripheral B cells is currently under investigation.

E1379

INTERRUPTION OF CCL20-CCR6 INTERACTION INHIBITS METASTASIS OF ADVANCED CUTANEOUS T-CELL LYMPHOMA

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Background: The expression of interleukin-22 (IL-22), chemokine receptor CCR6, and its ligand CCL20 is upregulated in advanced Cutaneous T-cell lymphoma (CTCL) (Miyagaki et al., Clin Cancer Res 2011). We have also shown that a non-coding RNA, microRNA-150 (miR-150), is silenced in advanced CTCL, and that the miR-150 downregulates CCR6 directly and CCL20 indirectly. Based on these data, we hypothesized that continuous CCR6 and CCL20 upregulation might lead to continuous CCL20-CCR6 interaction in CTCL cells and in turn, lead to metastasis to distal organs in a nutrition-dependent manner. We further found that IL22RA1, one of the IL-22 receptor subunits, was aberrantly overexpressed in CTCL, and that its knockdown decreased CCL20 production. These results suggested that the IL-22 produced by the CTCL cells might activate the IL-22 receptor in these cells, leading to the activation of downstream targets and subsequently increasing the transcription of CCL20 (Ito et al., Blood 2014). However, we could not determine the downstream cascade of IL-22 that mediates CCL20 transcription activation.
Aims: Aim of this study is to determine whether 1) CCR6-CCL20 interaction is actually functional in advanced CTCL cells representing metastatic capability, and 2) which transcriptional factor(s) might activate CCL20 activation.
Methods: Immunohistochemistry of p-STAT3 and CCR6 were performed for five patients with Mycosis Fungoides patients of early and advanced phase of the same individual. CTCL cell lines such as My-La, HH MJ and HUT78 were used for in vitro experiment. Transient knockdown of IL22RA1, CCR6, and CCL20 was conducted for detecting functional analysis of CTCL cells. To determine whether the transient knockdown of STAT3, CCL20, or CCR6 or treatment with neutralizing CCL20 antibody could reduce the migration ability of CTCL cells, we conducted an in vitro migration assay. To examine the *in vivo* effect of neutralizing CCL20 antibody, we used NOD/Shi-scid IL-2γnul mice inoculated with CTCL cells (namely CTCL mice). Written informed consent was obtained from all patients prior to collection of specimens, in keeping with all institutional policies and according to the Declaration of Helsinki. Samples were collected under a protocol approved by the Institutional Review Boards of Akita University and University of Tokyo.
Results: We demonstrated increased STAT3 expression during the progression of primary CTCL. STAT3 was spontaneously activated and mediated the transcription of CCL20 in CTCL cell lines. However STAT3 was not spontaneously activated in mantle cell lymphoma cell lines because these cell lines required upstream stimulation of IL-22 for activation of CCL20. In vitro migration assay demonstrated that all treatments reduced the nutrition-dependent migration activity of CTCL cells. Notably, treatment with neutralizing CCL20 antibody reduced the migration ability of the cells without decreasing the expression of CCL20 and CCR6. This demonstrates that the CCL20-CCR6 interaction is actually functional in metastatic CTCL cells. *in vivo* administration of neutralizing CCL20 antibody significantly prolonged the survival of the CTCL mice.

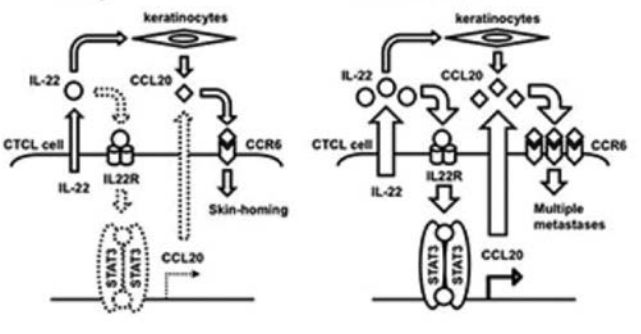

Figure 1.

Summary/Conclusions: These findings suggested that automatic activation of the STAT3/CCL20/CCR6 cascade was actually involved in advanced CTCL lymphomagenesis. On the other hand, activation of STAT3 might require upstream stimulation of IL-22 in early CTCL (see image Figure). Thus, disruption of CCL20-CCR6 interaction could be a key therapeutic strategy against advanced CTCL.

E1380

TUMOR GENE EXPRESSION PROFILES ASSOCIATED WITH SINGLE-AGENT COPANLISIB ACTIVITY IN HEAVILY PRETREATED PATIENTS WITH INDOLENT AND AGGRESSIVE NON-HODGKIN LYMPHOMA (NHL)

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Background: Copanlisib, a novel pan-class I PI3K inhibitor with predominant activity against α and δ isoforms, has shown promising single agent anti-tumor activity in a phase 2 study (NCT01660451, part A) in heavily pretreated patients with indolent and aggressive lymphoma.
Aims: Tumor gene expression profiling was used as a discovery tool to identify genes or pathways associated with copanlisib treatment outcomes and provide potential predictive markers and rationale for novel combinations.
Methods: Single gene multivariate approach and gene set enrichment analysis (GSEA) using RNA expression array data from archival tumors from patients enrolled in the phase 2 trial were performed to identify genes and pathways associated with copanlisib efficacy. A weighted gene expression score (WGS) reflecting overall expression level for each pathway was generated from logistic regression and Cox proportional hazards models to assess association with response status and progression-free survival (PFS), respectively.
Results: A total of 24 patients including both indolent and aggressive lymphoma were available for analysis. Patients had either complete response (n=3), partial response (n=5), stable disease (n=11), or progressive disease (n=5) as best clinical response; follicular lymphoma (FL, n=10), marginal zone lymphoma (n=2), mantle cell lymphoma (n=2), diffuse large B-cell lymphoma (DLBCL, n=5), transformed indolent lymphoma (n=2) or chronic lymphocytic leukemia (n=3). The GSEA gene sets ranked highest for correlation with response were those reflecting upregulated B-cell receptor (BCR) signaling and a PI3K signature (including both α and δ isoforms), with normalized enrichment scores of 1.92 and 1.62 and false discovery rates of 0.014 and 0.087, respectively. Response rates were increased in patients with high BCR and PI3K WGS compared to low (nominal p=0.06 and 0.07, AUC=0.81 and 0.75, respectively). PFS was longer in copanlisib-treated patients with high (above the median) BCR WGS (377 *vs* 62 days, HR=0.035, nominal p<0.0001) and in patients with high PI3K WGS (288 *vs* 104 days, HR=0.24, nominal p=0.02) compared to those with low. In contrast, upregulation of NFκB, IL6/JAK/STAT3, stromal and inflammatory process gene sets and the individual genes NOP10, MT2A, and CSTB were potentially associated with lower likelihood of response to copanlisib.
Summary/Conclusions: Durable response to single-agent copanlisib is associated with tumors with PI3K pathway activation and/or strong BCR signaling. A strong NFκB expression profile is associated with lower likelihood of response to single-agent copanlisib. The higher response rate in FL as compared to DLBCL is consistent with a predominant BCR/PI3K expression profile in FL, with low NFκB activation. These results suggest that FL even in advanced stage and prior extensive treatment can respond to single-agent copanlisib. Efficacy in DLBCL may require combination therapy, adding agents that target NFκB pathway to copanlisib.

E1381

PROGNOSTIC IMPACT, PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF CONCORDANT AND DISCORDANT BONE MARROW INVOLVEMENT IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: In the rituximab era, the biology and prognostic influence of bone marrow (BM) infiltration in patients with diffuse large B-cell lymphoma (DLBCL) has been hardly studied.
Aims: In this retrospective study, we aimed to investigate: i) the prognostic influence of concordant (DLBCL histology) and discordant (low-grade lymphoma) BM infiltration in patients with DLBCL; ii) the correlation of cell of origin (COO) of the DLBCL and the type of BM infiltration; iii) the clonal relationship between BM and lymph node (LN) tumor cells in the discordant cases; and iiii) the incidence of *TP53*, *CMYC*, *BCL2*, *BCL6* genetic alterations in the cases with BM infiltration.
Methods: All patients with histological diagnosis of DLBCL in our center from January 1, 1999 were included. Survival analysis was performed only in the patients treated with rituximab (R) plus curative chemotherapy. All BM samples were reviewed and classified as concordant or discordant infiltration according to histological criteria. COO was assessed by Hans method. Phenotypic description of discordant marrows was performed by flow cytometry (FCM). Clonal relationship between BM and lymph node (LN) was performed by VDJ sequencing according to BIOMED-2 protocol. *TP53*, *CMYC*, *BCL2*, *BCL6* were analyzed using fluorescence in situ hybridation (FISH).
Results: From 232 patients included in the study, 36 (15%) had concordant histological BM infiltration and 22 (9%) discordant. Phenotypic characterization of the discordant cases, including those detected only by FCM, was heteroge-

neous, representing different types of indolent B-cell lymphomas:CLL (7), FL (8), MZL (4) LPL (2), DLBCL (23), Composite (2), Not specified (8). Clonality studies from the 15 evaluable discordant cases, confirmed the same clone in both BM and lymphadenopathy in 12 patients (80%); we observed that the most frequent VH segment was VH4.34, only seen in patients with non-germinal center (non-GCB) COO phenotype. FISH analysis revealed a high rate of genetic aberrations, particularly in the concordant group of patients, as shown in table 2. *BCL2* translocations stood out in the GC phenotype group, while CMYC gains concentrated among those with discordant BM infiltration. *TP53* deletions, were mostly seen in the concordant non-GC group of patients. Survival analysis were conducted in the 189 patients treated with R plus curative chemotherapy. With a median follow up of 58 months, 5-year PFS was significantly worse in patients with concordant BM infiltration (30,4%) compared with those with discordant (64.8%, p=0.004) or without infiltration (67.8%, p=0.001). This negative influence of concordant BM involvement on PFS was independent of IPI in the multivariate analysis (HR=2.25, 95% CI 1.2 to 4.3, p=0.01). IPI was the only variable with independent influence on OS. By combining COO and type of BM infiltration, we observed that patients with discordant BM and non-GC COO were a group not previously described with decreased PFS (41.9% at 5 years) as compared with the non-infiltrated GCB group (78.3%, p=0.007) and the non-infiltrated non-GCB group (73%, p=0.05) (Figure 1).

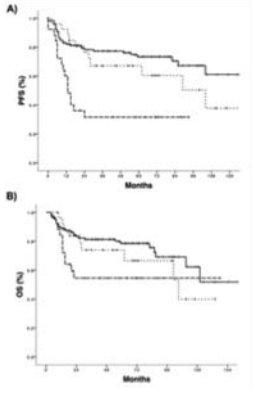

Figure 1. A) PFS of patients without BM infiltration (continuos line), discordant infiltration, dotted line (p=0.2) and concordant infiltration, discontinuous line (p<0.001). B) OS of the concordant BM infiltration group is significantly decreased (p=0.005) while there are no differences between the non-infiltrated group and those with discordant infiltration (p=ns).

Figure 1.

Summary/Conclusions: Our results confirm the adverse prognosis of concordant BM infiltration but reveal that the type of BM infiltration could be more informative when combined with DLBCL COO and FISH analysis, allowing better prognostic stratification of patients. Among discordant cases we find a high rate of clonal relationship between the two different histologies, which suggest that most cases are histologic transformations from a low-grade lymphoma. Larger prospective studies are needed to confirm these results.

E1382

MULTIPLE NF-KB INDUCERS PROMOTE C-MYC-DEPENDENT B-CELL LYMPHOMAGENESIS

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Background: Not only Burkitt lymphoma (BL) with the translocation of MYC, but also diffuse large B-cell lymphoma (DLBCL) by other mechanisms (mutation, amplification, promoter dysregulation…) are associated with dysregulation of c-Myc, the master transcription factor for proliferation. DLBCL's are classified in two subgroups: "Germinal center B-cell" (GCB) without and "activated B-cell" (ABC) with constitutive NF-kappa B activation. This constitutive activation of NF-kappa B can be the result of genetic alterations (MYD88, A20, TRAF2, and TRAF5) or the activation of B-cell receptor or CD40. These features raise the question of the synergy between NF-kappa B and c-Myc in ABC DLBCL. By inducing c-Myc and NF-kappa B, *via* in particular, the viral oncogene LMP1 (Latent Membrane Protein 1), Epstein-Barr virus (EBV) immortalized B cells are another example of the coexistence of these two transcription factors in continuous proliferating B-cells. Although BL are described without NF-kappa B activation, frequent association with EBV raises the question of the role of NF-kappa B activation during the early phase of the oncogenic process.
Aims: To study the synergy between NF-kappa B and c-Myc using the effect of several NF-kappa B drivers in the context of a continuous activation of c-Myc.
Methods: *In vitro*, study of an EBV and c-Myc double inducible human B-cell model and Burkitt lymphoma cell lines. *Ex vivo* and *in vivo,* study of TLR9 activation of B cells from the λ-c-Myc transgenic mouse model. *In vivo,* study of double transgenic mouse model with constitutive activation of CD40 and c-Myc (mouse CD40 / Myc). Techniques used are those for studying functional proliferation, apoptosis, B-cel-differentiation, expression of protein and regulation of genes.
Results: Our results show that induction of NF-kappa B in the context of overexpression of c-Myc, i) by EBV latency III program, provides a selective advantage to those cells (gene expression in favor of a high metabolism, intense proliferation and protection against apoptosis), ii) by TLR9 (*in vivo* and *in vitro* model) increases the survival and proliferation of B lymphocytes of λ-c-Myc mice (increase of activated B cells, splenomegaly, increased B cells proliferation), and iii) by CD40 in the double transgenic CD40/Myc mice have a very aggressive B lymphomagenesis with *in vivo* increased proliferation and tumorigenesis.
Summary/Conclusions: We concluded that, whatever the tested model, NF-kappa B and c-Myc are functionally synergistic for transformation B cells.

E1383

PROGRAMMED CELL DEATH 1 EXPRESSION IS ASSOCIATED WITH INFERIOR SURVIVAL IN PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: Programmed cell death 1 (PD-1) and its ligands PD-L1/PD-L2 have been shown to mediate immune evasion in various cancers, but their prognostic roles have not been studied in patients with primary central nervous system lymphoma (PCNSL).
Aims: To evaluate the prognostic role of immunohistochemical PD-1, PD-L1, and PD-L2 expression in immunocompetent patients with PCNSL.
Methods: We performed a retrospective, immunohistochemical study on 76 PCNSL patients at initial diagnosis initially treated homogenously with high-dose methotrexate (HD-MTX)-based chemotherapy, and evaluated the prognostic roles of high PD-1, PD-L1, and PD-L2 expression. The cut-off values for high PD-1 (≥70 cells/high power field [HPF]), PD-L1 (≥100 cells/HPF), and PD-L2 (≥100 cells/HPF) were determined by the area under the receiver operating characteristic curve.
Results: The median follow-up duration for surviving patients was 31.9 (range, 2.4–128.5) months. Sixteen (21.1%) patients received consolidative upfront ASCT after a median of 4 cycles (range 2–4) of HD-MTX-based chemotherapy. Expression of PD-1, PD-L1, and PD-L2 was high in 7.9%, 13.2%, and 42.1% patients, respectively. High PD-1 (HR: 4.95, 95% CI: 1.54–15.86, P=0.007) and MSKCC prognostic scoring (HR: 2.56, 95% CI: 1.17-5.64, P=0.019) were independently associated with inferior overall survival on multivariate analysis. High PD-1 also remained an independent prognostic factor for inferior progression-free survival (HR 2.73, 95% CI: 1.12-6.69, P=0.028), as did MSKCC prognostic scoring (HR: 1.56, 95% CI: 1.09-2.45, P=0.041) on multivariate analysis. However, there were no differences in survival according to the expression levels of PD-L1/PD-L2. Patients with high expression of PD-1 showed significantly shorter survival compared to those with low expression of PD-1 (P=0.008 for OS, and P=0.037 for PFS) (Figure A, B). In a subgroup analysis of 60 patients who did not receive upfront ASCT, high PD-1 expression tended to associate with inferior OS (P=0.145) and PFS (P=0.197) (Figure C, D). However, among 16 patients who received upfront ASCT, high PD-1 expression was significantly associated with inferior OS (P<0.001) and PFS (P=0.008) (Figure E, F).

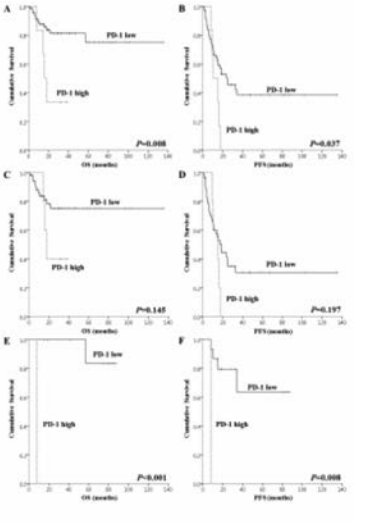

Figure 1.

Summary/Conclusions: We found that high PD-1 expression was associated with inferior survival in patients with PCNSL. PD-1 may be considered a biomarker and potential therapeutic target in PCNSL.

E1384

ARGINASE 1 IS A MARKER OF MYELOID-MEDIATED IMMUNOSUPPRESSION WITH PROGNOSTIC MEANING IN CLASSIC HODGKIN'S LYMPHOMA

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Background: The role of microenvironment in the pathogenesis of classic Hodgkin`s Lymphoma (HL) is well recognized. Our previous work showed that myeloid-derived suppressor cells have prognostic meaning, but their detection in peripheral blood is controversial due to lack of specific surface markers that could help to distinguish from mature polymorphonuclear neutrophils (PMN).
Aims: Evaluate if mature PMN in HL are different from healthy and in particular if they are immunosuppressive
Methods: Using oligonucleotide microarrays, we first evaluated the gene expression profile (GEP) of PMN at the steady state in 5 HL and 5 healthy subjects matched for sex and age, identifying arginase (Arg-1) among the first 10 genes differentially expressed in HL *versus* healthy PMN. Secondly, we investigated if HL-PMN could suppress T-cell activation. Thirdly, since we found that N-HL immunosuppression was due to the increased amount of Arginase-1, we prospectively measured Arginase (s-Arg-1) in sera collected from 118 HL patients, distinguished in a training set (N=40) and a validation set (N=78), and 35 healthy participants.
Results: Arg-1 was increased in HL-PMN (at both m-RNA and protein level), with an increased activity up to 15 times compared to healthy subjects matched for age and sex. In lymphocytes isolated from healthy subjects and PHA-activated, T-cell proliferation and expression of activation markers were down-regulated by co-culture of with HL-N at ratio 1:2, 1:4, and 1:8. s-Arg-1 was increased in HL patients compared to healthy subjects, and reduced after therapy. At diagnosis, s-Arg-1 was higher in patients with advanced stage (p=0.045), carrying B-symptoms (p=0.0048) and with a positive PET-2 scan after two cycles of chemotherapy (p=0.012). In the validation set, baseline levels of s-Arg-1 >200 ng/mL resulted in 92% sensitivity and 56% specificity to identify patients with positive PET-2 scan. Patients carrying s-Arg-1 more than 200 ng/mL had a shorter progression free survival. In multivariate analysis, PET-2 and s-Arg-1 at diagnosis were the only significant prognostic variables (respectively p=0.0004 and p=0.012). With these two prognostic variables, we were able to define three distinct groups based on PET-2 status after two cycles of chemotherapy and s-Arg-1 level at diagnosis. Patients with low s-Arg-1 and negative PET-2 scan (score 0, N=63) had 89.5% PFS at 36 months *versus* 67.6% of patients with at least one unfavourable prognostic factors (score 1, N=46) *versus* 37% of those patients with high s-Arg-1 and positive PET-2 (score 2, N=9, p=0.0004).
Summary/Conclusions: In HL, neutrophils are dysfunctional and immunosuppressive. They also produce Arg-1 that can be easily measured and represents a promising prognostic tool, to validate in larger prospective series.

E1385

A NOVEL DIGITAL PCR ASSAY FOR MYD88 L265P MUTATION DETECTION IN WALDENSTRÖM MACROGLOBULINEMIA: MINIMAL RESIDUAL DISEASE MONITORING AND CHARACTERIZATION ON CIRCULATING FREE DNA

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Background: MYD88^{L265P} mutation is the earmark of Waldenström Macroglobulinemia (WM) and might represent an ideal marker for minimal residual disease (MRD) monitoring. However, current diagnostic tools, as allele-specific quantitative PCR (ASqPCR), are not sensitive enough for MRD monitoring on peripheral blood (PB), harboring low concentrations of circulating tumor cells. Besides, cell-free DNA (cfDNA) analysis has been shown to be highly promising for mutational studies. However, more sensitive approaches are needed and droplet digital PCR (ddPCR) might thus represent a powerful tool in this setting.
Aims: Here we describe the applications of a new, highly sensitive, ddPCR assay for MYD88^{L265P} detection to assess: 1) its feasibility for MYD88^{L265P} mutation screening and MRD monitoring in bone marrow (BM) and PB; 2) its potential application for mutational studies on cfDNA isolated from plasma.
Methods: BM, PB and plasma samples from a local series of WM and IgG-lymphoplasmacytic lymphomas (LPL) and IgM-MGUS patients (pts) were collected at baseline and during follow-up. 20 healthy subjects were used as negative controls. Genomic DNA (gDNA) and plasma derived cfDNA were extracted, according to the blood or body fluid protocols, by Maxwell RSC automatic system (Promega). MYD88^{L265P} was assessed on gDNA (100ng) and cfDNA (5µl) by a custom ddPCR assay on a QX100 System (Bio-Rad). For comparison ASqPCR was assessed on gDNA (100ng), as described [Xu L, 2013]. MYD88^{L265P} cut-off was settled based on the healthy samples background level. IGH-based MRD analysis was performed as described [Ladetto M, 2000; Drandi D, 2015].
Results: Once optimized, MYD88^{L265P} ddPCR assay sensitivity was compared to ASqPCR on a ten-fold serial dilution standard curves. Whereas ASqPCR confirmed the reported sensitivity of 1.00E−03, ddPCR reached a sensitivity of 5.00E−05. Overall, 137 samples from 77 pts (68 WM, 6 LPL, 3 IgM-MGUS), 86 baseline (64 BM, 22 PB) and 51 follow-up (23 BM and 28 PB), were analyzed. Median values at baseline were: age 67 years (range: 38-88), IgM 2.2 g/l (0.3-10.8), IgG for LPL 1.9 g/l (0.8-3.4), B2M 2.6 mg/l (0.14-7.9), infiltration at BM biopsy 45% (0-90%), BM infiltration by flow cytometry 10% (range: 0-87%). 12 pts had splenomegaly and 15 adenopathies. 63/64 (98.4%) diagnostic BM samples and 19/22 (86.4%) diagnostic PB samples scored positive for MYD88^{L265P} (BM median 4.5%, range: 0.02-72.6%: PB median 0.15%, range: 0.01-27.8%) (all 3 negative PB had a positive BM match). To investigate the concordance between methods, 100 samples (60 BM, 40 PB) were tested by both ASqPCR and ddPCR. Overall a good concordance was observed (p=0.0005) with the majority of discordances found in the follow-up samples (13/60 ddPCR positive-ASqPCR negative, 11/60 ddPCR negative-ASqPCR positive). However, ddPCR was able to detect a higher number of mutated cases in diagnostic samples (38 *vs* 36). Moreover, to investigate whether MYD88^{L265P} ddPCR could be used for MRD monitoring we compared it to the gold standard IGH-based MRD assay. Preliminary results on baseline and follow-up samples (18 BM, 5 PB) from 5 pts, selected from 33/57 (57.9%) displaying an IGH rearrangement, showed highly superimposable results between methods. Finally, pivotal results on cfDNA from 33 pts showed 1 log higher median levels of MYD88^{L265P} mutation in cfDNA from plasma (0.7%, range 0-25,7%) compared to PB (0.037%, range: 0.01-20.0%).
Summary/Conclusions: MYD88^{L265P} ddPCR is a feasible and highly sensitive assay for mutational screening and MRD monitoring in WM, particularly in samples harboring low concentrations of circulating tumor cells (e.g. PB after immunochemotherapy). Moreover, cfDNA from plasma samples represents a promising tissue source and might be an attractive, less invasive, alternative to PB or BM for MYD88^{L265P} detection. Methodological validation against IGH-based MRD detection and flow cytometry, as well as correlations with clinical data, are currently ongoing.

E1386

THE ROLE OF PREGNANCY IN PROMOTING NON-HODGKIN LYMPHOMA GROWTH AND AGGRESSIVENESS

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Background: Lymphoma is the most common hematological cancer reported during pregnancy. Recent data suggest that unlike lymphoma occurring outside of pregnancy, pregnancy-associated non-Hodgkin lymphoma is characterized by an excessive involvement of reproductive organs, advanced disease stage at diagnosis and an aggressive course, potentially leading to a high death rate of mothers. However, the mechanisms facilitating this clinical phenomenon are not fully defined.
Aims: The current study aimed to explore the hypothesis that pregnancy-induced hormonal milieu could be an important mediator in the interaction between lymphoma cells and their associated microenvironment, which may potentially contribute to the growth and progress of lymphoma developing during pregnancy.
Methods: BALB/c pregnant and non-pregnant mice were subcutaneously (s.c.) inoculated with murine B cell lymphoma cells (A20). Primary tumor growth was measured biweekly in both groups. Several human lymphoma cell lines (Ramos, Raji and Bl2) and the human lymph node (LN) stromal cell line (HK) were assessed using flow cytometry, Western blot and qPCR analysis, for the expression of estrogen and progesterone receptors (ER α&β and PR, respectively). The direct effect of estradiol (E2) or progesterone on lymphoma cell proliferation was analyzed by trypan blue exclusion. The effect of E2 on the expression of growth factors VEGF-C and VEGF-D and their receptor VEGFR3 in lymphoma and stromal cells was analyzed by qPCR. Similarly, the expression of metalloproteinases MMP-2 and MMP-9 was evaluated.
Results: Pregnant mice showed a significantly accelerated tumor growth, as demonstrated by its increased volume and weight, following lymphoma cell inoculation compared to non-pregnant mice (Fig. 1). All the three lymphoma cell lines, as well as LN stromal cells were found to express ER α&β, but not PR. The Ramos lymphoma cell line treated with E2 demonstrated an increased proliferation rate. This effect was found to be mediated by ERα. In contrast, progesterone appeared to have no effect on lymphoma cell proliferation or viability. Furthermore, E2 induced the expression of VEGF-C and VEGF-D mRNA in lymphoma and LN stromal cells. Notably, the expression of their receptor VEGFR-3 was elevated in lymphoma cells. Likewise, E2 treatment resulted in

increased expression of MMP-2 and MMP-9 mRNA in lymphoma and LN stromal cells, respectively.

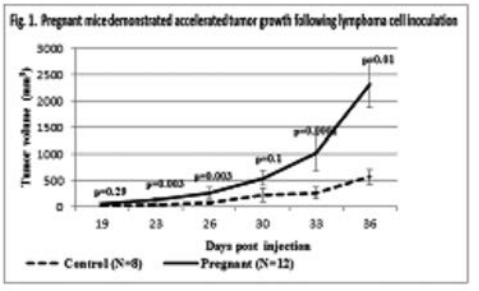

Figure 1.

Summary/Conclusions: The results of the current study have demonstrated for the first time that pregnancy significantly enhances lymphoma growth *in vivo*. This finding could be partially explained by a direct impact of pregnancy-induced estrogen on lymphoma cell proliferation through its effect on the expression of growth factors and metalloproteinases on lymphoma and LN stromal cells. These potential mechanisms need to be be further explored and validated.

E1387

ALK-POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA WITH THE VARIANT EEF1G-, RNF213- AND ATIC-ALK FUSIONS IS FEATURED BY COPY NUMBER GAIN OF THE REARRANGED ALK GENE

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Background: Molecular pathogenesis of ALK-positive Anaplastic Large Cell Lymphoma (ALK+ ALCL) is not completely understood. Approximately 80% of ALK+ ALCL cases harbor the t(2;5)(p23;q35)-associated *NPM1-ALK* rearrangement, while variant 2p23/*ALK* translocations involving at least nine partner genes have been identified in the remaining cases. The 5'*ALK* partners play a key role in the constitutive activation of the chimeric protein by mediating its oligomerization and its subcellular localization. In addition, they impact a range of biological activities of ALK chimeras, including proliferation, transformation and metastatic capacities. Comparative analysis of biological properties of ALK oncoproteins, however, is hampered by the relative low frequency of different variant ALK fusions.
Aims: The aim of the present study was to identify novel ALK-related fusions in ALK+ ALCL and to gain more insight into the molecular pathogenesis of this tumor.
Methods: Six cases of ALK+ ALCL with a cytoplasmic expression of ALK recently diagnosed in our institution were selected. The ALK fusions were characterized using 5'RACE PCR, low coverage full genome sequencing (LCFGS), FISH, array CGH and QRT-PCR. Functional studies were performed on the IL3-dependent Ba/F3 cell line transformation model.
Results: The ALK partner genes identified in the present cases included *EEF1G* (Eukaryotic translation elongation factor 1 gamma), a novel *ALK* partner located at 11q12.3 (one case), and the already known genes, *RNF213/ALO17* (17q25) (one case) and *ATIC* (2q35) (four cases). Notably, all six cases displayed a similar LSI ALK break-apart FISH pattern showing copy number gain of the rearranged *ALK* gene. The LSI 3'ALK/red signal was duplicated on der(11)t(2;11)(p23;q12.3) in the case with *EEF1G-ALK* and amplified in cases with the *RNF213-ALK* (5-7 extra red signals) and *ATIC-ALK* (2-4 extra red signals) rearrangements. FISH pattern in the ATIC-ALK cases suggests the presence of underlying inv(2)(p23q35) associated with one or two copies of the derivative i(2)(q10) carrying two additional *ATIC-ALK* loci each, as previously reported (PMID: 10706887). FISH findings were confirmed by array CGH in two available cases. These data provide a strong evidence that ALCL driven by at least three variant ALK fusions (EEF1G-, RFN213- and ATIC-ALK), but not by the classic NPM1-ALK, requires an increased gene dosage of the rearranged *ALK*. To assess whether this need is caused by the weaker promoter of *EEF1G, RFN213* and *ATIC*, compared to the *NPM1* promoter, we determined at first the relative mRNA expression level of the four *ALK* partner genes. The study revealed a significantly lower expression of *EEF1G, RNF213* and *ATIC* in nonmalignant lymph nodes when compared to *NPM1*. In the next step, we compared oncogenic potential of all four fusions in the murine hematopoietic IL-3 dependent Ba/F3 cell line. We found that *EEF1G-ALK, RNF213-ALK* and *ATIC-ALK* are less potent to transform BaF3 cells than *NPM1-ALK,* which showed the highest oncogenic potential.
Summary/Conclusions: ALK+ ALCL driven by three variant ALK fusions, EEF1G-ALK (novel), RNF213-ALK and ATIC-ALK, are characterized by copy number gain of the rearranged ALK. These lymphomas, but not the NPM1-ALK-positive ALCL, likely require an increased gene dosage of the rearranged *ALK* to compensate the relatively low and insufficient expression of the chimeric gene driven by the partner genes. Occurrence of the *ATIC-ALK* rearrangement in four out of six cases analyzed confirms that *ATIC-ALK* is the most prevalent variant fusion in ALK+ ALCL.

E1388

THE IMMUNE SURVEILLANCE CONSTRAINTS B CELL LYMPHOMAS WITH CONTINUOUS CD40 SIGNALING TO AN INDOLENT PHENOTYPE

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Background: LMP1/CD40 transgenic mice, with specific expression of the chimeric protein composed of the transmembrane domain of LMP1, the main Epstein Barr Virus oncoprotein, and intracellular signaling domain of CD40 in B cells, develop indolent lymphomas after one year in 60% cases. LMP1/CD40 expressing B cells are characterized by constitutive activation of the MAPK JNK and ERK as well as the non-canonical NF-kappa B signaling pathway (Hömig-Hölzel et al., 2008). Since (i) NF-kappa B can induce a strong immune surveillance of the tumor when activated by LMP1, such as in transgenic LMP1 mice (Zhang et al., 2012) or in EBV immortalized human B cells (Le Clorennec et al., 2006, 2008) and (ii) not all LMP1/CD40 transgenic mice will develop a tumor, we supposed that the LMP1/CD40 activation signal could also favor some immuno-surveillance of B cells.
Aims: We asked the question of the control by the immune system on B-cell lymphoproliferation related to the constitutive activation of CD40 since the activation signal is very similar to that of the EBV protein LMP1.
Methods: In order to test the effect of immunosuppression on the kinetics of tumor emergence, we injected every day for 3 months the immunosuppressive Cyclosporin A molecule (CsA) at a dose of 10 mg/kg to 8 months-old wild type (WT) or LMP1/CD40 mice.
Results: While cyclosporin A was toxic *in vitro* on B cells, *in vivo* lymphomagenesis B was accelerated with a greater splenomegaly and increased number of tumor cells in the blood in LMP1/CD40 mice treated with CsA in comparison to untreated mice. Morphologically, tumor B cells of mice treated with CsA were larger with a more immunoblastic phenotype, both on blood smears and spleen tissue sections. Proliferation was increased both ex vivo and *in vivo* (Ki67 and BrdU labeling). Similarly, the number of cells with an activated phenotype was increased in CsA treated LMP1/CD40 mice. Finally, we noted that the injection of CsA increases the expression of PDL -1/B7 -H1 and decreases the expression of CD95.
Summary/Conclusions: Despite direct toxic effect on B-cells in vitro, immunosuppressive treatment with CsA accelerates CD40 signal dependent B-cell lymphomagenesis *in vivo* by inducing the transformation of an indolent tumor into a tumor morphologically and phenotypically resembling to a Richter syndrome. This suggests that B-cell lymphomagenesis related to constitutive CD40 signaling is under partial control of the immune microenvironment, which may influence tumor morphology. This may give some clues explaining why indolent B-cells lymphomas may transform in aggressive form.

E1389

EXPLOITING OXIDATIVE PHOSPHORYLATION TO IMPROVE ANTIBODY THERAPY OF LYMPHOMA

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Background: Diffuse Large B Cell Lymphoma (DLBCL) is the most prevalent non-Hodgkin's lymphoma (NHL) in adults. Since the addition of the Type I anti-CD20 antibody Rituximab to chemotherapy, overall survival of patients has improved dramatically compared to the pre-Rituximab era. However DLBCL has the worst survival rates out of all NHLs with an average 5-year survival of 55%. Unfortunately 40% of all DLBCL patients relapse within 2 years, and those that relapse or have refractory disease tend not to respond well to antibody-based salvage therapies. This has prompted many to try and enhance the efficacy of anti-CD20 antibodies in order to improve first-line treatment of DLBCL, resulting in the evolution of Type II humanised anti-CD20 antibodies. Metabolism has been reported to be involved in Type II anti-CD20 antibody-mediated cell death, however the mechanism and extent of metabolic involvement remains unknown.
Aims: The aim of this investigation is to see whether oxidative phosphorylation can be exploited to improve the efficacy of Type II anti-CD20 therapy of DLBCL.
Methods: Here, we established a panel of DLBCL cells that consisted of two groups of cell lines; one group harbouring a gene expression profile (GEP) with an enrichment of genes associated with an oxidative phosphorylation (OxPhos) phenotype, and the other group without this gene signature. We used the XF Seahorse Mito Stress Test to reveal bioenergetic profiles of the

cell lines before and after treatment with a panel of anti-CD20 antibodies that included Type I and Type II anti-CD20 antibodies. This assay utilises the differential activity of four mitochondrial inhibitors to calculate basal respiration, ATP production, and maximal and spare respiratory capacity of the cell. Using the same method we then assessed whether chemically manipulating oxidative phosphorylation added to the effect on the bioenergetic profile observed following treatment with anti-CD20 antibodies. Finally, we performed clonogenic survival assays to assess whether cytotoxicity of anti-CD20 antibodies was enhanced by simultaneous treatment with Metformin, a well-established inhibitor of oxidative phosphorylation.
Results: We have observed that treatment with anti-CD20 antibodies has a significant effect on the bioenergetic profile of all DLBCL cells in our panel. Each of the antibodies in our panel had a differential ability to increase or decrease bioenergetic activity, in a cell-line specific manner. Further, we have shown that treatment with Metformin causes a significant reduction in the amount of energy produced by oxidative phosphorylation in both groups of cells in our panel. Finally, when analysing the clonogenic survival of cell lines we have found that the cytotoxicity of Type II anti-CD20 antibodies, was enhanced by simultaneously treating cell lines with an oxidative phosphorylation inhibitors.
Summary/Conclusions: With regard to clonogenicity of DLBCL cells, our data suggests that compounds that inhibit oxidative phosphorylation enhance the cytotoxicity of Type II CD20 antibodies. We believe that understanding the mechanism of loss of clonogenic survival will allow us to establish effective treatment combinations to significantly improve the efficacy of anti-CD20 antibody therapy in DLBCL.

E1390

CORRELATION OF PRE-TREATMENT SERUM CYTOKINE ABNORMALITIES AND BLOOD MARKERS OF IMMUNOSUPPRESSION IN PATIENTS WITH LYMPHOMA

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Background: Multiple studies have demonstrated that higher ratios of pre-treatment absolute lymphocyte counts (ALC) and absolute monocyte counts (AMC) are associated with improved outcomes in non-Hodgkin (NHL) and Hodgkin lymphoma (HL). Conversely, elevated serum cytokines at diagnosis are associated with inferior outcomes. Lymphocytes and monocytes have been implicated in immune surveillance, suppression of host anti-tumor immunity, and alterations of the tumor microenvironment supporting growth and survival of lymphoma cells. The relationship between pre-treatment serum cytokines and ALC and AMC remains unknown. We hypothesized that patients with elevated serum cytokines would be more likely to have suppressed ALC/AMC ratios.
Aims: To evaluate the relationship between pre-treatment serum cytokines and ALC/AMC ratios in patients with newly diagnosed diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), mantle cell lymphoma (MCL), T-cell lymphoma (TCL), and HL.
Methods: We studied pre-treatment samples from 725 patients with lymphoma (DLBCL=202, FL=216, MCL=88, TCL=69, HL=150) who enrolled in the University of Iowa / Mayo Clinic Lymphoma SPORE between 2002 and 2011 and were part of previous studies on cytokine secretion in lymphoma. Three-hundred seventy-six of these patients also had ALC/AMC ratios available, obtained from pre-treatment complete blood counts. Serum cytokine concentrations in patients and controls were measured using a standard ELISA (Invitrogen, Camarillo, CA) and analyzed using the Luminex-200 system. Data were acquired using STarStation software (Applied Cytometry, Dinnington, UK). Twelve cytokines passed quality control and were determined to have adequate measurements within the dynamic ranges of the assays: IL-1Ra, IL-2R, IL-8, IL-12p40p70 (IL-12), EGF, HGF, FGF-β, EOTAXIN, MIP-1β, MCP-1, IP-10, and MIG. The cytokines used for analysis were median-normalized to correct for plate effects. To assess the relationship between pre-treatment serum cytokines and ALC/AMC ratios, Spearman's rank correlation coefficients were calculated. P-values below 0.001 were considered statistically significant.

Table 1. Correlations between blood cytokines and ALC/AMC ratios by disease.

	All	DLBCL	FL	MCL	TCL	HL
	(n=376)	(n = 128)	(n = 101)	(n = 37)	(n = 37)	(n = 73)
IL-2R	**-0.37 ***	**-0.43 ***	**-0.33 ***	-0.24	-0.24	**-0.38 ***
IP-10	**-0.21 ***	**-0.43 ***	-0.17	0.10	0.01	-0.18
MIG	**-0.30 ***	**-0.38 ***	-0.22	0.03	-0.41	-0.38
IL-12	-0.16	-0.17	-0.23	0.06	**-0.53 ***	-0.03

Values denote Spearman's rank correlation coefficients (for p<0.001)*

Results: The median age at enrollment was 60 years (18 - 93), 407 patients (56%) were male. Ann Arbor stages were I-II (35%), III-IV (64%), or unknown (1%). Nineteen percent were experiencing B symptoms and 30% had bone marrow involvement. International prognostic index scores were 0-1 (49%), 2 (28%), 3 (16%), or 4-5 (6%). Table 1 shows the statistically significant correlations by lymphoma type.
Summary/Conclusions: Patients with NHL or HL with high pre-treatment serum cytokines tended to have lower ALC and higher AMC. Similar results were observed in all subsets except MCL. These data support the notion that high levels of serum cytokines are immunosuppressive and add to our understanding why the ALC/AMC ratio is of prognostic significance in lymphoma. It also provides further rationale to target immunosuppressive monocytes and the tumor microenvironment for therapeutic benefit.

E1391

KPT-8602, A SECOND GENERATION CLINICAL STAGE SELECTIVE INHIBITOR OF NUCLEAR EXPORT (SINE) COMPOUND SHOWS ENHANCED ANTI-TUMOR ACTIVITY WHEN COMBINED WITH VENETOCLAX OR BENDAMUSTINE IN DLBCL

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Background: Selinexor, the first-in-class oral selective inhibitor of nuclear export (SINE) compound, is currently in Phase 1/2 clinical trials for the treatment of solid and hematological malignancies, including relapsed/refractory diffuse large B-cell lymphoma (DLBCL; NCT02227251). XPO1, the target of selinexor, has been shown to export >200 cargo proteins from the nucleus including major tumor suppressors (TSPs). SINE compounds prevent the nuclear export of many of TSPs facilitating suppressor reactivation. KPT-8602 is a second generation clinical stage oral SINE compound currently undergoing a Phase 1/2 open label study in patients with relapsed/refractory multiple myeloma (NCT02649790).
Aims: In preclinical studies KPT-8602 demonstrated improved tolerability over selinexor, possibly due to its reduced brain penetration. The goal of this study was to test whether single agent or combination of KPT-8602, with either venetoclax (selective BCL2 inhibitor) or bendamustine (DNA damaging agent) can further enhance the anti-tumor effect of KPT-8602 in DLBCL.
Methods: The effects of KPT-8602, bendamustine and venetoclax as single agents and KPT-8602 in combination with either bendamustine or venetoclax on cell viability were tested on a panel of DLBCL cell lines (i.e. RL, DB, SUDHL4, SUDHL10, Pfeiffer, U937, and Farage including the double hit lymphomas Toledo, DoHH2, and SUDHL6) using MTT assays. Total RNA and whole protein cell lysates from DLBCL cells were extracted and analyzed by qPCR and immunoblots. DoHH2 sub-cutaneous xenograft in mice were treated with KPT-8602, venetoclax or bendamustine alone or in combinations of KPT-8602-bendamustine or -venetoclax. Percent tumor growth inhibition (%TGI) and overall survival were determined for each treatment condition. Tumors were collected and analyzed using standard immunohistochemistry (IHC) methods.
Results: Combinations of KPT-8602 with bendamustine or venetoclax were highly effective both *in vitro* and *in vivo*. Using an MTT assay, we showed KPT-8602 was potent against a panel of DLBCL cells (median IC_{50}: ~100 nM) and was synergistic/additive when combined with bendamustine or venetoclax. In the KPT-8602-bendamustine combination DoHH2 xenograft, treatment with each drug showed a%TGI of 52% (KPT-8602) and 76% (bendamustine) while the combination%TGI was 107%. Western and IHC analyses showed that KPT-8602 reduced the expression of key DNA Damage Response (DDR) proteins preventing treated cells from repairing the damage induced by bendamustine. In the KPT-8602-venetoclax *in vivo* study, the individual drugs had similar%TGI (52%; KPT-8602 and 56%; venetoclax). However, when the two drugs were combined, the treatment showed an additive effect (98%). Although, the anti-BCL2 proteins, Bax and Bim, were upregulated in KPT-8602 treated xenograft tumors, these pro-apoptotic pathway proteins were elevated to a greater extent in the combination-treated tumors suggesting the two drugs induced non-redundant mechanism of apoptosis activation.
Summary/Conclusions: KPT-8602 shows single agent activity as well as enhanced antitumor activity in combination with bendamustine or venetoclax through modulation of the DDR and BCL2 pathways in models of DLBCL (including double hits). These data provide rational support for the study of single agent KPT-8602 and in combinations with bendamustine or venetoclax in future DLBCL clinical trials.

E1392

ANALYSIS OF DIFFERENT BIOLOGICAL FACTORS IN PATIENTS AFFECTED BY PERIPHERAL T-CELL LYMPHOMAS NOT OTHERWISE SPECIFIED: GATA-3 EXPRESSION IS ASSOCIATED TO REFRACTORY DISEASE AND POOR OUTCOME

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Background: Peripheral T-cell lymphomas (PTCL) have an aggressive clinical course with a poor 5-year overall survival with conventional therapy. Autologous stem cell transplantation (autoSCT) and allogeneic stem cell transplantation (alloSCT) can improve long-term disease control in first and second remission, respectively. Prognostic factors are not able to discriminate patients with chemorefractory disease. Preliminary evidence from gene expression profiling (GEP) and immunoistochemical studies have suggested that the majority of PTCL-not otherwise specified (PTCL-NOS) can be subdivided in two different groups based on GATA3 and Tbet (transcription regulators of T-helper 2 and T-helper 1 lymphocytes) expression with a poorer prognosis in the former group.
Aims: Because GEP studies are not feasible in routine clinical practice, we investigated whether expression changes of those proteins could be evaluated by immunohistochemistry (ICH) and used to predict prognosis
Methods: We collected paraffin tissues from 47 consecutive patients (pts) with a diagnosis of PTCL-NOS treated at different Italian Institutions, and 38 were available for analysis. Histology was centrally reviewed. Sections were analyzed for Ki-67, GATA3, T-bet expression by IHC. Results were expressed as mean percentages of positive tumor cells. Cases were regarded as immunoreactive for GATA-3 and T-bet if at least 27% and 25% of neoplastic cells exhibited positive staining, respectively. In case of GATA-3, we performed a quantitative analysis [from score 0 (<1%) to score 4 (>80%)] combined with staining intensity [score 1 (weak) to score 3 (strong)] and we defined high score for a summary value of 6-7. Median age was 57 years (range,18-79 years); 13 (38%) pts were characterized by IPI>2; 29 pts (76%) were candidated to transplantation whereas 9 (24%) were not due to age >65 years (n=8) or limited stage/IPI (n=1). All the pts received an anthracycline-based induction chemotherapy followed by autoSCT in 9 patients (7 in first remission); 16 pts (42%) underwent alloSCT in first remission (n=3) or at relapse (n=13). The median follow-up of alive pts was 33 months.
Results: The mean value of Ki67 expression was 60% (range, 10%>95%). Only 5 of 38 (13%) pts were immuno-reactive for T-bet whereas 17 of 38 (45%) were positive for GATA-3. Only two (5%) pts were characterized by double expression of GATA-3 and T-bet. 5 pts (31%) had a high GATA3 score that was associated with a median PFS of only 6 months. We did not observe differences baseline clinical characteristics between pts with positive (n=17) and negative (n=21) immunostaining for GATA-3. Pts immunoreactive for GATA3 were characterized by poor response to anthracycline therapy: 10 of 17 PD (58%) as compared to 4 of 21 (19%) in the negative cohort (p=0.01). Cases with positive GATA3 expression were significantly associated with a reduced 5-year PFS as compared to those with no expression [PFS: 6% (95%CI:0%>22%) *versus* 39% (95%CI:18%>59%), (p=0,03); OS: 38% (95%CI:13%>63%) *versus* 58% (95%CI:30%>78%),(p=ns), respectively]. By multivariable analysis, GATA-3 and Ki67 retained prognostic value on PFS whereas both GATA-3 and IPI influenced significantly the OS.
Summary/Conclusions: Our analysis identifies GATA3 ICH expression as a strong prognostic and predictive biomarker among PTCL-NOS. Pts with positive GATA3 expression values were characterized by chemorefractory disease and poorer outcome even with transplantation strategies. Validation of this biomarker in a larger series of patients is ongoing.

E1393

IN MANTLE CELL LYMPHOMA, BCR SIGNALING AND WNT PATHWAY INDUCE B-CATENIN STABILIZATION AND CELLULAR RE-LOCALIZATION BY DIFFERENT MECHANISMS

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Background: Mantle cell lymphoma (MCL) cells survival relies on the B-cell receptor (BCR) signaling pathway that also facilitates the interactions with the microenvironment. In more than 50% of MCL cases, the Wnt/β-catenin pathway is activated and contributes to cyclin D1 and c-myc expression. As both pathways are important for cell survival as well as tumor cell adhesion, we hypothesized that a cross talk between BCR signaling and β-catenin could affect the cell homeostasis and could be targeted by specific inhibitors.
Aims: The aim of the project is to identify the role of β-catenin in MCL and how it is activated. In parallel, the inhibition of a cross talk between the BCR signaling and the Wnt pathway by ibrutinib is analysed.
Methods: Peripheral blood B cells from MCL patients (n=8) were pretreated with Wnt/ β-catenin inhibitors: XAV-939 (25 μM), promoting degradation of β-catenin through the Axin dependent destruction complex or PKF118-310 (500 nM) blocking the interaction of β-catenin with the transcription factor TCF4. Cells were pre-treated with quercetine (20μM) a PI3K inhibitor, or Ibrutinib (5μM) a BTK inhibitor. BCR signaling pathway was then stimulated with soluble anti-IgM (10 mg/ml). As a control, Wnt/β-catenin pathway was activated by the conditioned media from human bone marrow stromal cells secreting large amount of Wnts. Apoptosis, β-catenin -dependent genes expression and β-catenin subcellular localization were analyzed by flow cytometry, RT-qPCR and cell fractionation respectively.
Results: β-catenin expression is detected in all leukemic MCL samples in variable amount. The inhibition of β-catenin/TCF transcriptional complex by PKF118-330 induces tumor cells apoptosis, suggesting an important contribution of β-catenin to MCL cell survival. In parallel, β-catenin level increases rapidly in response to BCR stimulation and this stabilization is inhibited by a pretreatment with Ibrutinib showing the existence of a cross talk between these two survival pathways. Wnt stimulation stabilizes β-catenin and its translocation into the nucleus leads to an increase of the target genes *i.e.* axin2, cyclin D1 and LEF1. After BCR stimulation, even though β-catenin rapidly translocates into the nucleus it does not induce the same transcriptional response, suggesting a different role of β-catenin when activated by BCR or Wnt. Moreover, stabilization of β-catenin degradation complex by a pretreatment with XAV939 does not induce its degradation after BCR stimulation suggesting that BCR signaling interferes with the β-catenin degradation process. Thus, the impact on β-catenin by BCR signaling is likely independent from Wnt.
Summary/Conclusions: The BCR signaling pathway leads to β-catenin stabilization and nuclear translocation. However, this nuclear translocation translates into a different transcriptional response than the one induced by Wnt. Most likely, β-catenin associates with different nuclear partners driving the expression or repression of BCR specific genes. Since β-catenin can stabilize cell adhesion structures, β-catenin likely represents another player through which the BCR signaling impacts on the interaction of MCL cells with the microenvironment. Importantly, this cross talk can be efficiently interrupted by Ibrutinib, currently used in mantle cell lymphoma treatment.

E1394

METHOTREXATE ELIMINATION AND TOXICITY: THE ROLE OF MTHFR 677C>T POLYMORPHISM IN PRIMARY CNS LYMPHOMA PATIENTS TREATED WITH HIGH DOSE METHOTREXATE MONOTHERAPY

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Background: Methylenetetrahydrofolate reductase (MTHFR) plays a key role in metabolism and homeostasis of intracellular folate, and substitution of C >T is reported to be associated with decreased MTHFR enzyme activity, contributing to low folate level. The genetic association of MTHFR 677C>T in methotrexate (MTX) toxicity was evaluated in a number of studies, however the results were conflicting. The substantial heterogeneity within the study population could compromise the effect of MTHFR 677C>T polymorphism on MTX toxicity, and conflicting published results may have attributed to this.
Aims: The aim of this study was to evaluate the role of MTHFR 677C>T polymorphism in MTX toxicity within a homogenous study population by limiting cancer type to primary central nervous system lymphoma (PCNSL) and chemotherapy protocol to the first four cycles of high dose methotrexate monotherapy and fixed leucovorin rescue regimen (HD-MTX & HD-ARA regimen).
Methods: Data of patients diagnosed with PCNSL treated with HD-MTX & HD-ARA regimen were retrieved. The effect of MTHFR 677C>T polymorphism on the incidence of MTX toxicity was evaluated using a generalized estimating equation analysis.
Results: A total of 111 patients (402 cycles) was included in the analysis. The hematologic toxicity and nephrotoxicity were most frequently presented in patient with heterozygous variant genotype, with an incidence of 57 (29.1%) and 7 (3.6%). The incidence rate of hepatotoxicity and oral mucositis requiring treatment was highest in patient with wild genotype (hepatotoxicity: 7.3%, oral mucositis: 4.1%). None of the patients with homozygous variant genotype experienced the oral mucositis. Twenty eight point six percent of nephrotoxicity occurred in cycles with delayed elimination, and delayed elimination was most frequently seen in patients with homozygous variant genotype (3.6%). The risk for developing clinically meaningful hematologic toxicity was higher in patients with heterozygous variant genotype than wild genotype (odds ratio; OR: 2.60, 95% confidence interval; CI: 1.32-5.09, *P*-value=0.0055). No valid difference was observed between patients with homozygous variant and wild genotype in terms of hematologic toxicity. Other explanatory variables shown to increase the risk of hematologic toxicity were the presence of delayed elimination (OR: 10.06, 95% CI:2.87-35.31, *P*-value=0.0003), high serum lactate dehydrogenase level exceeding the upper range of normal at the time of diagnosis (OR: 2.04, 95% CI:1.09-3.81, *P*-value=0.0257) and concomitant administration of penicillin antibiotics with MTX (OR: 2.88, 95% CI:1.17-7.07, *P*-value=0.0213). No correlation between age, sex, Eastern Cooperative Oncology Group performance status, concomitant administration of proton pump inhibitor and hematologic toxicity was demonstrated. For hepatotoxicity, MTHFR 677C>T polymorphism was the only explanatory variable included in the model. The MTHFR 677C>T polymorphism was not shown to be correlated with risk of

hepatotoxicity (heterozygous variant genotype; OR: 0.75, 95% CI: 0.29-1.96, *P*-value=0.5575, homozygous variant genotype; OR: 0.80, 95% CI: 0.24-2.69, *P*-value=0.7176).
Summary/Conclusions: The MTHFR 677C>T polymorphism is shown to be a valid marker in predicting MTX associated hematologic toxicity. Clinically significant nephrotoxicity might occur in patients without delayed elimination, suggesting that factors other than serum MTX level could play a role. MTX induced hepatotoxicity and oral mucositis occur independently of the serum MTX level.

E1395

IMPACT OF TREATMENT ON RNA EXPRESSION OF IMMUNE PROFILE IN PATIENTS WITH CLASSICAL HODGKIN LYMPHOMA

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Background: Although immunosuppression has long been recognized in classical Hodgkin lymphoma (cHL), the underlying basis for the lack of an effective immune response against tumor remains unclear. Recently, our group showed increased frequencies of pro and anti-inflammatory cytokines, such as interleukins (IL)-6, IL-10, TNF-alpha and sCD25, in cHL patients and the impact of treatment on these cytokines. These cytokines participate in the Hodgkin and Reed-Sternberg (HRS) cells survival and immunological escape.
Aims: In this study we aimed to evaluate the immune gene expression profile in cHL patients at diagnosis and the impact of treatment on this profile.
Methods: Twenty consecutively diagnosed cHL patients, with whole blood RNA extracted at diagnosis and after treatment, were recruited for this study and prospectively evaluated. The general expression of 96 messengers RNAs present in the peripheral blood and involved in immune response was performed by a customized quantitative real-time PCR array (TaqMan® Low Density Array). We also included 7 healthy controls. The data was normalized with B2M mRNAs levels and relative gene expression was calculated by the 2^DDCt method, considering Wilcoxon test and Benjamini-Hochberg adjustment to correct p-values.
Results: At diagnosis we observed that blood of cHL patients presents higher expression of IL-10 (2.4 fold, p=0.013) and decreased expression of CCL2 (-5 fold, p=0.028), CD40 (-2 fold, p=0.013) and HLA-DRA (-2 fold, p=0.028) compared with healthy controls. On the other hand, after treatment, the mRNAs levels returned to normal and no immune-related gene studied was found in different amounts when compared to control subjects. Considering patients after treatment, an important reduced expression of BCL2 (1.5 fold, p= 0.043), CCL2 (3,7 fold, p= 0.003); CCL22 (1.9 fold, p=0.025); CCL5 (1.7 fold, p=0.025), CD40 (2.2 fold, p=0.007), CD80 (2 fold, p=0.015), CSF2(2,5 fold, p=0.007), HLA-DRA (1.7 fold, p=0.025), IL-2RA (1.7 fold, p=0.025), IL-4 (2.5 fold, p=0.007), IL-6 (3.7 fold, p=0.007), LTA(1.7 fold, p=0.038), RORC (1.6 fold, p=0.025) in comparison with cHL patients before treatment. Epidemiologic and clinical correlation are currently being done.

Table 1.

Pre X Control

Gene	Pre	Control	^^Ct mean	Fold Change	p adjusted
IL-10	↑	↓	-1,241	2,4	p=0.013
CCL2	↓	↑	2,445	-5	p=0.028
CD40	↓	↑	1,418	-2	p=0.013
HLA-DRA	↓	↑	1,050	-2	p=0.028

Pre X Post

Gene	Pre	Post	^^Ct mean	Fold Change	p adjusted
BCL2	↑	↓	-0,636	1,5	p=0.043
CCL2	↑	↓	-1,884	3,7	p=0.003
CCL22	↑	↓	-0,924	1,9	p=0.025
CCL5	↑	↓	-0,738	1,7	p=0.025
CD40	↑	↓	-1,144	2,2	p=0.007
CD80	↑	↓	-0,973	2	p=0.015
CSF2	↑	↓	-1,342	2,5	p=0.007
HLA-DRA	↑	↓	-0,744	1,7	p=0.025
IL-2RA	↑	↓	-0,739	1,7	p=0.025
IL-4	↑	↓	-1,342	2,5	p=0.007
IL-6	↑	↓	-1,885	3,7	p=0.007
LTA	↑	↓	-0,761	1,7	p=0.038
RORC	↑	↓	-0,699	1,6	p=0.025

*↑ (High), ↓ (Low)

Summary/Conclusions: In this study, we showed that, at diagnosis, cHL patients presented with more inflammatory gene expression and, after treatment, a more effector immunological profile is found. Interestingly, posttreatment immunological profile is similar to healthy controls. Understanding cHL associated immunosuppression and the immune reconstitution after treatment maybe the key to develop new prognostic factors and treatment strategies.

E1396

MIR-181C REGULATES BRK1, PART OF THE WAVE COMPLEX, AND IS INVOLVED IN T CELL FUNCTION

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Background: miRNAs are short endogenous non-coding RNAs of 18-25 nucleotides in length which play an important role as negative regulators of gene expression either through mRNA degradation or translational repression. miRNAs have been shown to be involved in many cell functions such as angiogenesis, cell adhesion, cell differentiation, cell proliferation and cell survival. Thus, their aberrant expression implicated in various types of cancer including haematological malignancies. In this project, we studied miR-181 family which has been reported to play a crucial role in haematopoiesis, including megakaryocytic, erythroid and myeloid differentiation as well as B and T cell development and differentiation. Most of the available methodologies for miRNA target identification are based on computational algorithms. Therefore, there is a relatively high incidence of false positive target identification. To enable the identification of biologically relevant miRNA targets, a novel functional assay was developed in our lab, which is based on positive/negative selection (Gaken et al, 2012). By using this assay, we identified two functional targets of miR-181c which are *BRK1* and *DHTKD1*. Due to the well-known role of WAVE protein (BRK1 is a component of the WAVE complex) in actin polymerisation in T cells, and the published studies on the function of *BRK1* in cell migration in neuroblastoma, squamous cell lung carcinoma, renal carcinoma and osteosarcoma, we focused on studying the biological roles of BRK1 in T cells. As a model system, we used Jurkat T cells and investigated the role of *BRK1* in actin polymerisation.
Aims: The aims of this project are to identify the potential targets of miR-181 family by using a dual selection functional assay and to study the roles of *BRK1* (identified target of miR-181c) in actin polymerisation and T cell functions, in association with WAVE2 complex.
Methods: Using a functional assay, we identified *BRK1* and *DHTKD1* as novel targets of miR-181c/d and validated these targets by quantitative RT-PCR and western blot analysis of cells transfected with miR-181c/d as well as miR-181c and d inhibitors. We focused on ascertaining the roles of *BRK1* in T cell functions. We demonstrated a targeted reduction of *BRK1* in Jurkat T cells through shRNA (lentiviral strategy) or siRNA knockdown. The effects of *BRK1* knockdown on actin polymerisation and Jurkat T cells functions including changes in total cellular F-actin content, T cell proliferation, T cell activation, lamellipodia formation and T cell spreading as well as cell migration were assessed.
Results: By using the novel functional assay, two targets of miR-181c/d have been identified which are *BRK1* and *DHTKD1*. Both of the genes were validated as targets for miR-181c at protein level. To study the roles of *BRK1* in Jurkat T cell functions, we first showed that knockdown of *BRK1* in Jurkat T cells reduced the expression of other proteins (WAVE2, Abi1, Sra1 and Hem1) that are part of the WAVE2 complex and this indicated that any effects on T cell functions due to *BRK1* knockdown is indeed a result of dissociation of WAVE2 complex. Stable knockdown of *BRK1* in Jurkat T cells showed changes in total cellular F-actin content prior and after T cell activation by F-actin staining using Rhodamine phalloidin. Next, we showed that stable *BRK1* knockdown decreased cell proliferation by trypan blue staining. Also, *BRK1* knockdown showed reduced CD69 expression, indicating defects in T cell activation. Besides that, we also demonstrated that knockdown of *BRK1* caused defects in lamellipodia formation and cell spreading upon Jurkat T cell activation. Knockdown of *BRK1* also resulted in defects in cell migration in Jurkat T cells.

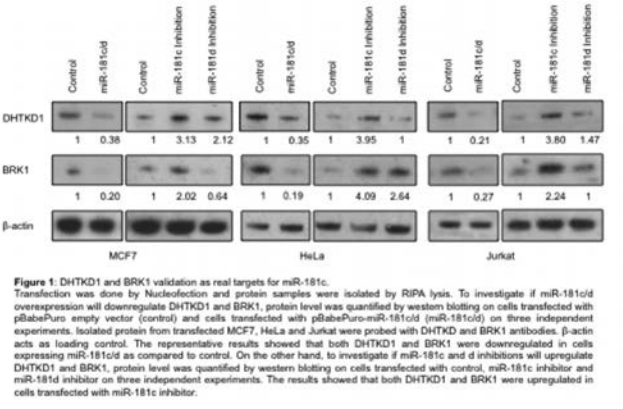

Figure 1: DHTKD1 and BRK1 validation as real targets for miR-181c.
Transfection was done by Nucleofection and protein samples were isolated by RIPA lysis. To investigate if miR-181c/d overexpression will downregulate DHTKD1 and BRK1, protein level was quantified by western blotting on cells transfected with pBabePuro empty vector (control) and cells transfected with pBabePuro-miR-181c/d (miR-181c/d) on three independent experiments. Isolated protein from transfected MCF7, HeLa and Jurkat were probed with DHTKD and BRK1 antibodies. β-actin acts as loading control. The representative results showed that both DHTKD1 and BRK1 were downregulated in cells expressing miR-181c/d as compared to control. On the other hand, to investigate if miR-181c and d inhibitions will upregulate DHTKD1 and BRK1, protein level was quantified by western blotting on cells transfected with control, miR-181c inhibitor and miR-181d inhibitor on three independent experiments. The results showed that both DHTKD1 and BRK1 were upregulated in cells transfected with miR-181c inhibitor.

Figure 1.

Summary/Conclusions: This study showed that miR-181c regulates *BRK1* by translational repression and *BRK1* plays a crucial role in Jurkat T cell functions including cell proliferation, T cell activation, cell spreading and cell migration mediated via actin polymerisation.

E1397

BIOMARKERS IN RELAPSED/REFRACTORY DLBCL AND FL PATIENTS TREATED WITH POLATUZUMAB VEDOTIN: RESULTS FROM THE PHASE II CLINICAL TRIAL (ROMULUS)

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Background: Polatuzumab vedotin (PoV) is an antibody drug conjugate (ADC) comprised of a monoclonal antibody targeting the B-cell marker, CD79b, linked to the microtubule-disrupting agent, monomethyl auristatin E (MMAE). PoV has shown clinical activity in patients with relapsed/refractory (R/R) non-Hodgkin lymphoma. Target expression, CD79b, and expression of anti-apoptotic factors, such as BCL-2, have the potential to mediate sensitivity and/or resistance to ADCs. Moreover, diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease in which distinct subtypes defined by gene expression profiles and, in some cases, prevalence of genetic variants, have been defined.
Aims: Perform a retrospective biomarker analysis on tumor samples at baseline (archival biopsies) from patients in the phase II trial (ROMULUS) who received PoV with rituximab, to correlate the activity of PoV with biomarkers in R/R DLBCL and follicular lymphoma (FL).
Methods: We evaluated target expression (CD79b) and potential for PoV resistance driven by BCL-2 expression by immunohistochemistry (IHC) and quantitative reverse transcription polymerase chain reaction (qRT-PCR) in 34 DLBCL and 19 FL patients with sufficient tissue for analysis. In DLBCL, cell of origin (COO) was evaluated by signature gene expression and a linear predictor score method described previously.[1] Additionally, mutations (i.e., *CD79b*, *CARD11*, *MYD88* and *EZH2*) enriched in COO DLBCL subtypes, and that may confer resistance to anti-lymphoma therapies, were evaluated using a multiplexed gene panel by next-generation sequencing.
Results: CD79b expression was comparable in DLBCL and FL by qRT-PCR and IHC. Consistent with the role of CD79b as a B-cell lineage marker, expression was seen in all samples. There was a range of CD79b expression, but ~90% of patients had expression at higher levels (IHC2/3+). Response to PoV was independent of CD79b expression, as responses were seen in patients with both high and lower levels. For BCL-2, there was no significant difference in expression across indications by qRT-PCR. However, while high BCL-2 expression (IHC 2/3+) was observed in ~90% of FL patients, expression was more distributed across levels in DLBCL. Importantly, there was no relationship between BCL-2 expression and response to PoV by IHC or qRT-PCR. PoV showed activity in both GCB and the more aggressive ABC DLBCL subtypes. Moreover, we observed mutations associated with COO, including *EZH2* (n=2) in GCB patients and dual *CD79b*/*MYD88* (n=2) in ABC patients. Tumor shrinkage and clinical response to PoV were observed in these patients. Similarly, mutations, such as *MYD88* (n=2), associated with resistance to ABC targeting agents were also observed but clinical response was variable.
Summary/Conclusions: These analyses demonstrate broad activity of PoV in R/R DLBCL and FL. CD79b was expressed across B-cell malignancies; while a majority of samples expressed high levels of CD79b, even minimal expression was sufficient for PoV activity. Though anti-apoptotic regulators, like BCL-2, have potential to drive resistance to microtubule-disrupting agents, this was not observed with PoV. While the DLBCL treatment landscape is evolving to account for biologic subtypes, PoV showed activity independent of subtype, in contrast to other targeted therapies. Overall, the robust and expansive activity observed with PoV may position it as an effective component of therapy across DLBCL subtypes and FL. Studies of PoV combined with other anti-lymphoma agents in multiple indications, including in newly diagnosed DLBCL patients, are ongoing.

Reference
1. Pfeifer, et al. *Leukemia*. 2015;29:1578-86.

E1398

ROLE OF BORTEZOMIB IN COMBINATION WITH AEROBIC OXIDATION INHIBITOR ON BURKITT LYMPHOMA CELLS

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Background: Caner and non-malignant cells vary vitally with respect to their metabolic pathway. Consuming elevated levels of glucose from a frequently nutrient-poor environment in order to satiate anabolic respiratory reactions, is a sufficiently prevalent phenomenon of cancer cell metabolic adaptions, which has been conceptualized as the "Warburg effect". However, the molecular mechanism underlying this metabolic reprogramming is obscure. Recent investigations propose that the oncogene-directed metabolic reprogramming, rather than the permanent malfunction of mitochondrial oxidative phosphorylation (OXPHOS), may have profound effects on aerobic glycolysis. The biochemical aspects of the Warburg effect outline a strong explanation for the cause of cancer cell proliferation. However, the more reliant of malignant cells manifest on glycolysis, the more vulnerable to drugs targeting this pathway they will be. Prognosis of Burkitt lymphoma, the most aggressive B-cell lymphoma, is poor even in the Rituximab era. Therefore, targeting the aberrant metabolic pathway rather than illimitably increase the dose of chemotherapy prompts a highly desirable strategy for treating this aggressive lymphoma. Bortezomib is the first proteasome inhibitor approved for treating multiple myeloma and mantel cell lymphoma. In addition to the well-known mechanism on proteasome inhibition, bortezomib is supposed to have effects on the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway, which is deemed crucial to underpin the glycolysis of malignant cells. Given the central role of glycolysis in cancer metabolism, we sought to explore the effect of bortezomib on tumor cell metabolism in Burkitt lymphoma. Considering the fact that even counteracting glycolysis, cancer cells shows low-glucose resistance, which may associate with an increased compensatory upregulation of mitochondrial OXPHOS, the present study was undertaken to investigate the potential of using Bortezomib in combination with OXPHOS inhibitor as a novel therapy for Burkitt lymphoma.
Aims: To investigate the effect of bortezomib (BTZ) in combination with aerobic oxidation inhibitor oligomycin (OM) on proliferation and apoptosis of Burkitt lymphoma cell line Raji, and to explore its possible molecular mechanism.
Methods: Raji cells were treated with different concentrations of BTZ (0, 5, 10, 15, 20, 25 and 30 nmol/L) alone or in combination with OM (0.05 μg/mL). Cell proliferation was detected by CCK-8 method. The mRNA and protein expression levels of oncogene C-myc, hypoxia inducible factor-1α (HIF-1α) and its target genes vascular endothelial growth factor (VEGF) and glucose transporter 1 (GLUT1), as well as key enzymes and proteins related to glycolysis pathway including hexokinase II (HKII), lactic dehydrogenase (LDHA) and succinate dehydrogenase (SDHA) were detected by real-time fluorescent quantitative PCR and Western blotting, respectively. Glucose consumption and lactic acid generation were examined by Glucose (hexokinase, HK) Assay Kit and Lactate Assay Kit, respectively. Apoptosis and cell cycle distribution were analyzed by FCM.
Results: The result of CCK-8 method showed that treatment with different concentrations of BTZ could inhibit the proliferation of Raji cells in a dose-dependent manner (all $P<0.01$), the inhibition effect was significantly enhanced at lower initial concentrations of BTZ (5, 10, 15 and 20 nmol/L) in combination with OM (all $P<0.01$). The expression levels of C-myc, HIF-1α, VEGF, GLUT1, HKII, LDHA and SDHA mRNAs and proteins were suppressed by BTZ, and the expression levels were further down-regulated due to treatment with BTZ in combination with OM (all $P<0.05$). The inhibition of glucose consumption and lactic acid generation induced by BTZ in combination with OM was significantly enhanced as compared with those induced by BTZ alone (both $P<0.05$). The result of FCM showed that BTZ could induce apoptosis of Raji cells in a dose-dependent manner, and the cell cycle was arrested in G2/M phase under a relatively high concentration of BTZ (25 nmol/L). Furthermore, when BTZ was used in combination with aerobic oxidation inhibitor OM, significant synergistic effect was observed; besides, the cell cycle was arrested at G0/G1phase instead.

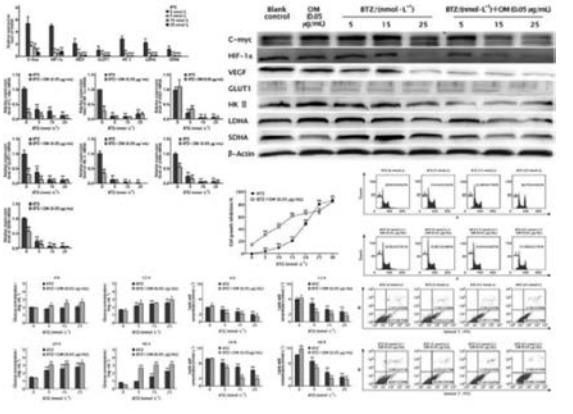

Figure 1.

Summary/Conclusions: BTZ can hinder the glycolysis pathway in Burkitt lymphoma cell line Raji, which can also be enhanced by the involvement of aerobic oxidation inhibitor OM, acting as a synergistic inhibition. The mechanism of this synergy may be interpreted as inhibiting the dual metabolic pathway, namely glycolysis and aerobic oxidation. This inhibition of dual metabolic pathway may imply a novel approach to the treatment of Burkitt lymphoma.

E1399

CONSTITUTIVE GSK-3B ACTIVATION INDUCED B-CATENIN DEREGULATION IN CLASSICAL HODGKIN LYMPHOMA PATIENTS

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Background: Glycogen synthase kinase-3 beta (GSK-3β) is a serine/threonine kinase involved in glycogen metabolism, in cell cycle progression, differentiation and embryogenesis. One of the major biological functions of GSK-3β is to inhibit β-catenin by sequestration and promotion of its proteasomal degradation in the Wnt canonical pathway. Aberrant GSK-3β has been implicated in the pathogenesis of many disorders such as diabetes, Alzheimer's and Parkinson's disease and cancer. The biological role of GSK-3β in classical Hodgkin lymphoma (cHL) has not yet been clarified.
Aims: The aim of this study is to clarify the biological relevance of GSK-3β in the regulation of the β-catenin in cHL.
Methods: Three tissue microarrays (TMA) for immunohistochemical studies were obtained from formalin-fixed paraffin-embedded samples collected at diagnosis from 100 cHL patients. TMA sections were investigated by antibodies reactive with pY216 and pS9 GSK-3β and β-catenin. Three samples of hyperplastic lymph nodes were added to investigate the expression of the same markers in the reactive lymphoid tissue. Immunohistochemical preparations were visualized and images were captured using Olympus Dot-slide microscope digital system equipped with the VS110 image analysis software.
Results: Our results showed that the pY216 GSK3β, which is the stimulatory form of the kinase, was observed in 100% of cHL cases with a range of positivity in the neoplastic population from 8% to 100% and a mean expression of 56%. In 78/100 cases, we noticed that the kinase was predominantly relocated in the nucleus of the Hodgkin and Reed-Sternberg cells (Figure A), in which it is expected to be highly active. Conversely, the germinal centres of the reactive follicles showed a weak cytoplasmic positivity of the stimulatory pGSK-3β. Moreover, 20 samples were assessed positive for the inhibitory form pS9 GSK-3β with a range of positivity from 1% to 58% and a mean expression of 8%. β-catenin was detected only in 12% of the cases with a nuclear localization (Figure B). Interestingly, a statistically significant association between the presence of β-catenin in the nucleus and the inhibitory pGSK-3β expression was observed (P=0.013).

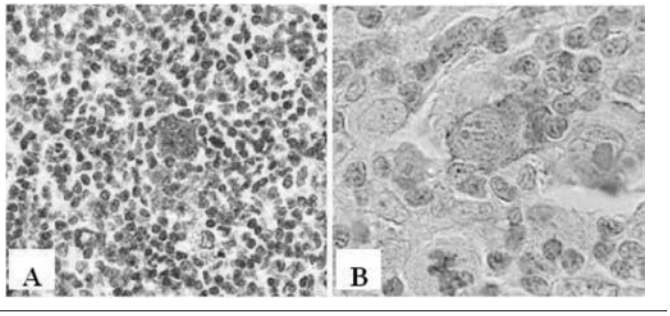

Figure 1.

Summary/Conclusions: Our results reported the constitutive activation of the stimulatory pGSK-3β that plays a previously unrecognized important role in the negative regulation of β-catenin transcription activity in cHL cells, by affecting its binding to the promoters of a subset of related-target genes. GSK-3β is an intriguing protein that seems to be involved in the pathogenesis of cHL, but many questions remain unanswered and the role of GSK-3β and its potential application in this disease become an interesting aspect to clarify. These data suggest GSK-3β as a promising novel target for therapeutic intervention in cHL.

E1400

AUTOLOGOUS STEM CELL TRANSPLANTATION MODULATES T-CELL RECEPTOR PROFILES IN HIV-INFECTED AND -UNINFECTED PATIENTS WITH NON-HODGKIN LYMPHOMA

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Background: Treatment of HIV$^+$ lymphoma patients has been greatly improved by the combination of highly active antiretroviral therapy with high-dose chemotherapy and autologous stem cell transplantation (ASCT). It has been demonstrated that the T-cell recovery after ASCT does not differ between HIV$^+$ and HIV$^-$ patients with non-Hodgkin lymphoma (NHL), because the two groups present with a similar thymic output, as measured by means of T-cell receptor excision circles (TRECs) quantification. Nevertheless, whether T-cell repertoire (TCR) modifications may occur at the same extent in the two group of patients receiving ASCT has not been described yet.
Aims: To better elucidate the extent of T-cell recovery and its effects on the TCR repertoire in HIV$^+$ and HIV$^-$ patients with NHL who underwent ASCT.
Methods: Eleven ASCT-treated HIV$^+$ and nine HIV$^-$ NHL patients were followed for 2 years (T0: pre-ASCT; T6, T12 and T24: 6, 12 and 24 months from ASCT, respectively). The number of TRECs was measured by quantitative real-time PCR. Recent T emigrants (RTE), the cells that were recently produced in the thymus, were identified as CD4$^+$CD45RA$^+$CCR7$^+$ lymphocytes expressing the CD31 molecule. The analysis of TCR beta variable (TCRBV) families was performed by complementarity determining region 3 (CDR3) spectratyping. The deviation of CDR3 size distributions from a theoretical Gaussian curve in each TCRBV family was analysed both qualitatively and quantitatively using the generalized Hamming distance method. Results were compared to that of age matched healthy controls (HC).
Results: Starting at T6, both HIV$^+$ and HIV$^-$ patients presented with a gradual but significant increase in naïve CD4 lymphocytes (difference between means: T12 − T6=27 cells/ml, p=0.011), RTE (T12 − T6=23 cells/ml, p=0.003) and TRECs$^+$ cells (T12 − T0=1355 TRECs/ml, p<0.001) that reached the top at T24. TCR repertoire analysis showed that, compared to HC, the mean proportion of TCRBV families with shifted, restricted and mono/oligoclonal profiles of TCRBV families was significantly higher in samples obtained pre-ASCT (HIV$^+$ − HC=32.3%, p<0.001; HIV$^-$ − HC=39.8%, p<0.001) and post-ASCT (HIV$^+$ − HC=29.8%, p=0.002; HIV$^-$ − HC=24.2%, p=0.012) in both groups of patients. Quantitative analysis showed that the mean perturbation of CDR3 distributions was higher compared to HC in infected and uninfected patients both pre-ASCT (HIV$^+$ − HC=10.5%, p<0.001; HIV$^-$ − HC=12.8%, p=0.001) and post-ASCT (HIV$^+$ − HC=13.1%, p<0.001; HIV$^-$ − HC=12.6%, p<0.001). However, in both HIV$^+$ and HIV$^-$ patients, the pattern of TCR repertoire distortions changed 24 months after ASCT, as supported by modifications in the number of perturbed TCRBV and within the CDR3 distribution profiles, leading to either enlargement or restriction of the TCR heterogeneity.
Summary/Conclusions: Using different approaches, we confirmed that new CD4$^+$ cell production is similarly increased in HIV$^+$ and HIV$^-$ patients with NHL that received ASCT. The production of new lymphocytes was not sufficient to induce a general enlargement of the TCR repertoire but, nevertheless, led to relevant modifications of TCRBV family profiles, not related to HIV infection. Because TCR repertoire is usually extremely stable in HC, these modifications cannot be merely due to a physiologic repertoire "drift" over time, but are likely due to a repertoire re-assortment occurring after ASCT. Modulation of TCR repertoire may have important implications for immune system-mediated anti-infective and anti-tumor activities.

E1401

DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) STUDY REVEALS BIOLOGICAL HETEROGENEITY BETWEEN ETHNICALLY DIVERSE COUNTRIES; VALIDATION OF 6-GENE PROGNOSTIC SCORE IN AN INTERNATIONAL COHORT

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Background: Biological variation may arise from ethnic diversity or environment which may influence disease or host response. The International Atomic Energy Agency sponsored a prospective cohort study of diffuse large B-cell lymphoma (DLBCL) in countries from 5 United Nations-defined geographical regions to test this hypothesis.
Aims: The molecular analysis of DLBCL was conducted aiming to use the pre-

viously reported 6-gene score (*LMO2, BCL6, FN1, CCND2, SCYA3* and *BCL2)* (Lossos *N Engl J Med* 2004; 350: 1828–37) as a predictor of prognosis in DLBCL and validation of the utility of this scoring system in an international cohort.
Methods: Consented patients with DLBCL (n=162) in Hungary (n=28), Chile (n=27), India (n=27), Thailand (n=27), Brazil (n=18), Turkey (n=15), Philippines (n=13) and S Korea (n=7) were treated with R-CHOP between 2008 and 2013. RNA from formalin fixed paraffin embedded (FFPE) diagnostic tissue was shipped to a central laboratory. Expression of the 6 genes were assayed, using either high volume or low volume (Brazil) cDNA, by Taqman QPCR and relative quantification assigned based on expression ratio to normalised copy number. In a multivariate analysis, as described in the following equation: mortality-predictor score=(-0.0273x*LMO2*)+(-0.2103x*BCL6*)+(-0.1878x*FN1*)+(0.0346x*CCND2*)+(0.1888x*SCYA3*)+(0.5527x*BCL2*), variation in gene expression by country was investigated using analysis of variance, while variation in event-free (EFS) and overall survival (OS) for the whole cohort was investigated using Cox proportional-hazards regression models.
Results: There was significant inter-country variation for all 6 genes individually ($p<0.0001$), and when combined in the 6-gene model ($p<0.0001$). The variation in 2y EFS between countries ranged from 56% (Turkey) to 85% (Chile) With the analysis of the cohort as a whole the 6-gene score returned a hazard ratio of 0.35 (95% CI 0.17–0.74) for OS, demonstrating the score can stratify relative survival risk. To further develop the prognostic value, patient mortality-predictor scores were ranked into 2 groups with lower or higher risk of death. In a univariate Kaplan–Meier model of these 2 groups [low (n=81), and high (n=81)], defined by the prediction model based on the weighted expression of the 6 genes (Malumbres *Blood* 2008; 111:5509-5514), there was no significant difference in EFS between low and high risk groups (p=0.18); however, a significant difference in OS between low and high risk ($p<0.01$) was observed (Figure 1A). When patients with a high international prognostic indicator (IPI) (3-5), n=51, and low IPI (0-2), n=111 were analysed separately, the 6-gene score did not add prognostic value to the low IPI cases (p=0.08), but did add additional predictive value for OS, though not EFS, in high IPI cases (Figure 1B, $p<0.05$). There was no significant difference between patients treated with CHOP (n=28) or R-CHOP (n=132) for EFS or OS. *Conclusion:* The analysis of this cohort showed that the 6-gene model added prognostic information for overall survival in high IPI cases, thereby validating its utility for predicting outcome in an international setting.

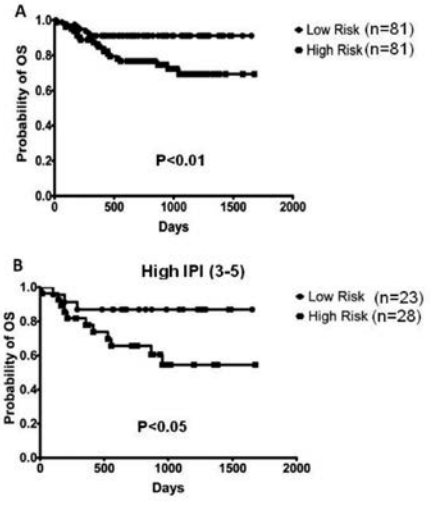

Figure 1. Kaplan-Meier curves showing 6-gene predictor score correlating with overall survival (OS) and adding a prognostic value to a high International Prognostic Index (IPI).

Summary/Conclusions: The analysis of this cohort showed that the 6-gene model added prognostic information for overall survival in high IPI cases, thereby validating its utility for predicting outcome in an international setting.

Non-malignant hematopoietic disorders

E1402

EVALUATION OF IRON OVERLOAD IN THE HEART AND LIVER TISSUE BY MAGNETIC RESONANCE IMAGING AND ITS RELATION TO SERUM FERRITIN AND HEPCIDIN CONCENTRATIONS IN PATIENTS WITH THALASSEMIA SYNDROMES

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Background: Thalassemias are inherited disorders characterized by the presence of hypochromic microcytic anemia due to the reduced or absent production of one or more globin chains in hemoglobin. Individuals with thalassemia are classified according to genotype and the severity of anemia as thalassemia major, intermedia, or minor. Life expectancy is dependent on lifelong blood transfusions and iron-binding therapies. Iron overload (IOL) and associated tissue damage are the most important factors that determine mortality and morbidity.
Aims: We aimed to evaluate iron accumulation in the heart and liver by MRI in thalassemia major, thalassemia intermedia, and S-ß thalassemia patients and to examine its association with ferritin and hepcidin levels.
Methods: Serum ferritin and hepcidin levels were recorded. Iron overload (IOL) in the liver and heart parenchyma was determined based on the standardized R2 and T2* values calculated on MRI. The results were evaluated considering the tissue iron overload, serum ferritin and hepcidin levels.
Results: Comparing the 109 patients with the 30 healthy controls showed the mean age: 24.4±11 *vs* 31.2±5 years, and, median levels of serum ferritin and hepcidin: 1693 *vs* 40 ng/mL and 1.94 *vs* 0.355 ng/mL; (p=0.0001), respectively. In order of the disease diagnosis, the median cardiac T2* and hepatic R2 values were: 25.3 msec, 31.4 msec, 28.2 msec, and, 4.49 msec, 8.14 msec, and 10.35 msec on MRI, respectively. Comparing age, serum ferritin and hepcidin levels in the patients with or without iron overload, only ferritin was significantly higher (p=0.004) in the patients with IOL by means of cardiac tissue, and, age and ferritin were significantly higher (p=0.036 and $p<0.001$) in the patients with IOL by means of liver tissue. Positive correlations between serum ferritin levels and the severity of IOL were found (p=0.002 and $p<0.001$).
Summary/Conclusions: In the present study, enhanced intestinal iron absorption in the intervals between successive transfusions characterized by decreased serum hepcidin levels were considered to result in iron accumulation in our patients.

E1403

HYPERFERRITINEMIA AND SERUM INFLAMMATORY CYTOKINES IN ADULTS WITH GAUCHER DISEASE TYPE 1

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Background: Gaucher disease type 1 (GD1, OMIM #230800) is a rare multisystem lysosomal storage disorder (autosomal recessive). The storage of glucosylceramide in macrophages produces an inflammatory response with iron recycling dysregulation and a release of cytokines. Patients with GD1 suffer from an increased susceptibility to malignancies (*e.g.*, multiple myeloma), and while the underlying mechanisms are not known, it is postulated to be associated with macrophage dysfunction and immune dysregulation.
Aims: This study was undertaken to evaluate ferritinemia, iron metabolism profiles and inflammatory cytokine concentrations in Swedish patients with GD1.
Methods: The study included 16 adults with GD1 aged 20–86 years. All but one patient (94%) carried at least one allele with c.1226A>G (N370S) mutation in the *GBA1* gene. Zimran's severity score index (SSI) was calculated for all patients at the time of their inclusion in the study. The following laboratory variables were collected from fresh blood for analysis: iron profile (s-ferritin, p-iron, p-TSAT); *HFE* gene mutations; s-hepcidin; LFTs; CRP; serum IL-1β, IL-6, IL-8, IL-10, TNF-α; serum sIL-2Rα. Assessment of the aforementioned variables was performed at baseline (the first blood sampling at Karolinska) and at follow-up (every 6–12 months).
Results: Hyperferritinemia >500 µg/L was present in all but 3 patients (81%). Values of P-transferrin, P-Fe, and P-TSAT were within normal limits for all but one patient. There was no correlation between hyperferritinemia and patient's sex, spleen status, and clinical status as defined by the Zimran's SSI. *HFE* gene mutations were analyzed in 11 patients: 4 pts were heterozygous for

His63Asp mutation, one pt was heterozygous for both His63Asp and Cys282Tyr mutations, and 6 patients had no mutation in the *HFE* gene. No obvious correlation between ferritinemia and *HFE* genotype was detected in the studied group. Serum hepcidin concentrations were analyzed in 10 patients; 7 pts had normal hepcidin results; one pt with a normal ferritinemia (75 µg/L) had a low hepcidin concentration at 4 µg/L and 2 pts with a hyperferritinemia of 1910 and 833 µg/L had elevated hepcidin at 50 and 55 µg/L, respectively. *Cytokine and inflammatory marker analysis* The serum concentrations of IL-1β, IL-8, IL-10 were normal in all studied GD1 patients. The mean and median serum concentrations of TNF-α, IL-6, sIL-2Rα, and CRP were within normal limits in all studied patients. However, in 5 of 11 patients (45%) TNF-α was moderately increased. Additionally, 2 patients with the highest TNF-α concentrations (28.4, and 30.4 ng/L) showed mildly elevated IL-6 concentrations (5.9 and 10.7 ng/L, respectively). In the first aforementioned patient, CRP was increased to 12 mg/L but was normal in the second patient. Of note, the first patient was diagnosed with concomitant chronic lung infection (*Mycobacterium avium* and *Aspergillus fumigatus*). Serum levels of IL-6 were within normal limits in the remaining 14 patients (87%). Serum sIL-2Rα concentrations were within normal range in 62% of patients (10/16). *Longitudinal assessment of hyperferritinemia and inflammatory cytokines in treated patients with GD1* Eight patients with at least 36 months' follow-up data were eligible for a longitudinal assessment of ferritinemia, TNF-α, IL-6, IL-8, and sIL-2Rα. Four patients were untreated when their baseline sampling was performed and began GD1 therapy. All remained on unchanged therapy throughout follow-up. Longitudinal assessments of the studied variables for the treated patients are presented in the figure.

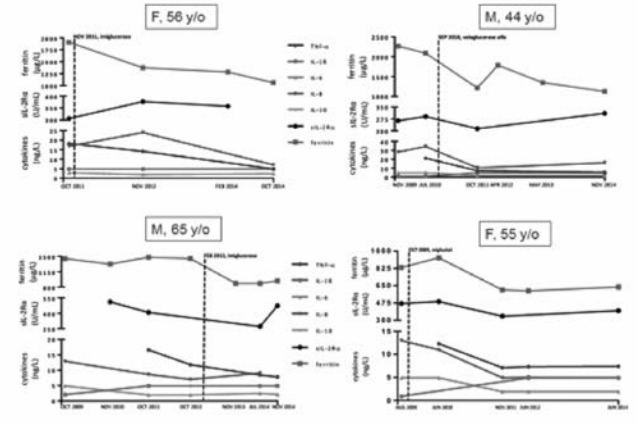

Figure 1.

Summary/Conclusions: The study revealed that hyperferritinemia is common in Swedish GD1 patients. Unlike hemophagocytic lymphohistiocytosis, hyperferritinemia in GD1 is not associated with high serum levels of sIL-2Rα. In some, but not all patients TNF-α and IL-6 levels could be mildly elevated. GD1 therapy has a potential to improve ferritinemia and cytokine levels.

E1404

PEG-HBCO MEDIATED CARBON MONOXIDE AND OXYGEN TRANSFER TO HYPOXIC RED BLOOD CELLS PREVENT, SLOW, AND/OR REVERSE SICKLING IN VITRO

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Background: In Sickle Cell disease (SCD), a mutation promotes HbS polymerization causing abnormal cell morphology that results in occlusion of small blood vessels and increased circulatory inflammation. PEGylated-carboxyhemoglobin (PEG-HbCO; SANGUINATE) is a novel therapeutic agent designed to release carbon monoxide (CO) and then transfer oxygen (O_2) to hypoxic tissue and cells. PEG-HbCO was shown to mediate transfer of either a CO or O_2 and restore normal morphology to hypoxic, sickled RBCs *in vitro*. Studies are now focused on the potential therapeutic implications of delaying sickling, which should maintain normal blood flow through hypoxic microvasculature.
Aims: Current *in vitro* studies examined the effects of time and dose of PEG-HbCO to not only reverse, but also prevent or delay sickling by transferring CO and expedite atmospheric O_2 transfer to the sickled RBCs.
Methods: Reversal of sickling were conducted by deoxygenating RBCs from healthy and SCD volunteers followed by treatment with either PEG-HbCO, fully oxygenated PEG-Hb (PEG-HbO_2) or PEG-BSA. For prevention of sickling studies, fully oxygenated RBC suspensions were treated with increasing amounts of PEG-HbCO and then subjected to hypoxia for 3 hours. Time-dose effects were quantified by area under the curve (AUC) analysis. O_2 transfer studies were conducted by treating hypoxic, sickled RBCs to increasing concentrations of PEG-HbCO and raising the pO_2 from 3.8mm to 40mm. In all studies, the fractions of CO-Hb, O_2-Hb and reduced Hb were determined by co-oximetry and sickled RBCs were quantified by imaging flow cytometry of fixed RBC specimens.
Results: PEG-HbCO -mediated delivery of either CO or O_2 unsickles SCD RBCs. The sickle reversion time-course studies showed differential kinetics between the CO and O_2 capacity to cause unsickling. AUC analysis at 20 minutes demonstrated that both CO and O_2 reversed sickling by 41% and 42%, respectively. PEG-HbO_2 was able to exert substantial unsickling by 5 minutes, where PEG-HbCO showed a delayed, more pronounced effect, peaking approximately 20 to 40 minutes post-treatment. When fully oxygenated SCD RBCs were pretreated with PEG-HbCO prior to oxygenation, sickling was inhibited with an IC_{50} of 2.5±0.6 mg per mL in deoxygenated saline. In addition, treatment concentrations below IC_{50} values had increased time-dose AUC values indicating that sickling was delayed. Oxygen transfer facilitation studies indicated that PEG-HbCO increased the rate of unsickling as measured by AUC by 50% and 15% at 4 and 2 mg per mL, respectively. These levels are within the expected therapeutic dosage of PEG-HbCO.
Summary/Conclusions: RBCs from patients with SCD undergo morphological changes. It is only when the fraction of oxygenated HbS reaches a sufficient level that reversion to normal morphology occurs which promotes vascular perfusion. These experiments showed a concentration and time-dependent effect of PEG-HbCO ability to deliver both O_2 and CO to sickled RBC. Additionally since ASH 2016, PEG-HbCO was shown to improve rheology and down-regulate inflammation in a hemorrhagic shock model; these pathologies also exist in SCD. These data suggest that PEG-HbCO is a promising gas transfer agent that has the potential to improve sickle cell morphology by reversing sickling; the underlying pathology of sickle cell disease co-morbidities as well as decrease hypoxia-induced inflammation. SCD patients with hyperhemolysis and acute chest syndrome have been treated with PEG-HbCO under eINDs. Phase 2 trials with PEG-HbCO are underway in SCD vaso-occlusive crisis.

E1405

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: RETROSPECTIVE ANALYSIS FOR PROGNOSTIC FACTORS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a life- threatening systemic inflammatory condition. Due to rarity of the cases, it presents difficulties in diagnosis and management. Survival remains poor despite aggressive chemotherapy. Patients with fever of unknown origin, lymphadenopathy, deteriorating performance status, encephalopathy and elevated systemic inflammatory parameters frequently show no clear cut diagnostic criteria specific for HLH. Among adults, late onset of inherited HLH is possible, but acquired HLH triggered by infection, malignancy or autoimmune disease is more frequent than in pediatrics.
Aims: Patient outcomes varied markedly despite standardize therapy. Reliable prognostic disease markers may help to tailor intensity of therapy and predict long term outcomes. We attempt to look for variables associated with difference in mortality within 30 days of diagnosis.
Methods: We performed a retrospective search on mayo clinic patient database for the patients with the diagnosis of HLH from 2005 to 2015. HLH-04 criteria were used to select the study population.[1] Patients were divided in two groups based on survival after the diagnosis. We analyzed different clinical and laboratory parameters to detect significant difference between patients expired within 30 days of diagnosis or survived longer than 30 days. Chi-square analysis was performed between two groups of patients: 1- Time to death from diagnosis<30 days. 2- Time to death from diagnosis >30 days. We assessed difference in various clinical and laboratory parameters.

Table 1.

Clinical variables	Survival < 30 days	Survival > 30 days	P value
Age (years)	62	40	0.0004
Median time to start treatment (weeks)	40.7	8.6	0.23
Baseline ferritin (mcg/L)	32866	10667	0.01
Peak ferritin (mcg/L)	45870	29894	0.26
Albumin (g/dL)	2.3	2.6	0.41
LDH (U/L)	1271	720	0.51
Bilirubin (mg/dL)	8.9	4.5	0.14
Triglyceride (mg/dl)	260	276	0.70

Results: Demographics: 40 patients were included in the analysis who met HLH- 04 criteria. Mean age was 49 years, 40% (16/40) were female and 60% (24/40) were male. Underlying HLH etiology was malignancy 37% (15/40), infection 20% (8/40), rheumatological 17% (7/40), idiopathic 20% (8/40). Two patients were peripartum and one with Kikuchi syndrome. Fever was present in 90% (36/40) and splenomegaly was found in 75% (30/40) of the patients.
Labs: Average ferritin at diagnosis 19546 mcg/L, ferritin peak 36284 mcg/L, ALP 226U/L, AST 274U/L, T. bilirubin 6.3mg/dL, LDH 937 U/L, triglyceride 269

mg/dL, albumin 2.4g/dL and EBV DNA PCR were positive in 32% (13/40) of the patients. **Treatment:** Median time from onset of symptoms to start the treatment was 8.1 weeks. Steroids were used in 92% (37/40), etoposide was used in 55% (22/40), and HLH 04 protocol (Etoposide/dexamethasone/cyclosporine) was used in 40% (16/40) of the patients. IVIG was used in 13% with underlying rheumatological process. Mean follow up was 57 weeks (0.1 to 336 weeks) for the whole group. Total 40% (16/40) died within 30 days of diagnosis.
Outcomes: Risk of 30- days mortality was significantly higher in the patients with ferritin >5000 mcg/L at the time of diagnosis and age >55 years. Out of total 40 patients, 54% (12/22) died in ferritin >5000mcg/L group and 22% (4/18) died in ferritin<5000 mcg/L group within 30 days. (p- 0.03) Death rate within 30 days was 65% (11/17) with age >55 years and 22% (5/23) with age<55 years at the time of diagnosis of HLH. (p-0.05). We did not find any significant difference in the following factors: 30 day mortality was 31% (4/13) in EBV positive group and 44% (12/27) in EBV negative group (P-0.40); 50% (8/16) in malignant group and 33% (8/24) in non- malignant. (P-0.29). No difference between the groups in terms of gender, etoposide use, and time to start treatment was found in 30 days mortality. Table 1 summarizes the findings.
Summary/Conclusions: Elevated ferritin at the time of diagnosis and older age are associated with significant risk of 30 day mortality from HLH. In combination with other prognostic factors age and ferritin can be used to risk stratification at the time of diagnosis. These factors can be incorporated in future clinical trials to choose different treatment pathways.

E1406

CLINICAL RELEVANCE OF LOW PERCENTAGE OF GLYCOSYLATED FERRITIN IN MANAGEMENT OF ACQUIRED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A SINGLE CENTER EXPERIENCE

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare and life-threatening condition characterized by excessive chaotic and uncontrolled inflammation leading to multiorgan failure and death. HLH can be either primary (familial), when caused by a genetic mutations affecting cytotoxic function, or secondary (acquired), when due to infectious, malignant, rheumatologic or metabolic conditions. Although diagnostic scores have been proposed such as HLH-04 criteria, which were validated only in pediatric patients, and the HScore, HLH diagnosis is still challenging owe to lack of solid criteria allowing differential diagnosis with other multisystem illnesses characterized by fever, hepatic failure, and neurologic symptoms. Recently, a percentage of glycosylated ferritin (GF)<20% has been proposed as highly specific of HLH and useful in the differential diagnosis from other hyperinflammatory syndromes, such severe sepsis and flare of a secondary underlying disease.
Aims: To validate the usefulness of glycosylated ferritin levels in identification of patients with HLH and to evaluate its potential role in predicting treatment outcome.
Methods: Over a 2-year period, nineteen adult (>18 years) patients with a clinical and laboratory pattern consistent with HLH were included in the study. HLH-04 criteria and HScore were applied for HLH diagnosis. The percentage of glycosylated ferritin was measured by an in-house assay (Marinova et al, in press). Specificity and sensitivity for each approach was then calculated.
Results: Ten out of 19 (53%) subjects had clinical and laboratory findings consistent with HLH. In all cases an underlying disorder was identified: infection (n=1), autoimmune disease (n=1), malignancy (n=1), Hodgkin or non Hodgkin lymphoma (n=1 and n=6, respectively). All but one patient (90%) fulfilled the HLH-04 criteria, whereas a high probability HScore (>90%) was present in 7 out of 10 subjects (70%). Nine out of 19 patients (47%) in whom HLH was ruled out, underlying disorders were autoimmune disease (n=1), infection (n=1), AML (n=1), non-Hodgkin lymphoma (n=5). Among these 9 patients, 2 (22%) met at least five HLH-04 criteria and no one had a HScore probability >90%. Ferritin levels failed to differentiate patients with HLH (11565±95161 *vs* 4669±3541 µg/L, HLH *vs* non-HLH, p=0,18). When GF was measured in our cohort, a percentage of GF level<20% was observed in 9/10 patients with HLH, whereas all unaffected patients had GF level >20%. We found a negative linear correlation between HScore and GF percentage (p<0,05, r=-0,54) and between HLH-04 criteria and GF percentage (p<0,05, r=-0,47). All three methods were able to significantly discriminate affected patients from unaffected (all p<0,05), but GF level<20% showed higher sensitivity against HScore (90% *vs* 70%) and better specificity against HLH-04 (100% *vs* 78%). Moreover, GF shows higher negative predictive value compared to the other diagnostic methods (GF: 90%). Five patients had GF levels measured over the course of the disease. Although the very limited number of patients does not allow to draw any conclusion, two patients who did not show recovery of GF levels >20% within one week after treatment initiation died within one month, while 3 patients who experienced a rise in GF >20% showed a resolution of HLH-related clinical picture and longer survival (median 6 months).
Summary/Conclusions: In our study a fraction of glycosylated ferritin<20% showed high concordance between HLH-04 criteria and HScore and it seemed of help in identifying patients affected by HLH when scores are contradictory as well as in excluding false positive cases. Monitoring GF levels over the course of HLH may also allow to predict response to therapy.

E1407

PROSPECTIVE STUDY OF SPLENECTOMY IN AUTO-IMMUNE CYTOPENIA OF CHILDREN

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Background: Chronic immune thrombocytopenic purpura (cITP), autoimmune hemolytic anemia (AIHA) and Evans syndrome (ES) are rare autoimmune diseases in children, characterized in 20-70% of cases by recurrent outbreaks and treatment dependence. In AIHA and ES, the effectiveness of splenectomy has never been clearly established, as it was in ITP. Since 2004, the OBS'CEREVANCE cohort has been recording all the cases of auto-immune cytopenias in children treated in the 30 French pediatric hematology units.
Aims: This prospective observational national study reports the practice, efficacy and safety of splenectomy performed for all the patients of this cohort.
Methods: On 1st January 2016, patients recorded in this cohort who underwent splenectomy in second-line after immunoglobulin and/or steroid failure, before the age of 18, were analyzed in 3 homogeneous groups according to the target: isolated cITP, isolated AIHA, and ES.
Results: Among the 1094 children included in the cohort, diagnosed between 1986 and 2014 (556 isolated cITP, 356 isolated AIHA and 172 ES), 181 underwent a splenectomy. 86 of those splenectomies were performed in third or more line in heterogeneous patients. 95 splenectomies were carried out on second-line, 80 for isolated cITP, 8 for isolated AIHA and 7 for ES. Laparoscopy was realized in 79% of cases. The median age at splenectomy was respectively 11.5, 5.9 and 8.4 years. The median delay from diagnosis to splenectomy was respectively 2.3, 0.7 and 1.8 years. The complete response achievement rate was similar in the 3 groups. With a median follow-up of 5 years post splenectomy, there was no need for further immunosuppressive treatment for 64/80 cITP (80%), 4/8 AIHA (50%) and 1/7 SE (15%). Of these 69 patients, 84% were still in continuous complete remission at the last follow-up (54 cITP, 4 AIHA, 0 ES). Comparative relapse-free survival curves are presented. Three of the four deaths are possibly related to the weight of the treatments. Infections and thrombosis were more frequent in AIHA and ES than in cITP.
Summary/Conclusions: This prospective national study provides the first evidence-based comparative data on children with early splenectomy for those rare diseases. The benefit-risk ratio of splenectomy seems lower in AIHA and SE than in cITP. Some underlying immune deficiencies as autoimmune lymphoproliferative syndrome are formal contraindications. Prospective pediatric studies will refine alternative immunomodulatory strategies aiming to delay splenectomy as late as possible in children with AIHA or ES.

E1408

A RETROSPECTIVE REVIEW OF BACTEREMIA AND OSTEOMYELITIS IN SICKLE CELL PATIENTS PRESENTING WITH FEVER IN LEBANON

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Background: Patients with sickle cell disease have higher risk of infections with encapsulated bacteria due to immature immune response and functional asplenia manifested by impaired complement activity. This has led historically to serious bacterial infections which include bacteremia with *Streptococcal pneumonia* and osteomyelitis mainly caused by *Salmonella* species.
Aims: In this study we aim to review the bacteremia rates in sickle cell patients in the era of vaccination against *Streptococcus pneumonia* and osteomyelitis rates with the causative organisms. The purpose is to prove an emergence of gram negative organisms, other than *Salmonella*, as the cause of osteomyelitis; and the vast decrease in *Streptococcal pneumonia* bacteremia rates after vaccination.
Methods: We conducted a retrospective chart review of 158 sickle cell patients registered at our database. Every patient presenting to ER with fever had his chart reviewed for blood cultures, pus cultures and MRI results for osteomyelitis over a period of 13 years. 16s ribosomal DNA was also performed to detect organisms not recovered by regular cultures.

Results: Sickle cell patients reviewed included 117 patients with SS type (74%), 36 patients with SB thalassemia type (23%), 2 patients with SC type (1%), 2 patients with SD type (1%), 1 patients with SO Arab type (1%). Patient ages ranged between 3 years and 40 years, with mean of 16 and median of 15. In the time period of the study, 105 patients (66%) presented to ER with fever leading to 581 febrile episodes. 341 episodes were in males (59%) and 240 in females (41%); 384 patients had SS type (66%), 171 had SB thalassemia type (29%), 26 had SD(5%). A total of 912 blood cultures were obtained. None of those grew *Streptococcus pneumonia* (0%), 14 cultures grew *Staphlococci* coagulase negative (1.5%), 1 culture grew *Salmonella paratyphi B*, and 3 cultures grew *Salmonella group C* (in same patient). There was a total of 9 episodes of osteomyelitis documented by imaging among patients who presented with fever (1.5%). 4 of the patients were females (44%), 5 were males (56%), 7 patients had sickle type SS (78%) and 2 had SB thalassemia type (22%). Ages ranged from 6 years to 28 years, with mean of 14 years. 4 patients (44%) had positive cultures and 5 patients (56%) had negative cultures. Cultures (puss/tissue) obtained included 1 with *Enterobacter cloacae*, 1 with *Bacteroides*, 1 with *Pseudomonas aeruginosa* (by 16s rDNA) and the fourth was a blood culture growing *Salmonella* group C. 16s rDNA was performed on tissue samples of 4 patients with osteomyelitis. Two of those patients had negative 16s rDNA and cultures. The remaining patients had *Bacteroides* detected by both modalities in one patient, and *Pseudomonas aeruginosa* detected only by 16s rDNA, not by culture, in the other. Osteomyelitis occurred in the spine in 3 patients (33%), in lower extremities in 3 patients (33%) and in upper extremity in 3 patients(33%). All our patients received pneumococcal vaccination, 104 patients (63%) were on hydroxyurea, and 86 patients (47%) were on oral penicillin prophylaxis.
Summary/Conclusions: Immunization against *Streptococcus pneumonia* has virtually eliminated pneumococcal bacteremia as was evident in our patient population. However, there was emergence of gram negative organisms, other than *Salmonella* as the cause of osteomyelitis. This has important implications for the empiric antibiotic coverage in sickle cell patients with osteomyelitis. Molecular testing by 16s rDNA is to be considered to detect causative organisms of osteomyelitis on tissue/ pus cultures when cultures fail to grow any.

E1409

FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN OMAN: AN UPDATE ON UNIQUE CLINICAL AND MOLECULAR FEATURES

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Background: Familial hemophagocytic lymphohistiocytosis (FHLH) is a rare life-threatening autosomal recessive disorder. Due to high rate of *consanguineous* marriage in Oman, the incidence of HLH is relatively higher in this part of the world. Clinically, HLH is characterized by unexplained high-grade fever and splenomegaly associated with bi/pancytopenia, hyperferrtinemia, hypofibrinogenemia and hypertriglyceridemia.
Aims: To review the clinical features, laboratory findings, molecular characteristics, course and outcome of all cases of FHLH in Oman.
Methods: A retrospective/prospective data analysis of all patients diagnosed with FHLH in Oman during the period from January 1997 to February 2016 was done. Flowcytometry has been used as a fast method to detect perforin expression at diagnosis. Direct DNA sequencing was done to specify mutations in patients and their families. Our results have been initially confirmed and validated by Karolinska Institute, Sweden.
Results: Forty-eight patients (26 males & 22 females) with FHLH were identified. Their median age was 88 days with a range of (3 days– 13 months). Thirty-seven patients (77%) had positive consanguinity, while 25 (52%) had positive family history. Forty-one patients (85.4%) presented with fever and 44 (91.6%) had splenomegaly. Twenty-seven cases (56.25%) had evidence of central nervous system involvement detected either at diagnosis or during the course of therapy. Atypical rare clinical presentations included capillary leak syndrome in 5 cases (10.5%), severe hypertrophic obstructive cardiomyopathy in 2 siblings after receiving high dose dexamethasone, and bilateral renal infiltration as a unique clinical feature in a 2 month-old male infant (Figure 1). Of note, renal involvement in this patient was completely reversed after 4 weeks of chemotherapy. Bicytopenia was found in 39 (81.3%) infants, hypertriglyceridemia in 28 (58.3%) and hypofibrinogenemia in 45 (93.8%). All patients had serum ferritin>500 ng/ml. At molecular level, the most commonly detected genetic abnormality was perforin gene mutation (FHLH2), identified in 14 out of 17 studied families (82.4%). Syntaxin 11 mutation (FHL4) was detected in only one patient, while MUNC 13-4 mutation was not found. Two families did not show any mutations. The high incidence of perforin gene mutations may denote a common genetic pool of FHLH cases in Oman. Twenty-three (47.9%) cases passed away. Fifteen infants were referred late after being treated with antibiotics in peripheral hospitals as presumed cases of sepsis. Our standard of care is HLH-2004 protocol, followed by hematopoietic stem cell transplant (HSCT). Twenty-one patients were successfully transplanted in our center. It is noteworthy that three of them have received haploidentical HSCT.

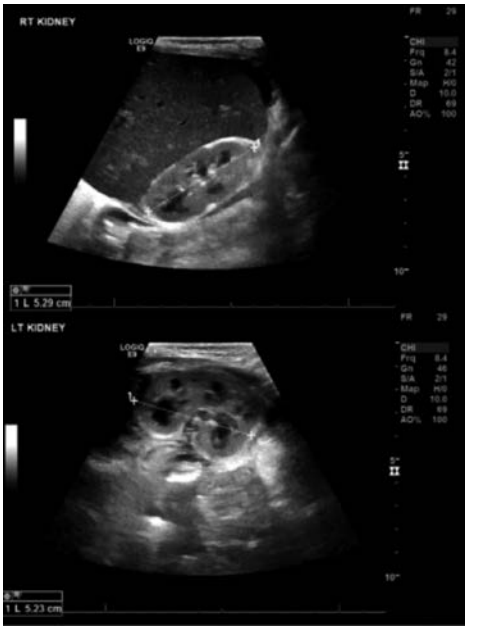

Figure 1.

Summary/Conclusions: We report an updated registry of FHLH cases in Oman, including typical and atypical unique clinical presentations, characteristic molecular features, course of the disease and treatment outcome. Taken into consideration the relatively higher incidence of FHLH in this part of the world, we recommend premarital screening, implementation of pre-implantation genetic diagnosis (PGD) for high risk families and raising the awareness of health care professionals in order to suspect and refer new cases as early as possible.

E1410

CLINICAL PRESENTATION AND OUTCOME OF BENIGN HISTIOCYTIC DISORDERS IN CHILDREN

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Background: Langerhans cell histiocytosis (LCH)& Hemophagocytic lymphohistiocytosis (HLH) are the most common benign histiocytic disorders. The clinical picture of LCH varies from single-system to multisystem disease which carries a potentially poor prognosis. HLH is referred to as either a"primary" form which is hereditary, or a "secondary" form associated with an underlying illness. The disease is invariably fatal if untreated.
Aims: Our goal was to evaluate the different clinical presentations of histiocytic disorders, mainly LCH and HLH. and to determine their outcome and survival in relation to the predisposing factors and the current treatment protocols
Methods: Demographic, clinical, laboratory and radiographic data of patients diagnosed with LCH and HLH over a period of 10 years was retrosecpectively collected. Protocol of treatment was evaluated including the 1 and 3 year event free and overall survival rates
Results: This study included 19 LCH patients (median age 2.3 years) with a males to female ratio of 1:1.7. All LCH patients had multisystem affection, 16 (84.2%) had risk organs involvement. Seven patients(36.8%) had Bone marrow infiltration; 13 (68.4%) patients had hepatosplenomegaly and 14 (73.7%) had lung infiltrations in their CT. In our study 19(94.7%) of LCH patients received LCH III protocol, while only one (5.3%) patient received LCHII protocol of treatment. Six (31.6%) had received salvage line, half of them (3 patients) needed only one treatment protocol, 1patient needed 3 salvage and the last 2 patients received 2 salvage treatment. Six patients died, the median duration from diagnosis till death duration was 1.9 year. Their 1,3 years overall survival was 78.9% and 46.1%. Twenty six patients with HLH were registered; their median age was 3.4 yrs with a male to female ratio of 2:1. Ten patients had HLH secondary to another disease (2 patients (7.7%) had ALL, 2 (7.7%) had Chediak Hegashi, 3(11.5%) had LCH, one had Wolman, one with myelodyplasia and one with ALPS). Sixteen (61.5%) patients did not have an underlying disease, 4 of them(15.4%) had MUNC13-4 gene mutation, 1(3.8%) had RAB27A gene mutation and 1(3.8%) patient had LYST gene mutation, and 2 patients had no common HlH mutations. Thrombosis was reported in 2(7.7%) of the patients and (19.3%) of them presented initially with skin lesions, moreover five (19.3%) patients had CNS manifestations but only 2 of them had positive findings in their MRI and CT brain. Most of the patients were treated according to HLH 2004 protocol, while only 2 received HLH 94 protocol. twelve patients (46.2%) had reactivations, 6 needed only one reinduction, 4 had 2 rein-

ductions and 2 had 3 reinductions. We report the death of 18 patients, with a median duration since the diagnosis till death of 3.36months.
Summary/Conclusions: All our LCH patients had multisystem (MS) risk organ affection, hence the high reactivation and the low overall survival rate. The increased awareness of the HLH criteria for diagnosis, allowed early and accurate detection of cases, yet the death rate is still high.

E1411

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: CLINICAL FEATURES AND OUTCOME IN 124 PATIENTS EVALUATED IN A SINGLE CENTER

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Background: The Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired disorder of hematopoietic stem cells characterized by intravascular hemolytic anemia, thrombotic phenomena and marrow failure. It is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) X-linked gene, responsible for a deficiency in GPI-anchored proteins (GPI-AP). The lack of the GPI-AP complement regulatory proteins (CD55 and CD59) leads to the main manifestations of the disease. The Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired disorder of hematopoietic stem cells characterized by intravascular hemolytic anemia, thrombotic phenomena and marrow failure. It is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) X-linked gene, responsible for a deficiency in GPI-anchored proteins (GPI-AP). The lack of the GPI-AP complement regulatory proteins (CD55 and CD59) leads to the main manifestations of the disease. The Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired disorder of hematopoietic stem cells characterized by intravascular hemolytic anemia, thrombotic phenomena and marrow failure. It is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) X-linked gene, responsible for a deficiency in GPI-anchored proteins (GPI-AP). The lack of the GPI-AP complement regulatory proteins (CD55 and CD59) leads to the main manifestations of the disease. The Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired disorder of hematopoietic stem cells characterized by intravascular hemolytic anemia, thrombotic phenomena and marrow failure. It is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) X-linked gene, responsible for a deficiency in GPI-anchored proteins (GPI-AP). The lack of the GPI-AP complement regulatory proteins (CD55 and CD59) leads to the main manifestations of the disease. The Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired disorder of hematopoietic stem cells characterized by intravascular hemolytic anemia, thrombotic phenomena and marrow failure. It is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) X-linked gene, responsible for a deficiency in GPI-anchored proteins (GPI-AP). The lack of the GPI-AP complement regulatory proteins (CD55 and CD59) leads to the main manifestations of the disease. The Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired disorder of hematopoietic stem cells characterized by intravascular hemolytic anemia, thrombotic phenomena and marrow failure. It is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) X-linked gene, responsible for a deficiency in GPI-anchored proteins (GPI-AP). The lack of the GPI-AP complement regulatory proteins (CD55 and CD59) leads to the main manifestations of the disease. The Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare acquired disorder of hematopoietic stem cells characterized by intravascular hemolytic anemia, thrombotic phenomena and marrow failure. It is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIG-A) X-linked gene, responsible for a deficiency in GPI-anchored proteins (GPI-AP). The lack of the GPI-AP complement regulatory proteins (CD55 and CD59) leads to the main manifestations of the disease.
Aims: The objective of this study was to describe the clinical features at diagnosis and outcome of PNH patients followed-up at a single institution.
Methods: Demographic, clinical, haematological and biochemical data were collected from patients followed-up at the our centre. The diagnosis of PNH was established using flow cytometry.
Results: 880 patients were tested for PNH. A hundred forty patients had PNH clone. Of these, 124 had complete records and were included in this study (61 male and 63 female). The median age at diagnosis was 29.5 years (range 10-72). Ninety one patients (74%) had a previous diagnosis of Aplastic Anemia, 5 patients had MDS (4%) and 28 (22.5%) patients had classical PNH. The most common symptom at diagnosis was fatigue present in 109 patients (88%). 63/124 (51%) had bleeding and / or hemorrhagic suffusion, 18 (14%) jaundice, 23 (18%) hemoglobinuria, 13 (10%) abdominal pain, 11 (9%) thrombosis, 4 (3%) renal failure and 34 (27%) fever / infection. Median hemoglobin at diagnosis was 7.9 (2.6 to 14.0), median neutrophils / mm3 was 1.050 (100-8400) and median platelet 21,500 / mm3 (1000-494000). 55/124 (44%) had LDH>1.5 times or more the upper limit of normal. Fifty one (41%) patients had PNH clone>50% in granulocytes. During evolution 31/124 (25%) patients had thrombosis, 33/124 (27%) had hemoglobinuria and 15/124 (12%) had renal failure. Patients with classical PNH received supportive treatment with red blood cell transfusions, folic acid, prophylactic anticoagulant (14 patients with PNH clone>50%) and long-term anticoagulation (31 patients with thrombosis). Two patients have been treated with eculizumab. 23/80 (28.6%) patients with AA/PNH have undergone bone marrow transplantation and the others (n=57) were immunosuppressed with cyclosporine and antithymocitic globulin. 15/57 patients after immunosuppressive treatment, developed in a median period of 3.4 years (2-11 years), large clones (>50%) behaving like classical PNH. In this period 8 patients with classical PNH died. The causes of death were thrombosis in 4 patients and infection in the others.
Summary/Conclusions: This study has a limitation because it was retrospective. Despite of that our results are similar to those found in the literature except in the occurrence of abdominal pain (less than expected). Eculizumab is not available for all patients in our centre. Because of that we have prophylactically anticoagulated patients with PNH clone above 50% and no contraindications for the procedure.

LB2263

EPSTEIN-BARR VIRUS-ASSOCIATED T-LYMPHOPROLIFERATIVE DISORDERS IN ADULTS: A CASE SERIES OF 13 PATIENTS IN A SINGLE INSTITUTION AND REVIEW OF LITERATURE

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Background: Epstein-Barr virus (EBV) usually infected B lymphocytes, sometimes also infected T- or natural killer (NK)-cells. EBV-associated T/NK cell lymphoproliferative disease (EBV-T/NK-LPD) represents a continuous spectrum of diseases, ranging from benign hyperplasia of reactivity to clonal and malignant lymphoproliferations. Epstein-Barr virus-associated T/NK-cell lymphoproliferative disorder in children and young adults is a systemic disease, and features with EBV infection of T or NK cells cloned proliferation.
Aims: To understand the clinical features of EBV-associated T/NK cell lymphoproliferative disease and its poor prognosis, recognize the importance of early diagnosis and timely treatment.
Methods: Retrospective analysis of 13 patients with clinical features, EB virus spectrum, treatment and prognosis
Results: we report 13 adult cases of systemic Epstein-Barr virus-positive T-cell lymphoproliferative disorder including 9 males and 4 females with a median age of 41 years old (range,24-75). the most common clinical symoptoms were fever 92.3% (12/13), splenomegaly 100% (13/13), hepatomegaly 30.8% (4/13), lymph node enlargement 38.5% (5 /13), blood cells decrease 92.3% (12/13), abnormal liver function 76.9% (10/13), elevated lactate dehydrogenase 76.9% (10/13), coagulant function abnormality 61.5% (8/13). All patients had significantly increased serum EBV-DNA levels (3.41×10^4-6.61×10^6 copy/ml). EBER were positive by Fluorescence in situ hybridization (FISH) (5 cases lymph node and 8 cases bone marrow). T lymphocytes were involved in this group, 7 cases were diagnosed as chronic active EB virus infection (CAEBV), and 6 cases were diagnosed as EBV-associated hemophagocytic lymphohistiocytosis (HLH).One paient with CAEBV had no fever and blood cells decreases, keeping stable disease with prednisone treatment; and another one patient with CAEBV progress for aggressive NK cell leukemia after 4 months diagnosis, with EPOCH regimen combined L-asparaginase chemotherapy. The remaining 11 patients were treated with immunochemotherapy containing etoposide, but the effect is not good. In a median follow-up time of 18 months, one patient was free of disease, one was alive with disease, and eleven died of disease in 5 months, respectively.
Summary/Conclusions: Adult systemic EBV + T/NK-LPDs was relatively rare disease and the prognosis is poor. Our report adds to the understanding of these rare disease with early diagnosis and intervention.

LB2264

MICROVASCULAR NETWORK IN BONE MARROWS OF TREATMENT-NAÏVE PATIENTS WITH TYPE 1 GAUCHER DISEASE: SKEWED ANGIOGENIC SIGNALS IN AN INFLAMMATORY MILIEU

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Background: Gaucher disease (GD) is characterized by accumulation of glucocerebroside-storing macrophages, called Gaucher cells (GC) in various organs, including bone marrow (BM). Since macrophages play a major role in chronic inflammation, it can be assumed that macrophage proliferation in GD can elicit or modify local inflammation-associated phenomena, including angiogenesis.
Aims: The study was aim to evaluate microvascular network in BM samples from untreated patients with GD type 1, and compare them to samples from healthy adults.
Methods: Bone marrow core biopsies from 11 treatment-naïve patients with GD type 1 (3 women, 8 men, median age 68.5 years), followed at the Hematology Center, Karolinska University Hospital, were analyzed retrospectively. Biopsies from 36 subjects (18 women, 18 men, median age 63.6 yrs) with no hematological diseases, were used as controls. In the immunohistochemically

and immunofluorescent stained samples, the following parameters were analyzed: cellularity, vessel morphology, microvascular density (MVD), vessel wall pericyte coverage and expression of proangiogenic growth factors (VEGF, ANGPT1 and 2). In GD biopsies, analyzes were performed separately in areas with greater and lower percentage of infiltrating GC (≥50% and <50% in a high power field).

Results: In GD patients, hematopoietic tissue was hypercellular for age, and MVD was 2.6-fold higher than in controls (p<0.001). In GC rich areas, MVD was 1.4-fold higher (p=0.026) and vessel architecture was abnormal as compared to GC poor areas. Moreover, 30±17% of GD BM vessels were pericyte-coated, significantly fewer than in controls (48±16%; p<0.001). Expression of ANGPT1 and 2 was significantly higher in BM vessel walls in GD samples than in controls (7.2 and 13.2 fold higher, respectively), whereas VEGF expression was 20-fold lower in GD (p<0.05 for all parameters). MVD values correlated with BM cellularity, particularly in GC rich areas. Gaucher cells stain moderately for ANGPT1 and 2, and variably for VEGF.

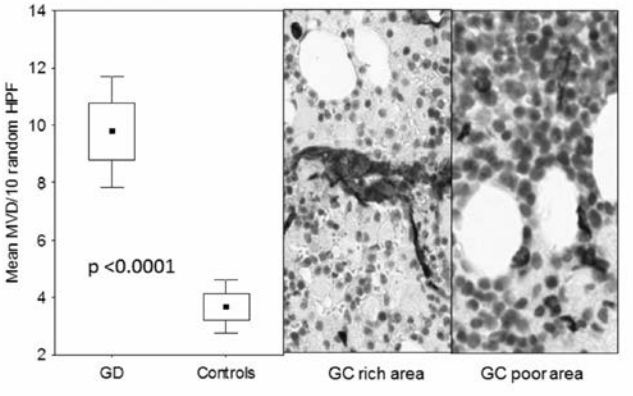

Figure 1.

Summary/Conclusions: Bone marrow in patients with GD type 1 demonstrates abnormal angiogenesis, with defective pericyte coating, and skewed VEGF/angiopoietin balance, which is presumably related to local accumulation of Gaucher cells.

Platelets disorders

E1412

POINT OF CARE PLATELET REACTIVITY TESTING AND DOSE-TITRATION IN A DOUBLE-BLIND STUDY OF PRASUGREL IN CHILDREN WITH SICKLE CELL ANAEMIA

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Background: Platelet activation and aggregation induced by release of ADP from haemolysed sickle erythrocytes may contribute to vaso-occlusion and ischaemic pain, a hallmark of sickle cell anaemia (SCA). Anti-platelet agents such as prasugrel (Pras) that target the P2Y12 ADP receptor may thus provide benefit. However, the potential increase in haemorrhagic stroke risk with high levels of platelet inhibition in SCA makes dose titration imperative. Previous studies suggested that a PRU (P2Y12 reaction unit, a measure of ADP-induced platelet aggregation) range of 231-136 would be appropriate to determine the risk / benefit ratio of prasugrel in SCA, and could be achieved by individual dose adjustment based on platelet testing - a challenge in a double blind study.
Aims: Attenuation of platelet reactivity to a pre-defined target range in patients with SCA, by dose titration using point-of-care platelet testing.
Methods: The DOVE study was a Phase 3 double-blind, placebo (Pbo) controlled study of Pras in pediatric patients with SCA. Treatment assignment was balanced according to hydroxyurea (HU) use, age group and country. Baseline PRU values were determined by Verify*Now*® P2Y12 (VN-P2Y12), a point-of-care platelet aggregation test which reports both PRU and% platelet inhibition (% inhibition) values. Over the subsequent 6 weeks patients returned for repeat VN-P2Y12 assessment and, if necessary doses were adjusted to titrate patients into a PRU range of 231-136, with a lower PRU value indicating greater platelet inhibition. The final dose was fixed for the remainder of the study, adjusted only if deemed necessary by changes in body weight. The majority of patients returned after 9 months for a final VN-P2Y12 measurement. All VN-P2Y12 data were encrypted and entered into an interactive voice response system which instructed the investigator to maintain or adjust the current dose for patients in both the Pras and Pbo (mock titration) arms, thus maintaining the blinding of the study.

Table 1.

	PRU LS Mean (SE)			% Inhibition LS Mean (SE)		
	Prasugrel	Placebo	p-value	Prasugrel	Placebo	p-value
Baseline	276.3 (3.8)	276.1 (3.8)	0.97	2.8 (0.4)	2.0 (0.4)	0.17
On final dose	203.1 (4.2)	275.5 (4.2)	<0.001	21.9 (1.0)	2.1 (1.0)	<0.001
9 Months	214.3 (5.3)	281.2 (5.4)	<0.001	19.4 (1.3)	1.3 (1.3)	<0.001

LS=least square; LS mean and p-values from ANOVA analysis with treatment, HU and age group as factors.

Results: 170 patients were assigned to the treatment arm and received Pras; of these, 160 were titrated to a final dose. Baseline (predose) PRU values were similar for both Pras and Pbo groups (Table). After titration to the final Pras dose, PRU values dropped to 203 (from 276 at baseline) and remained low through 9 months. In contrast and as expected, PRU values in the Pbo group did not change after mock titration or at 9 months. Baseline VNP2Y12-reported% inhibition levels were low in both Pras (2.8%) and Pbo (2%) groups. Mean% inhibition in the Pras group increased to approximately 22% after titration to the final dose and was maintained at this approximate level (19.4%) through 9 months of treatment. In the Pbo group, there was no change from baseline in% inhibition at either timepoint.
Summary/Conclusions: Although primary efficacy endpoints were not met in the DOVE study, the results of platelet function studies indicate that in the majority of patients, Pras at the final dose administered effectively reduced ADP-mediated platelet reactivity with a corresponding increase in platelet inhibition. This was achieved using encrypted point-of-care testing. Additionally, results demonstrated that safety was not compromised on the treatment arm, raising the question as to whether higher levels of platelet inhibition might provide a more optimal benefit/risk ratio.

E1413

THE STUDY OF GENETIC MUTATIONS THAT INFLUENCES THROMBOPOIESIS IN PATIENTS WITH NEOPLASMS: THE ROLES OF ANKRD26, RUNX-1 AND ETV6

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Background: The phenotype of inherited thrombocytopenia is characterized by different sizes of platelets, different degrees of thrombocytopenia, the presence or absence of platelet function defects, and an association with syndromes involving other cell lines or other organ functions. In particular, some forms of inherited thrombocytopenia secondary to 5' UTR of *ANKRD26, RUNX-1*, and *ETV6* mutations are associated with neoplasms of haematological or non-haematological type.
Aims: Study of genetic mutations in *ANKRD26*, *RUNX*-1 and *ETV6* that influences thrombopoiesis in patients with myelodysplasia (MDS).
Methods: We study the presence of mutations in the 5'UTR of the *ANKRD26, RUNX-1*, and *ETV6* gene in the following cohorts of patients: a) 27 patients affected by sporadic and familial thrombocytopenia and normal platelet volumes, b) 12 patients with myelodysplasia and normal platelet count, c) 9 patients with myelodysplasia with prevalent thrombocytopenia.
Results: In the cohort of the studied patients we found mutations in the 5'UTR of the *ANKRD26*, *RUNX-1* and *ETV6* genes only in 6 patients with thrombocytopenia and MDS of the group c. The mutations found are 5. One deletion in exon 1 of the *ANKRD26* gene (c.60_62del AGA), three mutations in the *RUNX-1* gene: a missense mutation with a modification in the amino acid polarity in exon 4 (c.76C>G), a deletion (c.934del A) and an insertion (c.1214_1215insTG), respectively, in exon 8 and 9. Finally, a deletion in intron 1 of the *ETV6* gene (c.28+192delC). All the mutations found were not present in public databases.
Summary/Conclusions: The preliminary data demonstrated that mutations in the *ANKRD26*, *RUNX-1* and *ETV6* genes were present in patients with MDS and prevalent thrombocytopenia. Even if the involvement of these genes in megacaryopoiesis is well known, the exact mechanism responsible for thrombocytopenia is not yet clear. As the pathology is extremely rare, it is difficult to find data for genotype-phenotype analyses. However we hypothesized that these mutations are responsible for the low platelet counts in patients with myelodysplasia. Greater knowledge about the pathogenesis of these forms of thrombocytopenia will facilitate finding new therapies for them. This will have important clinical implications since platelet transfusion is the only therapeutic option for these patients. Defining the molecular mechanism of thrombocytopenia in these patients could constitute the first step on the path leading to new therapeutic possibilities with TPO mimetics as has been demonstrated in hereditary form of thrombocytopenia.

E1414

DECREASED PLATELET FUNCTION IN ACUTE MYELOID LEUKAEMIA AND MYELODYSPLASIA DETECTED BY A NEW TEST OF PLATELET AGGREGATION OF VIABLE CELLS USING FLOW CYTOMETRY

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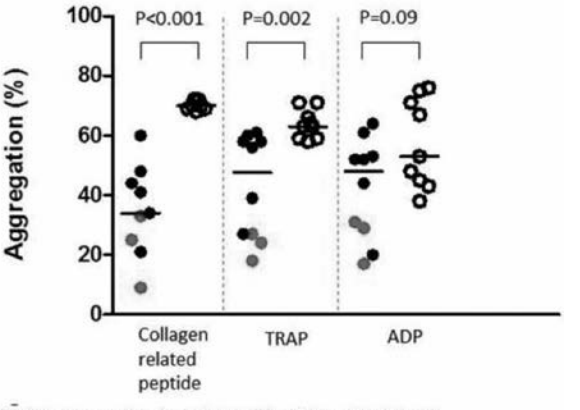
Background: It is unknown why only some thrombocytopenic patients bleed despite equally low platelet counts. Platelet function is likely to be of importance, but is difficult to access as results from existing platelet aggregation methods have major methodological limitations at low platelet count.
Aims: The aim was to establish a method for measuring platelet aggregation of viable platelets in thrombocytopenic patients. Secondly, we investigated platelet function in thrombocytopenic patients with acute myeloid leukaemia (AML) or myelodysplasia (MDS).
Methods: Washed platelets were split in two and differently labelled with fluorescent dye (Calcein AM and Calcein Violet) and added to AB-positive donor plasma in a 1.5 mL tube. Agonist was added (collagen-related peptide, adenosine diphosphate (ADP) or thrombin-receptor activator peptide-6 (TRAP)), samples were shaken for 5 min and subsequently analyzed using flow cytometry. Double-colored events in stimulated samples were defined as platelet aggregates. Effect of addition of platelet inhibitors (abciximab, ticagrelor or vorapaxar) was determined. Using flow cytometry, platelet activation after addition of agonist to whole blood was evaluated by fl ow cytometry using antibodies to detect alpha-granule release (P-selectin(CD62p)), dense-granule release (CD63) and activated fibrinogen receptor (PAC1 binding), respectively. Surface receptor levels were quantified using antibodies against GPIIb/IIIa (CD41/CD61), GPIb(CD42b)/GPIX(CD42a)), and CD49b. Participants were ≥18 years, healthy individuals or patients with AML or MDS. Participants who received platelet inhibitors within two weeks, platelet transfusions, or major surgery within one week were excluded. Informed consent was obtained. Comparisons were made with Wilcoxon ranksum test.
Results: Presence of aggregates in unstimulated samples was low (≤4%). The intra-assay coefficient of variation was 1-3%. Increasing inhibition of platelet aggregation at increasing dose of platelet inhibitors was observed. Enrolled were ten patients (n=8 AML/n=2 MDS) and nine healthy controls with platelet count ranging 6-47 x 10^9/L *versus* 145-337 x 10^9/L, respectively. The inter-individual variation among healthy persons were low (Figure 1). Collagen-related peptide and TRAP-induced aggregation were lower for patients compared with controls, while no difference in ADP-induced aggregation was found. Three patients reported bleeding episodes within the past one month and comprised three out of four persons with platelet aggregation below 34% for any agonist. No difference was observed in platelet count in patients with *versus* without bleeding episodes: 23 x10^9/L (12-24 x10^9/L) *versus* 25 x10^9/L (6-47 x10^9/L), p=0.51. Platelet activation was lower for patients *versus* controls for all agonists. There was a strong correlation between results from the platelet activation method and platelet aggregation method e.g. for PAC1-binding *versus* collagen-related peptide induced aggregation, r=0.96, p<0.001. No difference in expression of platelet surface receptor levels was found.



Platelet aggregation in patients with AML or MDS and platelet count <10 x10^9/L (full circles) compared with healthy individuals (open circles). Red circles represent patients with a history of bleeding within the past month. Horizontal lines indicate the median aggregation in each group.

Figure 1.

Summary/Conclusions: Based on the presented method for testing platelet aggregation of viable cells, our study shows that low platelet aggregation by the method identifies thrombocytopenic patients with bleeding problems among AML/MDS patients with thrombocytopenia. This platelet aggregation method may be useful for providing indications for prophylactic platelet transfusions or for evaluating indication of platelet inhibitor therapy in thrombocytopenic individuals. Further, it may facilitate the important distinction between inherited thrombocytopenia from immune thrombocytopenia.

E1415

SEASONAL VARIATIONS OF PRIMARY IMMUNE THROMBOCYTOPENIA INCIDENCE IN ADULTS

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Background: Seasonal variations of primary immune thrombocytopenia (ITP) incidence have been suggested in cohorts of childhood ITP. The existence of such variations is not well known in adult ITP.
Aims: The aims of this study were to assess the variations of primary ITP incidence in a clinical cohort of adult patients and to detect differences in seasonal patterns about the outcome of patients, differentiating ITPs lasting less than 3 months (defined by complete remission before 3 months: stable normal platelet count without any bleeding, without any exposure to treatment active on ITP) and ITPs lasting more than 3 months (*i.e.* persistent and chronic ITPs).
Methods: We conducted a retrospective study in the patient database of the French National Referral Center for Adult Autoimmune Cytopenias in Créteil, France. ITP was defined in accordance with international guidelines. We restricted the population to adult patients (aged ≥15 years) with primary ITP whose onset occurred between 1990 and 2014. ITP onset was defined by the date of first abnormal platelet count (<100 x 10^9/L) in patients with previous normal platelet count. Demographics data (age and gender), platelet count at ITP onset, bleeding score excluding the age at ITP onset, first line treatment and ITP activity at 3 months were also extracted from the database. We, then, calculated the proportion of patients with ITP symptoms onset by calendar month, with their 95% confidence intervals.
Results: Out of 663 primary ITP patients included in the study, 127 (19.1%) were excluded due to missing record of calendar month of first abnormal

platelet count. Median age was 40.9 years (range: 15.0-94.2), and 358 (66.8%) were females. Median platelet count at ITP onset was 14.0x10^9/L and 131 (26.2%) patients had bleeding signs. Median Khellaf's bleeding score excluding the age was 3 (range: 0-36). Out of these 536 patients, 321 (59.9%) became persistent or chronic (lasted more than 3 months) and 184 (34.3%) lasted less than 3 months (31 patients with missing value for this data). In regards to first-line treatment, 452 (84.3%) were treated by corticosteroids and 193 (36.0%) were exposed to with IVIg. Overall, there was a significantly higher proportion of patients with ITP onset in January/February than in August (nadir). Variations were marked in ITPs lasting less than 3 months. In this population, two smaller peaks of incidence were also observed: in June and in October. By contrast, there was no significant difference of ITP incidence by calendar month in ITPs that became persistent or chronic.

Summary/Conclusions: This study tends to confirm seasonal variations of ITP incidence with peak in winter and nadir in August in adults, as suggested by a previous population-based study in France. This pattern corresponds to influenza outbreak in France. The two smaller peaks (in June and in October) observed in ITP lasting less than 3 months are superimposable to enterovirus outbreaks in France. Both influenza and enterovirus have been associated with ITP occurrence in children. Moreover, vaccine against influenza tended to be protective against ITP onset in adults in a recent case-control study in France. In conclusion, this study suggests that ITP lasting less than 3 months in adults may be timely linked with major viral outbreaks in France, unlike persistent and chronic ITP. Further prospective epidemiological and microbiological studies are needed to confirm this observation.

E1416

SAFETY AND EFFICACY OF LONG-TERM OPEN-LABEL DOSING OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Pediatric patients with chronic immune thrombocytopenia (ITP) that completed the romiplostim phase 1/2 or phase 3 study could enroll in this open-label long-term extension study.
Aims: To examine the long-term effects of romiplostim in children with chronic ITP.
Methods: All patients received subcutaneous romiplostim once weekly. The initial dose was the final dose from the parent study or 1 µg/kg (for patients previously receiving placebo or that had not received romiplostim for >24 weeks), adjusted weekly from 1-10 µg/kg to target platelet counts of 50-200×10^9/L.
Results: A total of 66 patients (parent study: phase 1/2, n=12; phase 3, n=54) entered the extension study; 1 patient withdrew consent before treatment. At baseline, median (min-max) age was 11 (3–18) years; 56% were female; 61% were white, 14% African American, and 14% Hispanic/Latino; 9.1% had prior splenectomy. Median (min-max) treatment duration was 57.9 (1–269) weeks. Median (range) average weekly romiplostim dose was 5.5 (0.1–10.0) µg/kg. Thirteen patients discontinued treatment: consent withdrawn (n=6), noncompliance (n=2), administrative decision (n=2), nonresponse (n=2), and per protocol (n=1). For 15 patients (23%), the first study week was the first week they were receiving romiplostim (ie, they received placebo in the parent study). Fifty-six (86%) patients (or caregivers) self-administered romiplostim. Twenty-one (32%) patients received rescue medications on 63 occasions (for low platelet counts [n=35], bleeding/bruising [17], pre- or post-procedure [9], and other [2]); treatments included IVIg (n=10), prednisone (9), aminocaproic acid (3), tranexamic acid (2), methylprednisolone (2), and platelet transfusion (1). Patients required rescue treatment during the first 3 months (27/63 instances), >3–6 months (9), >6–9 months (6), >9–12 months (7), and after 1 year (14) in the extension study. Five patients who previously received placebo received rescue medication in this extension, mostly during the first 3 months (10/14 instances). Three patients achieved remission (platelet counts ≥50×10^9/L for 24 weeks with no ITP treatments): 1) A 9-year-old boy with ITP for 8 years; after 4 years of romiplostim, he entered remission for the last 2 years as of this datacut; 2) An 11-year-old boy with ITP for 6 years; after 3 years of romiplostim, he entered remission for the last year; and 3) A 17-year-old girl with ITP for 8 years; after 6 years of romiplostim, she entered remission for the last 44 weeks of the study. Thirty-nine serious AEs occurred in 14 patients, including pyrexia (n=3), epistaxis (n=2), and thrombocytopenia (n=2); 3 were deemed treatment-related (anemia, epistaxis, and thrombocytopenia), and none led to discontinuation of romiplostim. Five patients had life-threatening AEs, including thrombocytopenia (n=2) and infection, decreased platelet counts, and subcutaneous abscess (n=1 each); none were fatal or deemed treatment-related. Bleeding AEs occurred in 47 patients; 3 were deemed treatment-related by the investigator (gingival bleeding, petechiae, and epistaxis). No thrombotic events were reported. There were no peripheral blood abnormalities suggestive of malignancy to warrant a bone marrow examination in any patient. Anti-romiplostim neutralizing antibodies were found in one patient at end of study after 50 weeks. This patient received rescue medications for much of the study.

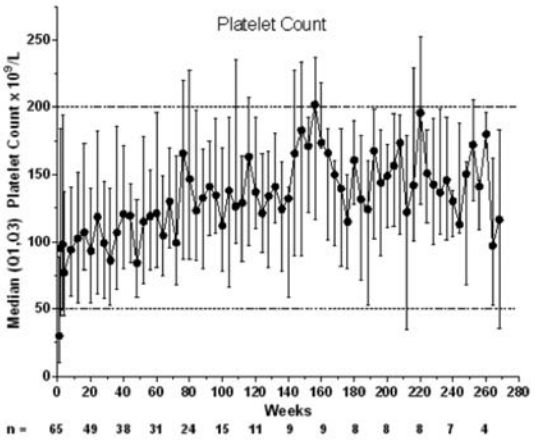

Figure 1.

Summary/Conclusions: In this ongoing open-label extension study of children with chronic ITP, romiplostim for ≤5.2 years maintained platelet counts with a safety profile similar to that seen in past studies.

E1417

SAFETY AND EFFICACY/EFFECTIVENESS OF SECOND-LINE TREATMENTS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA: A SYSTEMATIC REVIEW OF THE LITERATURE

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Background: Immune thrombocytopenia (ITP) is a rare disorder characterized by low platelet counts and an increased tendency to bleed. Typical first-line therapies include corticosteroids, intravenous immunoglobulin, and anti-D. Patients who fail initial treatment or relapse may require second-line treatment, yet definitive guidelines in this setting are lacking, presumably because of a paucity of relevant rigorous clinical research.
Aims: To systematically review studies evaluating the safety and efficacy/effectiveness of therapies used to treat ITP in the 2nd-line setting (splenectomy, azathioprine, cyclophosphamide, cyclosporine A, danazol, dapsone, eltrombopag, mycophenolate mofetil, rituximab, romiplostim, and vinca alkaloids), with a focus on randomized controlled trials (RCTs).
Methods: Using comprehensive search strings, Cochrane Central Register of Controlled Trials, the Cochrane Database of Systematic Reviews, PubMed, EMBASE, and clinicaltrials.gov were searched with no time or geographic constraints. Studies in which these agents were used as first-line therapy; with<20 patients; in populations of children, pregnant women, or patients with secondary thrombocytopenia; or not published in English were excluded. Studies were reviewed for several safety and efficacy endpoints. Twelve prospective, randomized, placebo- or standard-of-care (SOC)-controlled studies were further evaluated given their comparable study designs and levels of evidence provided. Outcomes common to multiple studies (bleeding, overall and complete platelet response, and use of rescue therapies) were summarized and indirectly compared using forest plots of calculated risk or response ratios for each outcome from each study.
Results: Final abstraction was performed on 165 articles meeting inclusion criteria. The majority of studies were observational in nature and none represented interventional studies providing head-to-head comparisons of the second-line therapies. Twelve prospective, randomized, placebo- or SOC-controlled studies evaluated the efficacy and safety of either one of two thrombopoietin-receptor (TPO-R) agonists (romiplostim [n=5] and eltrombopag [n=5]) or rituximab (n=2). Although aspects of study design, outcome definitions, and specific subject inclusion/exclusion criteria varied across the studies, patients receiving romiplostim, eltrombopag, or rituximab tended to demonstrate a reduced risk of bleeding and use of rescue therapy and an increased likelihood of platelet response compared with patients receiving either placebo or SOC almost without exception. These risk/response differences tended to be greatest in trials involving a TPO-R agonist (e.g., the range of response ratios for overall platelet response was 1.81-34.28 for romiplostim *versus* placebo/SOC; 1.40-14.00 for eltrombopag *versus* placebo; and 0.86-1.09 for rituximab *versus* placebo).

Summary/Conclusions: Data from rigorous RCTs are still currently limited for most second-line ITP treatment options. Twelve prospective, randomized placebo- or SOC-controlled studies were identified but covered only three (eltrombopag, rituximab, and romiplostim) of the several second-line medical treatment options available. These studies provide high-quality evidence regarding the efficacy and safety of these three therapies and, in the absence of head-to-head data, offer insights regarding how they compare to one another in terms of clinical outcomes.

E1418

THYROID DYSFUNCTION AND ITS RESPONSES TO TREATMENT ARE ASSOCIATED WITH THROMBOCYTOPENIA IN CHRONIC HEPATITIS B-INFECTED SUBJECTS WITH COMPENSATORY CIRRHOSIS

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Background: Many reports have demonstrated a significant relationship between thrombocytopenia and thyroid dysfunction and its treatment. However, the pathogenetic link between these conditions has not been clarified. Autoimmune theory has been well-supported hypothesis. Our previous studies have demonstrated that chronic hepatitis B (CHB) withcompensatory cirrhosis accompanied by thrombocytopenia is an important research subject. Additionally, we have observed many casesof hypothyroidism in CHBpatients with thrombocytopenia in our clinicalstudies. However, no information is available regarding the association between thyroiddysfunction and thrombocytopenia in CHBpatients with compensatory cirrhosis.
Aims: We investigated whether thrombocytopenia in patients diagnosed with CHB withcompensatory cirrhosis is related to thyroid dysfunction, and further explored the treatment and the platelet response to treatment.
Methods: Thrombocytopenia was defined as a platelet count of $<100\times10^9$/L. We divided all patients into two groups based onplatelet counts of $<50\times10^9$/L and $>50\times10^9$/L. The prevalence of thyroid dysfunction, including normal thyroid function, overt or subclinical hyperthyroidism, and overt or subclinical hypothyroidismwas determined in each group. Thyroid function and liver function were detected periodically. During treatment, platelet counts were assessed weekly during the first month and then every 4 weeks for one year. Treatment of thyroid dysfunction and treatment responses were also recorded.
Results: A total of 261 patients were enrolled in this observational study. Among the 30(11.49%) patients with abnormal serum TSH levels, there were 6 (2.29%) with hyperthyroidism, and 24 (9.20%)with hypothyroidism, and 60% were female. Patients with a platelet count $<50\times10^9$/L had a higher incidence of hypothyroidism, which developed in 11% of patients in group A (platelet count $<50\times10^9$/L) compared with 8.07% in group B (platelet count $>50\times10^9$/L) ($P<0.05$). Serum TSH was significantly higher but serum FT3,FT4,TT3, and TT4 were significantly lower in group A ($P<0.05$). We identified risk factors associated with platelet count before therapy. Logistic regression analysis showed that serum TSH, FT3, FT4,TT3 and TT4 levels were positively associated with the platelet count. We calculated the difference in platelet count from baseline for each time pointduring treatment. A greater change was exploredin group A than group B ($P<0.05$). In each group, the change in platelet count was higher in patients who received treatment for their thyroid condition ($P<0.05$).We further assessed factors influencing patients who had a platelet response compared with those who had no reponse. Highly significant variables associated with a platelet response, even in the multivariate analysis, included serum TSH, FT3, FT4, TT3 and TT4 levels.
Summary/Conclusions: This is the first studyto demonstratethat thyroid dysfunction, especially hypothyroidism, is common in patients with CHB accompanied by thrombocytopenia.Females are more likely to develop thyroid dysfunction. Thyroid function is associated with platelet counts. Additionally, a more dramaticplateletcountresponse was observed with administration of thyroid hormone therapy in patients with hypothyroidism, even in the multivariate analysis.It may provide a novel approach for thetreatment of thrombocytopenia in CHB patients with compensatory cirrhosis.

E1419

RITUXIMAB THERAPY AS PROPHYLAXIS AGAINST THROMBOTIC THROMBOCYTOPENIC PURPURA: COMPARISON OF STANDARD AND REDUCED DOSE REGIMENS

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Background: Acute antibody-mediated thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy with high morbidity and mortality. Rituximab is highly effective when given as prophylaxis in patients at high risk of acute TTP relapse, but the ideal dosing regimen is unknown.
Aims: A retrospective cohort study across two centres was performed to compare outcomes between TTP patients given rituximab prophylaxis at standard dose ($375mg/m^2$) *vs* reduced dose (200mg), weekly for 4 weeks.
Methods: Patients had all previously had at least one acute TTP episode, and were deemed at high risk of TTP relapse based on ADAMTS13 activity dropping to ≤15%, having previously been documented in the normal range. Outcome measures included normalisation of ADAMTS13, subsequent relapse rate. Comparison was made of time to re-treatment for patients given standard dose *vs* reduced dose rituximab.
Results: Rituximab was given in 51 patient episodes, to 32 patients (24F, 8M); in 19 episodes (14 patients) this was re-treatment where patients had received rituximab prophylaxis on at least one prior occasion. In 47/51 (92%) episodes, patients had a median pre-prophylaxis reduction in ADAMTS13 level of ≤15%. Patients received standard dose rituximab in 24 episodes and reduced dose in 27 episodes. Normalisation of ADAMTS13 occurred in 48/51 (94%) patients. Over a median follow up of 14.5 months (range 9-133 months), there were only 2 subacute relapses, both occurring in the reduced dose group. Of the 29 patients requiring re-treatment, 12/29 (41.4%) had received standard dose, and 17/29 (58.6%) reduced dose, at a median 22.5 months (range 10-112 months) in the standard dose group, and 12 months (range 9-33 months) in the reduced dose group, p=0.1351.
Summary/Conclusions: Rituximab therapy is effective as prophylaxis normalising ADAMTS13 and preventing acute TTP relapses in patients with immune TTP. Standard dose rituximab prophylaxis may be associated with longer treatment free survival than reduced dose.

E1420

MODELING AND SIMULATION (M&S) SUPPORT ELTROMBOPAG (EPAG) DOSAGE ESCALATION BASED ON PLATELET COUNT FOR PEDIATRIC PATIENTS WITH PERSISTENT OR CHRONIC IMMUNE THROMBOCYTOPENIA (CITP)

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Background: EPAG is approved for use in patients with persistent or cITP, chronic hepatitis C, and severe aplastic anemia. M&S supported EPAG dosing in adult populations (Gibiansky E et al. *J Clin Pharmacol* 2011; Hayes S et al. *J Clin Pharmacol* 2011; Wu K et al. *J Clin Ther* 2015; Zhang J et al. *Pharm Res* 2015).
Aims: To support initial EPAG doses and dose titration based on platelet count response in pediatric patients with cITP.
Methods: Plasma EPAG concentration and platelet count (PLTC) data collected in pediatric cITP patients in the PETIT & PETIT2 studies (Bussel JB et al. *Lancet Haematol* 2015; Grainger JD et al. *Lancet* 2015) were analyzed by nonlinear mixed-effects modeling. Patients received EPAG for ≥24 weeks. Doses were increased at 2-week intervals for PLTC<50 Gi/L, decreased for >200 Gi/L, and interrupted for >400 Gi/L. Serial pharmacokinetic (PK) samples were collected at Week 6 in PETIT. A single PK sample and PLTC were collected at all visits in both studies. A population PK (PPK) model was developed with PETIT data. Following successful external validation with data from PETIT2, data from both studies were combined to obtain final model parameter estimates. PLTC data were fitted alone using individual post-hoc PK parameter estimates. Simulations of PLTC for various starting doses and dose titration schedules were completed.
Results: The analyses included 168 patients (mean age 9.5 [1–17] years; weight 42.1 [11–135] kg; baseline PLTC 14 [1–38] Gi/L; 51% female; 20% East Asian; 6% splenectomized); 98% received prior ITP medication. Concurrent ITP medications were used at 17% of the PLTC assessments. PK was described by a 2-compartment model. Inter-individual variation (IIV) was included on apparent clearance (CL/F), intercompartmental exchange/clearance rate (Q/F), distribution volume of central compartment (Vc/F), and inter-occasion variability (IOV) on CL/F. Typical values were CL/F=0.612 L/h, Vc/F=2.74 L, Q/F=0.716 L, distribution volume of peripheral compartment (Vp/F)=21.5 L, and Ka=0.189/h. The final PPK/pharmacodynamics (PD) model was a 7-compartment life-span model, including 3 PK and 4 PD compartments. Zero-order production rate of platelet precursors (KIN) was fixed to the value from adult healthy volunteers (KIN=1.43 Gi/L/h). The increase in KIN due to EPAG was linearly related to plasma EPAG concentrations by the parameter SLOP (ie, linear coefficient of drug effect on KIN). SLOP, first-order maturation rate of platelet precursors (KOUT), and proportion of patients identified as responders (P1) were estimated; IIV was included on SLOP and KOUT. Typical PD parameter values were P1=0.96, SLOP=0.651 mL/μg in responders, and KOUT=0.0126/h, and increased with age. The average platelet half-life was 7 h. A majority of patients, as also seen in PETIT/PETIT2, required dose escalation to achieve target platelet response.
Summary/Conclusions: M&S supports eltrombopag starting doses and dose adjustments based on platelet counts in children with cITP. A majority of children

require dose escalation from eltrombopag starting doses to achieve platelet thresholds ≥50 Gi/L. Higher starting doses may need to be considered when clinically indicated such as in cases of severe bleeding or pending surgery. Studies NCT00908037/NCT01520909 were sponsored by GlaxoSmithKline; EPAG became an asset of Novartis AG as of March 2, 2015.

E1421

EXPRESSION OF GLUCOCORTICOID RECEPTOR ISOFORMS (A, B, Γ, AND P) IN ADULT EGYPTIAN PRIMARY IMMUNE THROMBOCYTOPENIA PATIENTS

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Background: Glucocorticoid (GC) resistance has been demonstrated in nearly 30% of primary immune thrombocytopenia (ITP) patients even managed with high dosages GC. The biological effects of GC are mainly mediated through activation of glucocorticoid receptors (GR). An insight into the molecular mechanisms underlying GC resistance is important to avoid GC treatment in patients contraindicated from steroid use.
Aims: We aimed at determining glucocorticoid receptor (GR) isoforms expression in adult ITP and its relation to glucocorticoid resistance.
Methods: Thirty three ITP patients from the Hematology unit, Alexandria Main University Hospital were the subject of the study. They were subdivided into two groups (sensitive and resistant) according to their response to 4 weeks GC treatment. 15 healthy volunteers of matched age were also included. Glucocorticoid Receptor α, β, γ and p gene expression were measured in cases and controls by real time Polymerase Chain Reaction. (QIAamp® RNA blood mini kit (cat no. 52304). Informed written consent was obtained from allpatients and the study was approved by theMedical Ethical Committee.
Results: The mean age value of glucocorticoids sensitive (GCS), glucocorticoid resistant (GCR) and control group was 33.4±11.6, 38.1±12.3 and 31.7±5.8 years respectively (F=1.496, p=0.235). Half of our female patients (n=14) were GC resistant while all the males (n=5) were GC sensitive. Statistically significant difference between GR alpha mRNA isoform and GRa/ GRβ ratio was detected between GC resistant and GC responsive group while GRp, GRγ and GRβ were insignificantly differed between groups. GRα is expressed in most human tissues and cell line. It functions as ligand-dependent transcription factors. There is a wide variability in median value of GRα between our GCS and GCR patients. The ratio of GRα/GRβ expression is critical to the glucocorticoid responsiveness of various cells. Higher ratios correlate with glucocorticoid sensitivity, while lower ratios correlate with glucocorticoid resistance. There was non-statistically significant correlation between GR α and age (r=-0.302, p= 0.087). GRα had a strong inverse correlation with GRβ and a significant direct correlation with GRγ and p. Fig. 1 shows ROC curve of different GR isoforms in predicting GC resistance in ITP. GRα/β ratio had the highest sensitivity (81.8%) and is the most accurate predictor of GC resistance (79.2%). GRγ had the highest specificity (86.7%). GRβ had the lowest sensitivity (9.1%) and the lowest specificity (26.7%).

GR isoform	Cases (n=33)		Test of significance
	Sensitive (n=19)	Resistant (n=14)	(p value)
GR α Median (Q1-Q3)	191.3 (5.3-689.8)	3.9 (1.7-101.1)	Z=2.058 P=0.038*
GR β Median (Q1-Q3)	0.003 (0.001-0.606)	0.006 (0.002-0.1)	Z=1.785 P=0.077
GR γ Median (Q1-Q3)	1.4 (0.1-13.5)	0.03 (0.003-2.2)	Z=1.931 P=0.055
GR p Median (Q1-Q3)	0.5 (0.1-34.3)	0.02 (0.01-2.3)	Z=1.748 P=0.084
GR α/GR β ratio Median (Q1-Q3)	35598.3 (866.9-134775.3	224.5 (0.9-53060)	Z=2.003 P=0.046*

Z: Mann Whitney test, *significant at P≤0.05

Table 2: Correlation between GR α expression and other GR isoforms among the studied ITP

Parameter	GR α		GR β		GR γ		GR p	
	r	P	r	P	r	P	r	P
GR α								
GR β	-0.479	0.035*						
GR γ	0.771	0.0001*	-0.585	0.0001*				
GR P	0.552	0.01*	-0.331	0.06	0.393	0.024*		
GRα/GRβ ratio	0.91	0.0001*	-0.739	0.0001*	0.847	0.0001*	0.496	0.003*

r: Spearman Rho correlation coefficient, *significant at P≤0.05

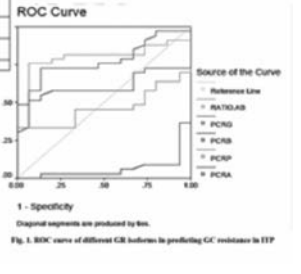

Fig. 1. ROC curve of different GR isoforms in predicting GC resistance in ITP

Figure 1.

Summary/Conclusions: In this study, we noted a statistical significant difference between GR alpha mRNA isoform and GRa/ GRβ ratio within ITP patients. GRa and GRa/ GRβ were higher among GC sensitive compared to GC resistant groups. GRα/β ratio had the highest sensitivity (81.8%) and is the most accurate predictor of GC resistance (79.2%). We concluded that the study of GRa and GRa/ GRβ ratio is recommended early in ITP assessment to avoid unnecessary glucocorticoids side effects during treatment.

E1422

ABNORMALITY OF CD8+ CD28- SUPPRESSOR T CELLS IN PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: Primary immune thrombocytopenia (ITP) is an acquired disorder characterized by autoantibody-mediated platelet destruction and insufficient platelet production. Multiple factors have been implicated in ITP pathogenesis, including loss of immune tolerance. CD8+CD28- suppressor cells (Ts), which have been identified in recent years, play important roles in maintenance of peripheral immune tolerance. Thus Ts cells are involved in immune escape of tumor and pathogenesis of several autoimmune diseases. Ma et. al. found steroid treatment selectively increased Ts proportion in both the passive and active mice models of ITP, suggesting Ts may be involved in ITP. However, until now, the role of Ts cells in ITP etiology was not clear yet.
Aims: To evaluate whether the number and the regulatory functions of Ts were abnormal in ITP patients, and found the reasons for these defects.
Methods: A total of 90 active ITP patients, 21 ITP patients in remission and 93 age- and sex-matched healthy donors (HCs) were enrolled in this study. And (1) the proportion of CD8+CD28- Ts cell was detected by flow cytometric analysis (FACS). (2) The CD8+CD28-Ts, CD8+CD28+/CD4+ effector cells and CD4-CD8- cells (as antigen presenting cells, APCs) were sorted by MACS, and then mixed lymphocyte reaction and BrdU method were used for evaluating the inhibitory rate of Ts cells. Here, Transwell (TW) experiments were also performed to identify soluble cytokines or direct cell-contact was involved in immune regulation of Ts cells. (3) The sorted Ts and APC were co-cultured with PHA and IL-2, then after 48 hours, CD80, CD86, ILT3 and ILT4 expressions on CD14+ monocyte were analyzed by FACS. And the proliferation of APC were detected by BrdU corporation after 72 hours. (4) Peripheral blood mononuclear cells were activated by PHA and IL-2 for 24 hous, following ICOS, PD-1 expression on Ts cells surface and IL-10 expression in Ts cells (were reactivated by PMA, BFA and Ionomycin for final 4 hours) were examined by FACS.
Results: (1) The percent of Ts cells in peripheral blood from active ITP patients were lower than those of HCs, although Ts proportion seems elevated in patients with remission, there was no difference between ITP patients in remission and active ITP patients or HCs. (2) Ts cells both from HCs and ITP patients inhibited CD8+CD28+/CD4+ effector cells proliferation in quantity dependent manner. The inhibition effect of Ts on CD4+ effector cells at ratio 1:2 and inhibition effect of Ts on CD8+CD28+ effector cells at ratio 1:4 were significantly lower in ITP patients than that in the HCs. (3) Ts cells from HCs and ITP patients suppressed effector cells proliferation both in the presence and absence of a TW; In addition, the suppressive degree of Ts cell from HCs is greater in the absence of TW, while no difference was found between presence and absence of TW groups in ITP patients. Thus, the results suggested that both the soluble mediators and direct cell-contact were involved in suppressive function of Ts cells, and the cell-contact regulated ability of Ts cells form ITP patients may be have defect. (4) The ICOS expressions on Ts cell surface with or without activation were both decreased in ITP patients than that in HCs. However, the PD-1 and IL-10 expressions of Ts cells were not different between ITP patients and HCs. (5) Ts cell inhibited proliferation of APCs both in HCs and ITPs. However, inhibition rate will decreased in ITPs than that of HCs. Moreover, Ts from HCs could down-regulated CD80, up-regulated of ILT3 and ILT4 expressions on CD14+ monocytes, but these abilities were disappeared in Ts cells from ITP patients.
Summary/Conclusions: The down-regulation of number and inhibitory function of Ts cells in active patients indicated that Ts defect was involved in pathogenesis of ITP. In addition, decreased ICOS expression on Ts cell surface and loss the ability of regulating co-stimulator expression on APCs were partly illustrated the reasons for defect s of Ts.

E1423

FIRST OBSERVATION OF TUBB1 GENE MUTATIONS IN TURKISH PATIENTS WITH MACROTHROMBOCYTOPENIA

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Background: Frequency of the rare bleeding disorders (RBDs) in the general population ranges from 1:500.000 to 1:2 millions. In countries with a high rate of consanguineous marriages RBDs occur more frequently, representing a significant clinical and social problem. Macrothrombocytopenia (giant platelet syndrome) is an autosomal dominant disorder and previous studies have demonstrated that Myosin Heavy Chain 9 (MYH9) mutations lead to this disorder. The pathogenesis of almost the half of diseases related to MYH9 gene is unknown. So its diagnosis is not possible. The membrane skeleton and the link between actin filaments of skeleton and microtubules contain the normal platelet morphology. The defects occurred on these systems results with macrothrombocytopenia. The relation between macrothrombocytopenia and the defect occurred on microtubules that consisted by alpha and beta tubulin heterodimers can be explained by the defect occurred on Tubulin Beta 1 (TUBB1) gene. According to HGMD (Human Gene Mutation Database) data; 52 mutations are defined in MYH9 gene between 2004-2014. 17 of these mutations are defined in patients with giant thrombocyte syndrome(macrothrombocytopenia). Only 2

mutations are defined related to TUBB1 gene, so the researches done on relation between TUBB1 and macrothrombocytopenia is limited. The guiding indicators on the treatment of this disease can be achieved in case of defining the effects of TUBB1 mutations on megakaryocytes-microtubule organization, proplatelet formation and platelet morphology

Aims: In this study, we aimed to analyze TUBB1 gene at Turkish population with clinically diagnosed macrothrombocytopenia.

Methods: A written informed consent for genetic analysis was obtained from the patients. The patient blood was collected from various hematology clinics in Turkey. In this study, TUBB1 gene mutation analysis was performed by using the blood of patients diagnosed with macrothrombocytopenia following the DNA isolation. Then samples were sequenced using a DNA sequencer (Beckman Coulter, USA). Sequencing are evaluated using with FinchTV program.

Results: TUBB1 gene analysis of Turkish patients with macrothrombocytopenia revealed a novel heterozygous Cytosine to Timin nucleotide change at 821 at exon4 (p.T274M/ Threonin to Methionine), at a child and his father in the same family, 99 nucleotide ahead which is at nucleotid 920, there is a novel heterozygous Guanine to Adenin nucleotide change, this situation caused to change of Arginine aminoacid to Histidine aminoacid at 307 position (p.R307H) in protein. These changes are conserved at evolutionary process, so we can say that these transitions are novel mutations. We also identified a double base pair substitution at nucleotide positions 130-131 at exon 2,this transition encodes the Q43P (p.Gly43Pro, p.Gly43His) mutant form of the protein. (nucleotide and aminoacid numbering as per m RNA GenBank entry NM_758664).

Summary/Conclusions: In this study, we identified the first TUBB1 mutation; combined 821C>T and 920 G>A mutations in a family and these mutations are not defined previously in Human Gene Mutation Database (HGMD). And Q43P single nucleotide polymorphism is defined firstly at Turkish population, so they are very important findings in terms of explaining the relationship of macrothrombocytopenia and TUBB1 gene.

Funding: This research is financially supported by Starting R&D Projects Funding Program (3001) of The Scientific and Technological Research Council of Turkey (TUBITAK)

E1424

PLATELET INCREASING STRATEGIES IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA REQUIRING LONG-TERM ANTITHROMBOTIC PROPHYLAXIS

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Background: As in the general population, patients with immune thrombocytopenic purpura (ITP) can develop pathologies requiring long-term treatment with anticoagulant or antiplatelet agents. Challenging situations in this setting are antithrombotic prophylaxis in patients with atrial fibrillation (AF), artery stenosis, previous cardiac or vascular surgery, percutaneous transluminal coronary angioplasty (PTCA) with or without stenting, arterial or venous thrombosis. Concomitant use of platelet increasing treatment is mandatory for acceptable safety.

Aims: To summarize a monocenter experience in the management of ITP patients requiring long-term antithrombotic treatment and concomitant platelet increasing treatment.

Methods: We analysed the medical records of 108 ITP patients referred to our Center since 2000 to 2015; we identified 18 patients with risk situations making necessary platelet increasing treatments (M/F 11/7, median age at the treatment 52 yrs, range 47-90). Antithrombotic treatments were categorized as vitamin K-antagonists (VKA), direct oral anticoagulants (DOACs), low molecular weight heparin (LMWH), antiplatelet agents (aspirin, clopidogrel, indobufene). Platelet increasing treatments were categorized as prednisone (PDN), high-dose dexamethasone (DEX), high-dose immunoglobulins (HD-IG), thrombopoietin receptor agonists (TPO-RA: eltrombopag ETP, romiplostim RPL). A platelet count >100 x10^9/L or >30 x10^9/L in at least 4 consecutive weeks defined complete response (CR) or partial response (PR). Antithrombotic treatments were started at a platelet count higher than 50 x10^9/L.

Results: All patients had previously received at least one line of treatment (one line=4, two lines=7, three lines=4, >three lines=3); five patients had had splenectomy. Four patients had AF and received AVK (n=2), DOACs (n=1), LMWH (n=1); two had carotid stenosis and received aspirin; one underwent aortic valve replacement and received after surgery AVK and then aspirin; one underwent angioplasty of popliteal aspirin plus clopidogrel; one patient had coronary bypass and received indobufene; three patients had coronary stenting and received AVK (n=1) and aspirin plus clopidogrel (n=2); four patients had deep venous thrombosis of the legs and received LMWH for 6 to12 months; two patients had caval vein and portal vein thrombosis, respectively, and received VKA; one patient had retinal vein thrombosis and received LMWH. The median platelet count before the platelet increasing treatment was 46 x10^9/L (range 13-67). Twelve patients received TPO-RA (ETP=10; RPL=2), 3 patients received PDN, 2 patients received DEX, and one received HD-IG and PDN. Nine patients receiving TPO-RA achieved CR, and three PR (62, 91, and 99 x10^9/L); on the other hand four patients receiving steroids achieved CR, and one PR (88 x10^9/L). The remaining patient with caval vein thrombosis had no response after HD-IG and PDN and a caval filter was placed. All the other patients received antithrombotic treatment at therapeutic doses. No thrombotic or bleeding event was recorded after starting antithrombotic treatment together with a platelet increasing treatment.

Summary/Conclusions: Antithrombotic treatments can be given safely in patients with ITP after having obtained an increased platelet count. TPO-RA and steroids are both effective as platelet increasing strategies, but possible long-term side effects due to prolonged steroid treatment should induce caution.

E1425

THE ROLE OF ROMIPLOSTIM IN CHEMOTHERAPY-INDUCED THROMBOCYTOPENIA TREATMENT

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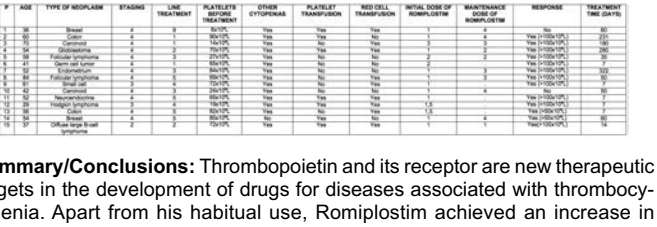
Background: The thrombocytopenia due to chemotherapy and/or marrow infiltration is a common complication in cancer patients. When it is moderate or severe, it can cause difficulties in the clinical management and the continuity of full-dose treatment. Romiplostim stimulates the thrombopoietin receptor inducing proliferation and differentiation of megakaryiocites, so administered at the right time, it could lead to a faster recovery or maintenance of platelet counts, avoiding delays and dose adjustments which may cause poor response to chemotherapy.

Aims: To evaluate safety and efficacy from Romiplostim in post-chemotherapy thrombocytopenia.

Methods: 15 patients with malignancies, 11 non-hematologic (2 breast cancer, 2 colon cancer, 2 carcinoid tumors, 1 glioblastoma multiforme, 1 neuroendocrine tumor, 1 endometrial cancer, 1 germ cell tumor and 1 small cell lung cancer) and 4 lymphomas (2 follicular lymphomas, 1 diffuse large B-cell lymphoma and 1 Hodgkin lymphoma) were treated with Romiplostim. The median age was 54 years (29-84) and the median baseline platelet counts was 69 x 10^9 L (8-90). All were receiving chemotherapy, 3 in first-line and the rest in second-line or later and 53% had marrow invasion. 14 patients develop cytopenia, requiring red cells transfusion (60%), platelet transfusion (47%) and the use of granulocyte-colony stimulating factor (G-CSF) (66%). The median starting dose of Romiplostim was 1 mcg/kg (1-3) and the median maintenance dose was 3 mcg/kg (1-4), for a median time of 50 days (7-322).

Results: 2 patients achieved no response, one of them requiring continued platelet transfusion support to carry on with chemotherapy. 87% of patients achieved response, in 11 cases with platelet counts of >100 x 10^9L. This allowed the use of full-dose chemotherapy and according to the initial scheme, except for dose adjustment in 2 cases due to platelet counts of<50 x 10^9L. Romiplostim treatment was well tolerated, with no treatment-related toxicities observed. No haemorrhagic events were reported over this period.

Table 1.



Summary/Conclusions: Thrombopoietin and its receptor are new therapeutic targets in the development of drugs for diseases associated with thrombocytopenia. Apart from his habitual use, Romiplostim achieved an increase in platelet counts in cancer patients, even after several treatment lines, making possible the use of full-dose chemotherapy and without delays, which could result in a therapeutic benefit. Despite the good results achieved, it would be necessary a larger number of patients to prove the thrombopoietin receptors agonists as equivalents to G-CSF and erythropoietin in post-chemotherapy cytopenias.

E1426

PRIMARY IMMUNE THROMBOCYTOPENIA TREATED WITH ROMIPLOSTIM IN ROUTINE CLINICAL PRACTICE: A RETROSPECTIVE STUDY FROM THE UNITED KINGDOM IMMUNE THROMBOCYTOPENIA REGISTRY

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Background: Immune Thrombocytopenia (ITP) is a rare disorder characterized by low platelet counts, leading to an increased tendency to bleed. Adult chronic ITP patients (pts) in Europe who are refractory to other treatments (e.g. corticosteroids, immunoglobulins) are eligible for treatment with romiplostim, a thrombopoietin-mimetic peptibody. Since ITP treatment-related decisions are principally dependent on clinical expertise or pt preference, observational studies can provide a better understanding of ITP treatment in routine practice.

Aims: To describe the demographic and clinical characteristics of patients with ITP receiving romiplostim in the UK, and to provide details on platelet counts, and the use and pattern of administration of romiplostim in routine clinical practice.
Methods: The United Kingdom Immune Thrombocytopenia (UKITP) Registry retrospectively and prospectively collects demographic and ITP-related clinical data on primary ITP pts enrolled by consent through a network of 68 centres throughout the UK. All adults (≥18yrs) within the UKITP registry who received at least one dose of romiplostim in routine clinical practice from October 2009 until 31st January 2015 were included in the analysis. Data described included demographic and clinical characteristics and ITP medications in patients since ITP diagnosis until end of follow-up and platelet counts before and after romiplostim initiation.
Results: At the time of data extraction from the registry (31st January 2015), a total of 1440 ITP pts were registered and 118 patients were treated with romiplostim. The median age at ITP diagnosis of the romiplostim cohort was 59 years (IQR: 36, 73). Members of the cohort had been diagnosed since 1980, with 50% diagnosed between 2010-14. The median time from ITP diagnosis to romiplostim initiation for those diagnosed between 2010-14 was 0.9 years (IQR: 0.3, 1.9). Almost three-quarters (73%) of patients initiated romiplostim ≥1 year from ITP diagnosis, and 12% of patients initiated romiplostim within 3 months of ITP diagnosis. There were some differences in baseline demographics between splenectomised and non-splenectomised patients (Table). Most patients (77%) had received at least three different ITP medications before romiplostim. The most common prior ITP treatments were corticosteroids (90%), intravenous immunoglobulin (IVIg) (77%), and rituximab (57%). The median maximum weekly dose of romiplostim was 3.1 mcg/kg (IQR: 2.0, 6.0). 82% of patients had 2 or fewer ITP medications after romiplostim initiation. The median platelet count within 2 weeks before romiplostim initiation was 17 x 10^9/L (IQR: 8, 41), which rose to 81 x 10^9/L (IQR: 31,155) within 1 month of romiplostim treatment and remained >50 x 10^9/L thereafter.

Table 1. Demographic and clinical characteristics of the romiplostim cohort overall, and by splenectomy status at romiplostim initiation.

	Total (N=118)		Splenectomised (N=26)		Non-Splenectomised (N=92)	
	N	%	N	%	N	%
Female	58	49.2%	15	57.7%	43	46.7%
Caucasian	96	81.4%	18	69.2%	78	84.8%
Age group at diagnosis						
18 to < 30	20	17.0%	4	15.4%	16	17.4%
30 to < 45	20	17.0%	5	19.2%	15	16.3%
45 to < 65	32	27.0%	11	42.3%	21	22.8%
≥ 65	46	39.0%	6	23.1%	40	43.5%
Time period of ITP diagnosis						
< 2010	59	50.0%	21	80.8%	38	41.3%
2010-14	59	50.0%	5	19.2%	54	58.7%
Year of romiplostim initiation						
<2011	6	5.1%	4	15.4%	2	2.2%
≥2011	112	94.9%	22	84.6%	90	97.8%
Disease phase (from ITP diagnosis to romiplostim initiation)						
Newly diagnosed ITP (< 3 months)	14	11.9%	1	3.8%	13	14.1%
Persistent ITP (3 months to < 1 year)	18	15.3%	1	3.8%	17	18.5%
Chronic ITP (≥ 1 year)	86	72.9%	24	92.3%	62	67.4%
< 3 prior therapies	27	22.9%	0	0%	27	29.3%
≥3 prior ITP therapies	91	77.1%	26	100.0%	65	70.7%

Summary/Conclusions: Limitations include potential selection bias into the registry and the small sample size and heterogeneous nature of the selected cohort. This retrospective analysis of secondary and tertiary care data from the UKITP registry provides a valuable insight in the real-world ITP pt population prescribed with romiplostim in the UK.

E1427

VARIATIONS IN CYTOKINE GENE POLYMORPHISM AMONG EGYPTIAN CHILDREN WITH PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: Childhood immune thrombocytopenia (ITP) is a common pediatric hematologic disorder with an association between the cytokine gene polymorphisms affecting the cytokine production and autoimmune diseases at the stage of formation of disease and in the course of disease and their responses to treatment.
Aims: To evaluate the possible role of cytokine genes as well as of their polymorphisms in Childhood ITP, we analyzed the allelic and genotypic frequencies of different cytokine gene polymorphisms known to be related to autoimmunity and inflammation (IL-6−174, IL-10−1082, IL-17F, TNF- α−308, 1RaVNTR) in Egyptian patients with ITP and healthy control subjects. In addition we assessed the potential role of these polymorphisms in relation to phases of ITP, disease progression and response to different treatment modalities.
Methods: A total of 50 (22 males, 28 females) pediatric patients with primary ITP (20 newly diagnosed, 30 chronic) and 50 healthy controls age and sex matched were investigated via polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis for cytokine gene polymorphism. Information on demographic characteristics, duration of disease, bleeding symptoms, life threatening bleeding, severity of bleeding, disease phases, type and response to different types of treatment were assessed.
Results: Compared to controls, all patients showed a higher frequency of IL-6−174 CC (P= 0.0001, OR=7.048, 95% CI=2.18-22.7), higher GA genotype of TNF-α(−308) (P= 0.001, OR=6.469, 95% CI=2.0-20.9), higher CC genotype of IL-17F (P= 0.0001,OR=55.545, 95% CI=14.4-213.2), higher GG of IL-10−1082 (P= 0.029, OR=3.6, 95% CI=1.08-12.18) and A1A2 genotype of IL- $1Ra^{VNTR}$ (P=0.039, OR=2.374, 95% CI=1.03-5.4). The frequency of IL-6 (-174) C allele (66% *vs* 49%, P=0.015), A allele of TNF-α−308 (24% *vs* 6%, P=0.001), A2 allele of IL-1RaVNTR (30% *vs* 14%, P=0.007), IL-17F C allele (89% vs.53%, P=0.0001) were significantly higher in all patients with ITP compared to controls. However, there was no significant difference revealed in allele distribution of IL-10(-1082) (p=0.67). IL-10 GA and IL-1Ra A1A1genotypes were higher among chronic patients (P=0.042, P=0.001 respectively) compared to newly diagnosed. There were no significant differences between different cytokine genotypes and the various clinical features including gender, age,disease duration, bleeding score, wet purpura, life threatening bleeding among newly diagnosed ITP patients (P>0.05). On the other hand, among chronic patients with ITP, 30% presented with mucosal bleeding (wet purpura) with highest prevalence among CC genotype (50%) followed by GC genotype (15.4%)and lastly GG genotype (0%) of IL-6 (P=0.048). Of note, the age of diagnosis of chronic patients was significantly higher than the newly diagnosed patients with ITP (2.84 *versus* 5.75 years(P=0.011). Best platelet response to steroid treatment was found among GC genotype of IL-6 (-174) and GG genotype of IL-10(-1082) in all patients with ITP with P value of (P=0.001 and P=0.002 respectively).
Summary/Conclusions: This suggests that previously mentioned cytokine gene polymorphisms possibly contribute to the susceptibility of acquisition of childhood ITP. Furthermore, GA genotype of IL-10 and A1A1 genotype of IL-1Ra polymorphisms are associated with increased risk of chronic ITP. IL-6 (-174) and IL-10 (-1082) genes might play a role in the effectiveness of steroid therapy among patients with ITP. However, the role of other genetic and environmental factors cannot be entirely ruled out.

E1428

LOW-DOSE RITUXIMAB AS PRE-EMPTIVE THERAPY IN MULTI-RELAPSING AND PRIMARY REFRACTORY PATIENTS WITH ACQUIRED IDIOPATHIC THROMBOTIC THROMBOCYTOPENIC PURPURA: A MONOCENTRIC RETROSPECTIVE STUDY

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Background: Acquired idiopathic thrombotic thrombocytopenic purpura (TTP) is a life-threatening microangiopathic disorder for which therapeutic plasma exchange (TPE) and steroids represent the primary standard of care. However, a significant proportion of patients still experiences refractory disease, with exacerbations shortly after a perceived remission, or relapse.
Aims: The aim of this retrospective study was to evaluate the long-term efficacy of rituximab in preventing early death in refractory/exacerbated TTP and avoid relapse in "relapsing" patients.
Methods: We collected retrospectively clinical data on all patients with acquired TTP, admitted to our Hematology Department between January 2005 and December 2015. Forty-eight patients [37 female and 11 male; median age: 42.5 years (range 17-80)] were registered and treated for TTP in this period.
Results: Thirty-six patients were included at their first episode of TTP, while 12 patients had a history of multiple relapses [median 2 (range 2-4)], in the previous 10 years (1995-2004). Nine of 48 patients (19%) were diagnosed with TTP during pregnancy. Median platelet and hemoglobin values at diagnosis were 13x10^9/l (range 5-49) and 7,9 g/dl (4,2-10.1),respectively. ADAMTS-13 concentration was measured at diagnosis or at relapse in all patients and was<10% in all cases [median 0% (range 0-6)]. Anti-ADAMTS13 autoantibodies were present in all 23 patients tested. All 48 patients received TPE associated to 1mg/kg methyl-prednisolone as frontline therapy. After a median of 10 TPE (range 8-18), 32 patients (67%) (20/36 naïve, 12/12 at relapse) achieved disease remission [normal platelet counts, increase of ADAMTS-13 activity [median 78% (range 50-215)] and disappearance of inhibitors), while 16 (33%) resulted refractory or experienced exacerbation. Rituximab (RTX), at the dose of 375 mg/m^2 weekly for 4 weeks, was then added to TPE in 27 patients (56%), including 15/36 patients at their first TTP episode, who resulted refractory to TPE or showed exacerbation, and in the 12 relapsing patients, to prevent subsequent relapse. One patient with TTP exacerbation died early, prior to RTX start. Complete response to RTX was documented in 12 of 15 refractory patients, and in all 12 relapsing patients. Three patients died early, before the

second RTX dose, resulting into 8% overall 30-day mortality rate (4 patients). Mainteinance treatment with low dose RTX (100 mg every three months for 2 years) was then administered to 23 patients (12 relapsing and 11 refractory), while 1 patient refractory to TPE refused maintenance. At a median follow up of 34 months (range 1-87) from the first dose of maintenance none of the patients relapsed, and we did not observe any infectious complications. This resulted into a significant reduction in the incidence of relapses after RTX treatment (HR 4.26, 95% CI 1.511-12.05, p 0.0089). ADAMTS-13 activity was stable at >50% and inhibitors were not detected during follow-up visits, performed every three months in all 23 patients.
Summary/Conclusions: Our results suggest that pre-emptive treatment with low dose rituximab, is safe and effective to maintain remission also in patients with primary refractory TTP or with a history of multiple relapse.

E1429

THE INTERLEUKIN-17F (7488T/C) GENE POLYMORPHISM AND THE RISK OF CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA IN EGYPTIAN PATIENTS

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Background: Chronic primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by both reduced platelet counts and suppression of megakaryocyte and platelet development.IL-17F is a novel inflammatory cytokine and plays an important role in some autoimmune diseases by inducing the expression of multiple chemokines, cytokines, and adhesion molecules. IL-17F (7488T/C) polymorphism influence IL-17 expression and activity. Thus, considering the abnormal percentage of T helper 17 cells and the reported high levels of circulating IL-17F in patients with primary immune thrombocytopenia suggests a possible role of IL-17F (7488T/C) polymorphism in development of chronic ITP.
Aims: We investigated the role of IL17F polymorphism (7488T/C) on the susceptibility and clinical features of chronic ITP in Egyptian patients and if it may be linked to response to treatment with glucocorticoides.
Methods: A cohort of 107 patients with chronic ITP and 100 healthy control were enrolled in this case control study.Genotyping of IL17F (7488T/C) gene polymorphism was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique
Results: Chronic ITP patients had a significantly higher frequency of the IL-17F 7488-TT genotype compared to controls (84.1% *vs* 70.0%; Odd ratio= 2.269; P-Value=0.015).Furthermore the IL17F 7488 TT genotype was significantly associated with poor response to corticosteroid therapy; 11.1% were steroid responsive *vs* 88.9% were not responsive (P value= 0.001)
Summary/Conclusions: These findings suggest that the IL-17F 7488 T allele is significantly associated with the development of chronic ITP, suggesting a role for IL-17F polymorphism in the pathogenesis of chronic ITP.

E1430

ELTROMBOPAG SAFETY AND EFFICACY IN OLDER THAN 65 YEARS OLD PRIMARY IMMUNE THROMBOCYTOPENIA PATIENTS IN CLINICAL PRACTICE

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Background: Eltrombopag is a a thrombopoietin receptor agonist (TPO-RA) approved for chronic primary immune thrombocytopenia (ITP) patients. However, ITP clinical practice in more than 65 years old patients could not reflect efficacy and safety of clinical trials results.
Aims: To evaluate the safety and efficacy results of eltrombopag in older than 65 years old patients in a real world setting
Methods: 81 equal or more than 65 years old primary ITP patients from 23 Spanish centers who had been treated with eltrombopag and included in the Spanish Eltrombopag ITP Registry were retrospectively evaluated.
Results: -The median age of the whole cohort was 75 (IQR, 69-79) years. Our case series included 16 newly-diagnosed, 16 persistent and 49 chronic primary ITP patients [78 (IQR, 73-81), 78 (IQR, 74-85) and 73 (IQR, 68-76) median years, respectively]. There were 51 women and 30 men. 23% of patients had a Charlson Comorbidity Index score of 2 or more at diagnosis. The median time from ITP diagnosis to eltrombopag initiation was 36 (IQR, 4-47) months [1(IQR,0-1.25), 4(IQR,6-8) and 57(IQR, 22-73) months, for each of the groups]. The median number of therapies before starting eltrombopag was 2 (IQR, 1-3), including rituximab (18%), romiplostim (17%) and splenectomy (10%). At the time of treatment start, 22 of 81 patients (27%) patients were receiving concomitant medication for primary ITP, mainly including corticosteroids (20%) or immunoglobulins (11%). 24 of 81 (30%) patients had bleeding symptomatology during the month preceding the starting eltrombopag. At eltrombopag initiation the median platelet count was 27 x 10^9/L (IQR, 7-26 x 10^9/L). 68 of 81 (84%) patients had a response (R) to eltrombopag treatment whilst 62 patients (76%) achieved a complete response (CR; platelet count >100 x 10^9/L). [R and CR rates were 94% and 87% in newly-diagnosed ITP; 75% and 68% in persistent ITP; 84% and 75% in chronic ITP]. Women reached 86% and 78% of R/CR while 80% and 73% of men. Responses were 91% in patients who received concomitant ITP medication at baseline and 64% in patients without other added treatments. The proportion of patients achieving a platelet response was quite similar regardless bleeding at starting eltrombopag (79% and 86% for patients with and without bleeding, respectively). Of the 82 patients, 20 (24.4%) experienced one or more adverse events during treatment with eltrombopag. Adverse events were mainly grade 1–2 in severity. The commonest adverse effects reported during eltrombopag treatment were diarrhea and headache. Eight percent of patients (7 of 82) had hepatobiliary laboratory abnormalities (HBLAs). On the other hand, we observed seven deaths: three of them were caused by ITP severity (cerebral bleedings), two were due to progression of COPD (chronic obstructive pulmonary disease), one was a gram-negative sepsis and one patient suffered an arrhythmia related sudden death. A subanalysis of primary ITP patients with more than 80 years (6 male and 14 female), revealed as high efficacy rates as younger patients (85% of CR with 75% of responses). This kind of patients are expected to be a very frail patients. Nevertheless, only 7 (35%) patients of this population reported adverse events.
Summary/Conclusions: Our case series describe the great efficacy and safety results observed with the use of eltrombopag in our more than 65 years old ITP patients. However more studies are needed to confirm the possible usefulness of TPO-RAs in this variety of primary ITP cases.

E1431

WERE THE MEASUREMENTS STANDARDIZED SUFFICIENTLY IN PUBLISHED PAPERS ABOUT MEAN PLATELET VOLUME?

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Background: Recently, mean platelet volume (MPV) aroused interest of the researchers and several studies about MPV changes in various pathological conditions were published.
Aims: The aim of this study was to evaluate the data accuracy of MPV measurements in these studies.
Methods: The study was performed using the data of 181 studies contain healthy control subjects within 1181 paper about mean platelet volume indexed by PubMed database since 2012.
Results: 81 studies (%44.7) were performed retrospectively. Healthy control groups included 80.52±70.51 subjects (mean±standard deviation). The distributions of gender in 16 (8.8%) and age in 12 studies (6.6%), and platelet counts in 28 studies (15.5%) were not reported. The gender and age groups were not differing significantly by the means of platelet counts (r= -0.40; p >0.05). EDTA, low (1:9) and high concentrate (1:4) citrate were used as an anticoagulant in 112, 7 and 2 studies, respectively and type of anticoagulant was not noted in 60 studies (33.1%). There was no study to compare the different anticoagulants. The instruments of Beckman Coulter, Sysmex, Abbott Cell-Dyn, Siemens ADVIA, Mindray BC-6800, HORIBA ABX Micros 60 and Diatron Abacus Junior B were used for the measurements of MPV in 53, 46, 32, 9, 2, 2, and 1 studies, respectively and the used technology in automated blood cell counting was not specified in 36 studies (19.9%). The MPV values measured with Sysmex was higher significantly than the MPV values measured with Beckman Coulter, Abbott Cell-Dyn and Siemens ADVIA. The MPV measurements varied up to 17.8% by the instruments. The measurement times between 15 minutes-2

hours was significantly different from the measurement times<15 minutes and >2 hours. The MPV measurement times from venipuncture were not indicated in 86 studies (47.5%). The MPV measurements by the MPV measurement times and plus the used instruments varied up to 17.8% and 27.7%, respectively. Both the MPV measurement times and used instruments were not stated in 29 studies (16.0%). Only 47 prospective studies (26.0%) enlightened about type of anticoagulant, used instruments for MPV measurement, MPV measurement time, platelet counts and MPV values.
Summary/Conclusions: As a result, the measurements were not standardized sufficiently in published papers about MPV. It may be explained the differences between the results of studies made same pathological conditions.

E1432

COMPARISON OF MEAN PLATELET MASS AND MEAN PLATELET COMPONENT IN IMMUNE THROMBOCYTOPENIA, HYPOPROLIFERATIVE THROMBOCYTOPENIAS, AND HEALTHY INDIVIDUALS
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Background: Immune thrombocytopenia (ITP) remains a diagnosis of exclusion. The Siemens ADVIA 120 has the capacity to calculate the mean platelet (PLT) component (MPC), a measure of PLT density, and the mean platelet mass (MPM), neither of which are currently employed in clinical decision making.
Aims: To determine if the MPC and MPM are significantly higher in ITP (reflecting increased PLT granules) than in hypoproliferative thrombocytopenia (HT) and in the healthy population.
Methods: Institutional review board approval was obtained. A prospective study was initiated in September 2013. This trial is registered at ClinicalTrials.gov. Patients at Mount Sinai Roosevelt Hospital with ITP, as defined by the ASH 2011 practice guidelines, and HT's (delineated in Results section) were included. A reference population was established. Patients with human immunodeficiency virus, hepatitis C, cirrhosis, pregnancy, and disseminated intravascular coagulation were excluded. Enrollment of 20 patients in each arm and 10 healthy individuals in the control arm was planned. Lavender tri-potassium EDTA tubes were filled and analyzed on the Siemens ADVIA 120 within a period not exceeding 2 hours from their collection. MPC and MPM values were compared for all 3 groups using one-way analysis of variance. The student's t-test was used to compare these parameters in the patient groups.
Results: Twenty patients with ITP, 20 patients with HT (4 aplastic anemia, 8 chemotherapy-induced thrombocytopenia, 3 myelodysplasia, 1 acute myelogenous leukemia, 1 hairy cell leukemia, 1 multiple myeloma, and 2 drug-induced thrombocytopenia (valproic acid, imatinib)), and 10 controls were enrolled. Baseline characteristics of the patient groups were similar. Median age, M:F ratio, and mean PLT count were 54 years, 0.67:1, and 62,500/µL (ITP) and 60.5 years, 1:1, and 53,900/µL (HT), p>0.05. MPC (g/dL, mean +/- SD) for each group was 26.7 +/- 1.89 (ITP), 24.2 +/- 1.89 (HT), and 28.09 +/- 0.74 (controls), p<0.01. MPM (pg, mean +/- SD) for each group was 2.43 +/- 0.451 (ITP), 1.95 +/- 0.210 (HT), and 1.91 +/- 0.10 (controls), p<0.01. Comparison of these parameters for the 2 patient groups showed statistical significance (p<0.01).
Summary/Conclusions: MPM is significantly higher in ITP than in HT and in controls, likely reflecting increased granular content. While MPC is significantly higher in ITP than in HT, it is highest in controls, etiology unclear. Future studies should further evaluate these parameters for distinguishing ITP from other thrombocytopenias.

E1433

ELTROMBOPAG LOW DOSES AS PROPHILAXIS OF CHEMOTHERAPY INDUCED THROMBOCYTOPENIA (CIT) IN CANCER PATIENTS TREATED WITH PLATINUM BASED CHEMOTHERAPY
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Background: Chemotherapy-induced thrombocytopenia (CIT) can cause delay or reduction in subsequent courses of chemotherapy. As reported in literature, thrombocytopenia occurred in 82% of those receiving only carboplatin, and in 58%, 64%, and 59% of those receiving combination therapies with carboplatin, gemcitabine or paclitaxel, respectively. Eltrombopag is an oral, non-peptide thrombopoietin receptor agonist that has been shown efficacy and safety in chronic immune thrombocytopenia (ITP) patients not responding to previous therapy by raising the platelets count in both continued long-term administration and in a repeated short-term administration. Eltrombopag bound the thrombopoietin receptor in the transmembrane region, an area different from where thrombopoietin or romiplostim bound, and activated the thrombopoietin receptor in a different fashion. Here, we report on a series of 18 patients at high risk of CIT because of platinum chemotherapy schedules who received low doses eltrombopag as prophilaxis
Aims: To prevent CIT in patients who cannot be supported by platelet transfusions and for whom the maintenance of dose intensity is crucial for remission or survival.
Methods: A total of 18 consecutive adult patients, female (60%), median age 47 years (range 28 - 65) were enrolled in the study.The reason of chemotherapy has been ovary cancer in 4 patients, colon cancer in 6 patients, relapsed DLBC lymhoma in 4 patients, TNBC in 2 patients, pancreatic cancer in 2 pts. All patients received eltrombopag 25 mg by mouth twice a weekly as soon as the platelet count falls below 80000 mmc, and continued on treatment until completion of cycles of chemotherapy.
Results: The mean platelet count nadir was 60000 mmc; the number of days with platelet count<80000/µL was 4 days; The maximum value reached was 270,000 mmc. No treatment-related toxicity was observe. The principal endpoints of the study: avoid nadir platelet counts<50,000/µL,platelet transfusions,bleeding events, chemotherapy dose reductions chemotherapy delays. were achieved in all patients.
Summary/Conclusions: In our opinion low dose eltrombopag prophilaxis can be an effective and safe strategy for preventing the CIT.

E1434

THERAPEUTIC APPROACH TOWARDS SYMPTOMATIC THROMBOCYTOPENIA IN DENGUE HEMORRHAGIC FEVER
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Background: Introduction: Immune-mediated destruction of platelets is thought to be the mechanism of thrombocytopenia seen after the viremic phase of dengue hemorrhagic fever (DHF). Immuno-suppressants such as steroids, immune globulin and Anti D immunoglobulin are effective in the treatment of this type of immune thrombocytopenic purpura.
Aims: To evaluate the efficacy of oral Prednisolone in the rate of resolution of thrombocytopenia and monitoring of complications in patients recovering from Dengue hemorrhagic fever.
Methods: A controlled study was carried out on diagnosed cases Dengue hemorrhagic patients presenting with sever thrombocytopenia and symptoms like confluent ecchymosis, epistaxis and purpuric rashes. This study was conducted in Ittefaq hospital (trust) in collaboration with KAU Jeddah, during the period of October to December 2013. Treatment group received steroids in two forms *i.e.* 1st line therapy prednisolone orally or as 2nd line therapy of initial I/V high dose (prednisolone) in pulse doses *i.e.* 40 mg bid for four days and later oral prednisolone as in 1st line therapy with omeprazole 20 mg bid in addition to standard treatment. Control group received standard supportive care only.
Results: A total of 341 suspected patients were admitted in hospital. Serological diagnosis was confirmed in 166 patients. CBC revealed platelet count ≤100x10^9/l in 106 patients. A group of symptomatic febrile patients have platelet count<20x10^9/l was selected for therapeutic intervention. 1st line therapy (oral prednisolone was stated in 43 patients. In Fourteen patients 2nd line therapy (high dose dexamethasone pulse) therapy was instituted. Seven of them attained complete response whereas two patients achieved partial response. Four patients were shifted to Anti D therapy. Three deaths occurred during our study. Rest of all the patients improved and were discharged in due course of time.
Summary/Conclusions: This small scale preliminary study shows promising results in reducing the morbidity of patients in a relatively serious stage but large scale double blinded randomized controlled studies are needed before making recommendations on use of steroids in symptomatic thrombocytopenic patients with dengue hemorrhagic fever.

E1435

WISCOTT ALDRICH SYNDROME IN A GIRL! WHAT IS THE MYSTERY?
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Background: Wiscott-Aldrich Syndrome (WAS) is an X-linked recessive inherited immunodeficiency; which is presented with micro-trombocytopenia, eczema, recurrent infections, and increased incidence of autoimmune diseases, and malignencies. WAS gene is located on the short arm of X chromosome, and encodes WAS protein (WASP). WASP is expressed in hematopoietic stem cell-lineages and responsible for cytoskeleton reorganization affecting the functions of T, B, NK- cell, granulocytes, dendritic cells and platelets. Mutation in the WAS gene ends up with X-linked thrombocytopenia (XLT) or classical WAS. The prevalence of WAS is, 1 in 100 thousand live births.
Aims: In this presentation we would like to share our experience about a girl diagnosed with WAS.
Methods: A 7 year old female patient was followed with trombocytopenia since she was born. She had been hospitalized for recurrent infections, gastrointesti-

nal bleedings, and CMV pneumonia. Bilateral ventilation tube regarding to persistent otitis media was also performed. Serum immunoglobulin levels were checked, Ig A and Ig E levels were increased, Ig M level was decreased and Ig G level was normal. Western blot studies confirmed the reduced WAS protein expression in peripheral mononuclear blood cells. The complete WAS gene was sequenced, one heterozygous mutation in Exon 7, leading to a premature stop codon p.G219*, c.655G>T was found.
Results: WAS is an X-linked recessive disorder, which is seen in male patients due to the transition. But, in case of X gene inactivation, it can also be presented in female patients.
Summary/Conclusions: The clinicians must be vigilant about the possibility of X linked diseases such as WAS in females.

Quality of life, palliative care, ethics and health economics

E1436

EFFECT OF EXERCISE AND COUNSELING INTEGRATED IN THE CLINICAL MANAGEMENT OF ACUTE LEUKEMIA ON PHYSICAL FUNCTION AND QUALITY OF LIFE DURING CONSOLIDATION CHEMOTHERAPY: A MULTICENTER RANDOMIZED TRIAL

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Background: Exercise and counseling interventions, widely used as rehabilitation strategies in cancer patients to remedy disease and treatment-related sequelae is novel in patients with acute leukemia due to the life threatening nature of the disease, the intensive treatment and safety monitoring requirements.
Aims: We assessed whether an exercise and counseling intervention could prevent and modulate physical and functional debilitation and improve health-related quality of life (HRQoL) in a two-center, randomized controlled trial of patients with acute leukemia during outpatient consolidation chemotherapy.
Methods: Patients were randomized to usual care or a twelve-week supervised exercise and counseling intervention. The primary outcome was 6 minute walk distance (6MWD), and secondary outcomes included submaximal VO_2 max, sit-to-stand test, biceps arm curl, and patient-reported HRQoL questionnaires. 70 patients were randomly assigned to the intervention (n=32) or control group (n=34), and 62 completed study requirements (88.6%).
Results: Changes in the primary outcome 6MWD differed significantly across randomization groups (85.5 m [51.2-119.8], $p<0.0001$) and physical capacity and functional outcomes were substantial (effect sizes 1 or above). Self-rated leisure-time physical activity levels, health-related quality of life (FACT-An) and levels of depression and anxiety differed significantly between groups, in favor of the intervention group. Eight patients experienced non-serious adverse events.
Summary/Conclusions: Exercise and counseling in patients with acute leukemia undergoing outpatient consolidation chemotherapy improved physical and functional capacity, physical activity levels, HRQoL and psychological well-being. The role of exercise and counseling integrated in the clinical setting has the potential of optimizing the management of acute leukemia and may facilitate resumption of everyday activities.

E1437

COST-EFFECTIVENESS ANALYSIS OF A DIRECTLY SELECTED CMV-SPECIFIC T CELL THERAPY FOR MANAGEMENT OF CMV DISEASE IN PATIENTS POST ALLOGENEIC STEM CELL TRANSPLANTATION: A UK ANALYSIS

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Background: Hematopoietic stem cell transplantation (HSCT) is a curative treatment option for a variety of malignant and non-malignant hematological diseases. However, cytomegalovirus (CMV) infection, an acknowledged complication after HSCT, is associated with significant morbidity, decreased patient survival, and considerable cost to health-care systems. Two randomized clinical studies, CMV~ACE/ASPECT and CMV~IMPACT (NCT01220895 and NCT01077908), assessed the efficacy and safety of a CMV-specific T cell therapy for prevention or treatment of CMV reactivation post allogeneic HSCT.
Aims: We assessed the cost-effectiveness of a CMV-specific T cell therapy for the management of CMV disease in patients after allogeneic HSCT in the UK.
Methods: A Markov model, reflecting current clinical guidelines, was developed to estimate cost-effectiveness of a CMV-specific T cell therapy for the management of CMV infections and CMV disease in patients after allogeneic HSCT in the UK from the perspective of the NHS in 2015. Here we report the results of this economic analysis in the CMV disease setting. Data sources, in the model, included the two randomized clinical trials for the CMV-specific T cell therapy, published literature, national costs, tariff lists, and a Delphi panel of 7 English hematologists. The model simulated clinical and economic outcomes associated with the a CMV-specific T cell therapy and standard treatment. The main comparators in the analysis depended on the positioning of the CMV-specific T cell therapy and were foscarnet in 2-line and cidofovir in 3-line. Clinical probabilities included response probabilities, mortality and recurrence. Inpatient costs included daily hospital stay, laboratory analyses, microbiological culture, specialists' visits, therapy acquisition and administration costs. Utilities were included for the Quality Adjusted Life Years (QALY).

Results: The use of the CMV-specific T cell therapy as 2-line CMV disease treatment increases costs from £27,882 to £30,656, which results in additional costs of £2,774, but leads to a gain in QALYs of 0.215 from 3.179 to 3.394. Consequently, the incremental cost-effectiveness ratio (ICER) is £12,902/QALY. The probabilistic sensitivity analysis (PSA) shows a probability of 79% that the ICER remains below £30,000/QALY. The use of the CMV-specific T cell therapy as 3-line CMV treatment increases total costs from £27,882 to £28,648 leading to additional costs of £766, but to a gain in QALYs of 0.031 from 3.179 to 3.210. Consequently, the resulting ICER is £24,710/QALY. The PSA shows the probability is 53% that the ICER remains below £30,000/QALY. The impact of risk adjustment for the clinical benefits of the CMV-specific T cell therapy *versus* standard therapy on the ICER was explored. The health economic outcomes were sensitive to a risk adjustment for mortality, but not for response and recurrence in the 2-line setting. In the 3-line setting, the ICER was sensitive to risk adjustment for mortality and response, but not to recurrence. The analyses also show that the health economic outcomes were quite robust for both 2-line and 3-line positioning of the CMV-specific T cell therapy for other variables. The favourable cost-effectiveness of the CMV-specific T cell therapy results mainly from the prevention of treatment failure and as a consequence the avoidance of mortality and the cost of salvage therapy.
Summary/Conclusions: CMV-specific T cell therapy is a cost-effective treatment for patients with CMV disease after allogeneic HSCT in 2-line and 3-line therapeutic treatment in the UK setting.

E1438

CLINICAL CHARACTERISTICS, TREATMENT PATTERNS AND HEALTH CARE RESOURCE UTILIZATION AMONG ITALIAN PATIENTS WITH RELAPSED REFRACTORY MULTIPLE MYELOMA: RESULTS FROM A PROSPECTIVE OBSERVATIONAL STUDY

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Background: Knowledge of clinical practice and healthcare resource utilization (HCRU) in relapsed refractory multiple myeloma (RRMM) can inform and improve national treatment strategies.
Aims: To describe clinical and treatment characteristics, HCRU and associated costs in patients (pts) with RRMM in Italy compared to a general European cohort.
Methods: PREAMBLE (NCT01838512) is a prospective, multi-region (US, Canada, EU: Italy, France, UK, Germany), observational study with up to 3 years of follow up per pt. Eligible pts have ≥1 prior therapy for MM with disease progression from the most recent therapy, and have initiated index therapy with immunomodulatory drugs (IMiDs) and/or proteasome inhibitors (PIs) within 90 days prior to or 30 days after study consent. Demographics, treatment and HCRU data were collected, including hospitalizations, visits to healthcare professionals due to MM, diagnostic tests, MM treatments and concomitant medications. Costs were calculated by assigning standardized specific costs from Italy and other EU countries. Descriptive statistics from an interim analysis are reported.
Results: At the cutoff date (December 7th 2015), 764 of 815 enrolled pts had received treatment (509 in Europe, including 197 in Italy), with a median follow-up of 15.1 (Q1–Q3: 8–24) months. Pts were mostly male with mean age 68 years, both in Italy and the EU. At study entry the majority of pts were relapsed (Italy 76%; EU 80%) or refractory (Italy 23%; EU 20%). In Italy most pts were ISS stage I (44%) or II (33%); EU pts were equally spread across severity stages. In both cohorts 44% of pts had prior transplantation. 72% of pts in Italy had comorbidities, commonly one (25%) or two (21%). Cardiovascular (40%) and metabolic (16%) disorders were most frequent. 39% of pts receiving PIs and 19% of pts receiving IMiDs had no comorbidities. In Italy for index therapy, 101 pts received IMiDs, 92 PIs and 4 IMiDs+PIs. The most common scheme amongst IMiDs was lenalidomide and amongst PIs bortezomib, both in combination with dexamethasone. Duration of index therapy varied between treatment types: median 8.8 (Q1–Q3: 3.9–14.2) months for IMiDs; 4.6 (2.8–6.2) for PIs; 4.4 (2.2–7.5) months for IMiD+PIs, with a similar pattern observed in the EU cohort. 52% of pts in Italy had received 1 prior line of therapy; most commonly bortezomib-based schemes. Average HCRU for RR MM patients during the first year of follow-up was lower in Italy compared to European cohort (Italy: mean 1.1 visit/year [max 27]; EU: 13.8 visits/year [max 112]). In both cohorts the number of visits decreased in subsequent years. In Italy HCRU differed by treatment: mean number of visits/year for pts treated with IMiD: 0.76, PI: 1.2, IMiD+PI: 7; the majority being hospital outpatient visits (68%) and all-cause hospitalization (17.5%). In the EU cohort, after outpatient visits (63%), most common were clinic/physician office visits (18%). The main reason for hospitalization was management of MM (Italy 78%; EU 67%). Cost distribution was heterogeneous among pts in both cohorts (median overall costs during index treatment: 116,000 (Q1–Q3: 78–152) euros in Italy; 88,000 (44–149) euros in the EU). Main cost drivers were MM treatments, concomitant medications (in Italy only for PI-treated pts) and hospitalizations.
Summary/Conclusions: Preliminary data from the PREAMBLE prospective observational study show variation in the management of RRMM pts in Italy having lower HCRU compared with the EU cohort. Routine management and disease progression continues to drive HCRU in MM.

E1439

EVALUATION OF PSYCHOMETRIC PROPERTIES OF EUROPEAN ORGANISATION FOR RESEARCH AND TREATMENT OF CANCER QUALITY OF LIFE QUESTIONNAIRE CORE 15 PALLIATIVE (EORTC QLQ-C15-PAL) IN MULTIPLE MYELOMA PATIENTS

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Background: The EORTC QLQ-C15-PAL was developed by shortening the QLQ-C30 from 30 to 15 items for easier administration to patients receiving palliative care. Its performance in measuring health-related quality of life (HRQoL) in relation to its parent version in patients with multiple myeloma (MM) has not been previously evaluated.
Aims: To assess the psychometric properties of the EORTC QLQ-C15-PAL in relation to the QLQ-C30 in patients with MM at different disease stages.
Methods: US patients with self-reported MM were recruited through an online patient-powered network, PatientsLikeMe. Upon completion of informed consent, study participants were asked to provide a brief MM treatment history and complete the EORTC QLC-C30. Domain scores for QLQ-C30 and QLQ-C15-PAL were calculated. Item-scale correlations, and floor and ceiling effects for all items on the QLQ-C30 were stratified by disease progression and palliative care status separately. To measure equivalent-form reliability, regression analysis was used to determine the proportion of variance (R-squared) using QLQ-C30 as the response variable and QLQ-C15-PAL as the explanatory variable for those domains shortened in the QLQ-C15-PAL. Intraclass correlation coefficients (ICCs) were calculated between the subscales shortened in QLQ-C15-PAL. The internal consistency of domains with 2 or more items was assessed using Cronbach α coefficients.
Results: A total of 199 patients completed the online survey, and 10 patients were excluded from the analysis because they had not received any MM treatment. Of the remaining 189 patients, the median age was 61 years (range, 37-82 years) and 57.6% were women. 51 patients (27.0%) received palliative care currently or previously and 65 patients (34.4%) switched treatment regimens at least once due to disease progression. With few exceptions, item-scale correlation, and floor and ceiling effects were in similar ranges for all items regardless of disease progression status or palliative care status. R-squared values ranged from 0.72 to 0.92 and ICCs varied from 0.87 to 0.96. Cronbach α coefficients for Physical Functioning, Emotional Functioning, and Fatigue were 0.64, 0.76, and 0.76, respectively.
Summary/Conclusions: The EORTC QLQ-C15-PAL appears to be a reasonable substitute to measure HRQoL in patients with MM at different disease stages.

E1440

CRYOPRESERVATION OF MATURE OOCYTES TO PRESERVE FERTILITY IS FEASIBLE WITHOUT TREATMENT DELAY IN YOUNG ADULT LYMPHOMA PATIENTS

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Background: Advances in the treatment of lymphomas have led to a substantial decline in the mortality rate and a remarkable improvement in the long-term outcomes, that has raised patients' expectations of a better quality of life, among which fertility preservation represents an imminent wish. However the majority of treatment protocols consist of combination chemotherapy (CT) which may lead to infertility due to severe injury to ovarian reserve, when alkylating agents –containing regimens, CT combined with radiotherapy (RT) on the pelvis, high-dose CT and autologous hematopoietic stem cell reinfusion and/or allogeneic bone marrow transplantation have to be given in the course of the disease. Attempts to prevent ovarian damage during CT±RT include a) the concomitant administration of Gonadotropin Releasing Hormone (GnRH) agonists, but results of randomized trials have led to controversial results, b) ovarian tissue cryopreservation, which requires two subsequent laparoscopic sessions, carries the possibility of malignant cells reimplantation and has produced only a limited number of published live births c) mature oocyte cryopreservation, d) embryo cryopreservation, which is not admitted by italian rules and requires the availability of a male partner.
Aims: To evaluate in a prospective observational study the acceptability and feasibility of the procedure of ovarian hyper-stimulation and mature oocyte retrieval and cryopreservation, in female patients with newly diagnosed Hodgkin (HL) and non Hodgkin lymphoma (NHL).
Methods: Female patients aged ≥18 years and ≤38 years with histologically proven untreated HL or NHL at any stage, diagnosed at Istituto Nazionale Tumori of Milan, without contraindications to ovarian stimulation, *i.e.* symptomatic rapid progressive disease, and/or superior vena cava syndrome, active

viral infectious disease and ovarian failure, were eligible for the study. After having addressed expected gonadal toxicity of CT±RT during their first haematological evaluation, they were referred to gynecological counseling. Ovarian reserve was assessed by antral follicle count regardless of the menstrual cycle. If the woman consented, she underwent ovarian hyper-stimulation according to a random start protocol. Briefly, gonadotropins were started the same day regardless of the menstrual cycle and the cycle was monitored through serial ultrasounds to tailor the initiation of GnRH antagonists and to identify the most proper time for ovulation trigger

Results: From July 2013 to December 2015 20 patients were enrolled into the study, median age was 27 years (range, 19-35), 16 patients were diagnosed with HL, 4 with NHL (2 DLBCL, 1 PMBCL, 1 follicular). Median time from first hematological visit and gynecological counselling was 2 days (range, 0-10), median time from diagnostic biopsy and gynecological counselling was 14 days (range, 0-29). 11 out of 20 (55%) patients were considered eligible and/or accepted to undergo ovarian stimulation; 6 patients refused due to personal reasons, 1 due to gynecological reasons (ovarian cysts), 1 due to symptomatic progressive disease after the first gynecological visit and in 1 DLBCL NHL patient stimulation was suspended due to initial superior vena cava syndrome. Median time from first gynecological visit and oocyte retrieval was 13 days (range, 12-16); median number of retrieved oocytes was 16 (range, 11-32) and median number of cryopreserved oocytes was 14 (range, 9-23). Median time from oocyte retrieval and CT start was 4 days (2-14).

Summary/Conclusions: Preliminary results of this study document that ovarian hyper-stimulation and oocyte retrieval using a random start protocol is feasibile in young females diagnosed with HL or NHL without a significant delay in CT start. The number of mature oocyte cryopreserved is adequate and comparable to non-cancer patients. Oocyte cryopreservation should be systematically considered in lymphoma patients of childbearing age before starting gonadoxic therapy and should be preferably performed during staging procedures.

E1441

PSYCHOMETRIC VALIDATION OF THE SF-36V2® HEALTH SURVEY IN AN AL AMYLOIDOSIS POPULATION

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Background: Light chain (AL) amyloidosis, a rare protein misfolding disease, leads to deficits in health-related quality of life (HRQoL). Patients with AL amyloidosis present with a wide variety of non-specific symptoms, organ involvement, and functional impairment. The SF-36v2® Health Survey (SF-36v2), a general HRQoL survey, has been used to quantify the impact of AL amyloidosis on HRQoL, though to-date there is no evidence of its psychometric validity for use with AL amyloidosis patients.

Aims: To document the psychometric properties of the SF-36v2 among AL amyloidosis patients, including tests of data quality, scaling success, reliability, and validity.

Methods: Adults (≥18 years old) with self-reported AL amyloidosis completed baseline (n=341) and one-month follow-up (n=252) surveys online to assess HRQoL, clinical and socio-demographic characteristics. Data quality evaluation (DQE) checks included item and scale distributions and a response consistency index (RCI). The online system did not allow out of range values or missing data. Scaling success was evaluated against assumptions of summated rating scales. Internal consistency reliability used Cronbach's α. Test-retest reliability used intra-class correlations (ICC) between baseline and one-month follow-up scores among a stable disease subgroup (n=180). Scale convergent and discriminant validity was tested and used correlations between scores from the SF-36v2, the Kansas City Cardiomyopathy Questionnaire (KCCQ-12), Patient Global Assessment of Functioning (GAF), and other surveys. Known-groups validity tests used ANOVA of scores across patient groups varying in disease severity (self-reported hematologic response status, and Patient Global Impression-Severity (PGI-S)).

Results: DQE showed excellent response distribution and RCI (94.1%). Scale reliability (Cronbach's α ≥ 0.780 across all 8 domains) and test-retest reliability (ICC ≥ 0.731) were acceptable. Tests of summated rating scale assumptions were satisfactory. Scale convergent and discriminant validity showed strong correlations with conceptually related measures. Tests for known-groups validity showed that the mean scores for respondents with self-reported complete hematologic response or remission were significantly greater than scores for respondents with no response to treatment (p<0.05 for all scores). Similarly, mean scores were also significantly associated with responses to the PGI-S (p<0.0001 for all scores).

Summary/Conclusions: This study provided robust evidence of the psychometric properties of the SF-36v2 in a diverse sample of patients with AL amyloidosis. Planned future analyses will assess responsiveness and confirm psychometric properties of the SF-36v2 in clinic-based samples of AL amyloidosis patients.

Study supported by: Prothena Biosciences Inc

E1442

MONOCENTRIC ASSESSMENT OF THE PROFESSIONAL PRACTICES IN THE MANAGEMENT OF PAIN DURING BONE MARROW ASPIRATION

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Background: Bone marrow aspiration performed for the diagnosis of hematologic malignancies is known for being painful. However, pain management is a current concern in the follow-up of patients with chronic diseases.

Aims: Within this overall dynamic of improvement of the analgesic gesture support, an evaluation of the professional practices was initiated by the Hematology Department of the European Georges Pompidou Hospital (Paris, France). The aim of this study was to evaluate the factors involved in the feeling of pain during bone marrow aspiration.

Methods: This monocentric cohort study included 480 patients from 2010 to 2015. They underwent bone marrow aspiration and filled out a standardized questionnaire-based survey including: demographic characteristics (patient's sex and age, medical department), clinical data (bone hardness, puncture site, diagnosis assumptions and final diagnosis), analgesia methods (lidocaine patch, infiltration with lidocaine, inhalation sedation and premedication) and processing (patient anxiety; intensity of pain evaluated after bone marrow aspiration quoted by the patient and the operator, using a visual analog scale (VAS) from 0 to 10; awareness of the gesture by the patient).

Results: Among the 480 patients, 58% were men. Patients' average age was 68.7 years. Most patients were from Nephrology (16%), Hematology (16%) and Internal medicine (30%) departments. The main indication of bone marrow aspiration was the diagnosis assessment of monoclonal gammapathies (30%). The most used puncture site was sternal site (88%) and in most cases the bone hardness was described as medium (64%). Bone marrow examination allowed us excluding a central origin for the hematologic anomaly in 43% patients. The foremost analgesia method was the use of lidocaine patch (80%) with an average exposure of 68 minutes. Half of patients declared not to be anxious before bone marrow aspiration. Pain was generally rated between 3 and 4 on a scale of 10 (43%), and lower than they had expected (58%). Overall, 97% of bone marrow aspirations were performed under analgesia.(Table 1.Pain's management). Analgesic treatment was found significantly effective compared with no analgesia? (p<0.001). Patients experienced less pain when they considered that they were well-informed (p<0.001).Women were significantly more sensitive than men (p<0.05). In our study, sternal site was more painful than superior iliac bone site (p<0.001). There were no relationships between pain intensity and patients' age, medical department, bone hardness, diagnosis assumptions, final diagnosis, a particular analgesia method and patient anxiety (p>0.05).

Table 1.

Puncture site	Sternal (n=421)	Iliac (n=58)
	n patients (visual analog scale mean)	
Lidocaine patch	345 (4.0)	2 (2.7)
Infiltration with lidocaine	17 (3.7)	48 (2.9)
Lidocaine patch + Infiltration with lidocaine	27 (2.9)	3 (1,9)
Premedication + Infiltration with lidocaine	1 (4.0)	2 (1.2)
Premedication+ Lidocaine patch	2 (/)	0
Inhalation sedation	1 (4.0)	1(2.0)
Inhalation sedation + Infiltration with lidocaine	1 (4.0)	0
Inhalation sedation+ Lidocaine patch	1 (1.0)	0
Inhalation sedation+ Lidocaine patch + Premedication	1 (3.5)	0
Premedication + Lidocaine patch + Infiltration with lidocaine	1(9.0)	
No analgesia	15 (5.1)	2 (3.8)

For 10 patients there was no available information

Summary/Conclusions: The perception of and reaction to pain are influenced by the psychological state of the patient. This is an undisputed fact that medical staff should provide information on the steps taken in daily practice and our study on bone marrow aspiration is consistent with this behavior. The prevention and management of pain require an integrated approach between its sen-

sori-discriminative component and its psychological dimension. In our study, we show the benefit of analgesia together with intelligible information in the management of pain during bone aspiration.

E1443

BURDEN OF ILLNESS ASSOCIATED WITH MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a cancer of plasma cells originating in bone marrow and affecting multiple organs. It accounts for 0.8% of all new cancers and is the 2nd most common haematological cancer in the US. It is an incurable disease with an average survival of 4-5 years. Median age at diagnosis is approximately 70 years. MM poses humanistic and economic burden by affecting the health related quality of life (HRQoL) of the patients and/or carers and by impacting healthcare budgets, respectively.
Aims: To review the humanistic and economic burden of MM.
Methods: Two targeted literature reviews were performed; one each for humanistic and economic burden. MEDLINE®, Embase and PubMed (for e-publications ahead of print) were searched to identify relevant publications from 2005 to date. EconLit and NHS Economic Evaluations Database (EED) were searched to identify articles specific to the economic burden. Search terms included disease, QoL and economic terms.
Results: A total of 1029 abstracts for the humanistic burden review and 629 abstracts for economic burden review were identified and screened. Twenty articles were included in the humanistic burden and 18 in the economic burden review. MM is associated with substantial burden of illness. Patients experience a high symptom burden that adversely affects their HRQoL. Bone pain and fatigue are the most common symptoms of the disease. Patients exhibit impaired physical, role, cognitive, emotional and social functioning. Coping with the disease and managing its consequences including loss of independence and being vigilant to prevent incidences of fracture emerge as the predominant factors of the disease. The causes of disease burden are similar in newly diagnosed and/or RRMM patients, with both populations experiencing significant symptom burden which adversely affects their HRQoL. However, the burden in RRMM patients worsens with disease progression such as increased bone pain, fractures and fatigue. Additionally, there is considerable burden on caregivers who report restricted social, leisure, professional/work activities. MM also imposes significant economic burden. The total cost varies depending on whether the disease presents as asymptomatic or symptomatic and by line of treatment. Non-drug costs, which include hospitalisation, autologous stem cell transplant (ASCT), adverse events and ambulatory costs account for approximately 54% of the total cost. Patients treated with novel agents or ASCT and patients who experience skeletal-related events incurred the highest spend for hospitalisation. Some adverse events associated with novel agent treatment required hospitalisation; anaemia and neuropathy were most common. These were associated with a mean length of stay of 9 to 14 days in R/R patients depending on the novel agent received. Costs of productivity loss due to MM were evaluated in one study. Patients who undergo ASCT incurred the highest costs of productivity loss based on working days and hours lost by the patients and caregivers, respectively. Improved outcomes associated with ASCT were not assessed in this study.
Summary/Conclusions: MM poses significant humanistic and economic burden; which increases with disease progression and subsequent lines of therapy. HRQoL in these patients diminishes with symptom severity and disease progression. The total cost of illness is driven by treatment line and choice. These findings warrant the need for more effective therapy with better safety profile delaying disease progression, reducing symptoms and improving patient's outcome.

E1444

WHAT IS THE EFFECT OF VENOUS THROMBOEMBOLISM AND POST-THROMBOTIC SYNDROME ON HEALTH-RELATED QUALITY OF LIFE? A SYSTEMATIC REVIEW AND META-ANALYSIS

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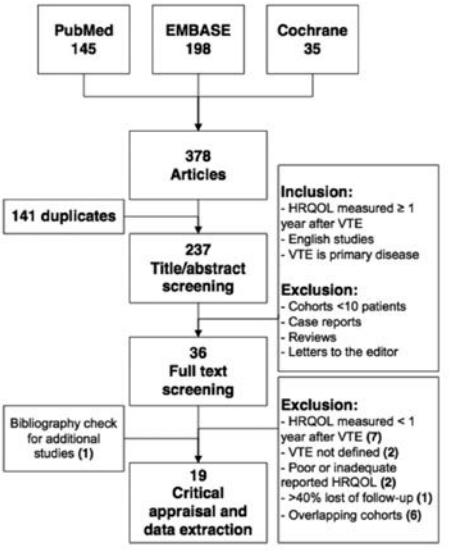
Background: There is limited insight for patients, their families, and their providers into the mid and long-term effects of pulmonary embolism (PE), deep vein thrombosis (DVT), and post thrombotic syndrome (PTS) on health related quality of life (HRQOL).
Aims: We conducted a systematic review and meta-analysis of the literature to (1) assess the HRQOL for patients with a minimum follow-up of one year after an episode of PE or DVT, and (2) to assess the HRQOL for patients with DVT who develop PTS.
Methods: PubMed, EMBASE, and the Cochrane Library were search from inception to October 12th, 2015. All studies measuring HRQOL at least one year after an episode of PE or DVT, with or without development of PTS, were selected. Data were extracted by two of the authors and pooled using random-effects meta-analysis. Heterogeneity was assessed with I^2 and Tau^2 tests. SF-12, SF-36, and VEINES-QOL were evaluated with pooled standardized mean difference (SMD) and 95% confidence intervals (CI).
Results: Fourteen studies were included for meta-analyses. In patients with PE physical health was impaired (SMD:-0.30, 95% CI: -0.46 to -0.15, Tau^2: 0.0, I^2:0%, 2 studies) and mental health was similar to population norms (SMD: 0.14, 95% CI: -0.01 to 0.30, Tau^2: 0.0, I^2: 0%, 2 studies). In patients with DVT physical health (SMD:-0.22, 95% CI: -0.53 to 0.10, Tau^2: 0.21, I^2: 94%, 9 studies), mental health (SMD: 0.03, 95% CI: -0.12 to 0.19, Tau^2: 0.03, I^2: 73%, 9 studies), and disease specific quality of life (SMD: 0.04, 95% CI: -0.10 to 0.18, I^2: 2.5%, Tau^2: 0.0, 8 studies) were similar to population norms. Patients who develop PTS reported worse physical health (SMD:-0.89, 95% CI: -1.21 to -0.57, Tau^2: 0.14, I^2: 82%, 7 studies), mental health (SMD:-0.27, 95% CI: -0.43 to -0.11, Tau^2: 0.01, I^2: 31%, 7 studies), and disease specific quality of life (SMD:-0.97, 95% CI: -1.35 to -0.59, Tau^2: 0.30, I^2: 84%, 10 studies) than population norms.

Table 1.



Summary/Conclusions: Patients who had a PE event report worse physical health. Quality of life after a DVT event is comparable to population norms, although those who develop a PTS have worse overall quality of life. These data can be used to inform patients in the rehabilitation process.

E1445

TREATMENT HISTORY, TOLERABILITY AND IMPACT ON HEALTH-RELATED QUALITY OF LIFE IN AL AMYLOIDOSIS

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Background: Light-chain (AL) amyloidosis is a rare disease characterized by misfolded amyloid protein deposits in tissues and vital organs. There are currently no FDA- or EMA-approved medications indicated for AL amyloidosis; however, chemotherapy, stem cell transplants (SCT), and immunomodulary drugs can reduce the production of amyloid-forming light chains. All existing regimens have tolerability problems due to treatment-related symptoms (TRSs). The SF-36v2® Health Survey (SF-36v2) is a widely-used general health-related quality of life (HRQoL) survey that can be used to describe and quantify the impact of many diseases and treatments.
Aims: To describe the history of treatments, past and current TRSs, and impact on HRQoL among a diverse sample of individuals with AL amyloidosis.
Methods: We report baseline data from an online non-interventional study was initiated in 2015 among patients with self-reported AL amyloidosis (n=341). Patients reported their current and prior treatment for AL amyloidosis. Aspects of TRSs were captured based on the following: 1) lifetime history of TRSs (dichotomous variable); 2) consequence of TRSs (discontinuation of a treatment; reduction of a treatment; or maintenance of treatment despite TRSs); and 3) ability to *tolerate the current AL amyloidosis treatment* (based on a 4 point scale from "extremely poorly" to "very well", higher scores indicate better tolerability). The prevalence of each treatment type and TRSs were estimated. The patients' ability to *tolerate the current treatment* was evaluated in relation to specific medications and HRQoL (as measured by the SF-36v2® Health Survey Physical [PCS] and Mental [MCS] Component Summary scores) using chi square tests for categorical variables and *t*-tests and ANOVA for continuous measures.

Results: The most commonly reported treatments were dexamethasone (81% reported ever being treated, 52% reported as the current treatment), bortezomib (72% ever, 36% current), SCT (53% ever, 24% current), melphalan (47% ever, 15% current), cyclophosphamide (46% ever, 20% current), and lenalidomide (28% ever, 15% current). Many patients reported combination treatments, including cyclophosphamide+bortezomib+dexamethasone (CyBorD, 17% current). Half of the patients (51%) had received three or more different treatments. Nearly three-quarters (71%, n=226) reported ever having problems tolerating AL amyloidosis treatment, of which nearly half (47%, n=107) had discontinued at least one treatment. Nearly half (46%) of those currently being treated reported some tolerability issue (less than very good tolerability). Tolerability varied across the common treatments from a low of 3.22 (SD=0.90) for cyclophosphamide to a high of 3.61 (SD=0.52) for SCT. Problems with tolerating current medications corresponded with decrements in HRQoL (both MCS and PCS, $p<0.001$).
Summary/Conclusions: Lifetime history of TRSs was high. Discontinuation of life-saving AL amyloidosis treatments was fairly common, though most patients were able to tolerate their current regimen. The high prevalence of treatment discontinuation and history of multiple AL amyloidosis treatments suggests that physicians and patients try a variety of treatments to balance tolerability and efficacy. TRSs are associated with decrements in HRQoL, over and above the burden of AL amyloidosis. These findings highlight the importance of assessing HRQoL during treatment for AL amyloidosis to better understand tolerability, and the need for more treatment options for AL amyloidosis, particularly those with favorable tolerability.
Study supported by: Prothena Biosciences Inc.

E1446

ICU AND LONG-TERM MORTALITY ANALISIS OF HEMATOLOGOGICAL MALIGNANCIES ADMITTED IN ICU. A UNICENTRIC SEVEN YEARS REVIEW IN A SPANISH HOSPITAL

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Background: Improvements in survival in patients with haematological malignancies (HM) admitted to the intensive care unit (ICU) has largely been reported in uncontrolled cohorts. Newly diagnosed patients should be admitted, since their prognosis is still to be defined. Nevertheless the admission of the remaining patients remains a matter of substantial controversy.
Aims: To analyse the survival of HM patients admitted to ICU over a 7-year period (from 2008 to 2014) in a single high complexity Spanish hospital
Methods: We conducted a detailed retrospective study of sequential adult ICU admissions with HM in a single centre, considering numerous variables with regard to their influence on ICU and mortality. Overall survival (OS) was defined as the time from ICU admission to death from any cause, and surviving patients were censored at last follow-up. OS were calculated using the Kaplan–Meier method estimates and the differences assessed by the log-rank test. All *P*-value less than 0.05 were considered significant.

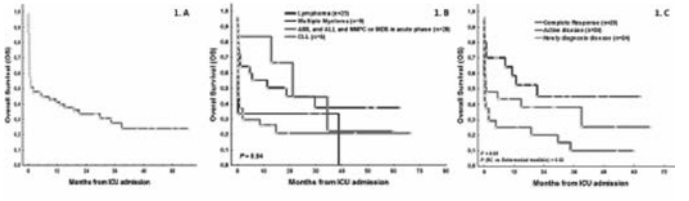

Figure 1.

Results: Overall, 67 HM patients were included, 62% were male, with a median age of 59 years (IQR: 19–82). The median APACHE II was 22 (IQR: 12-49). The hematologic diagnosis was as follow: 25 lymphomas (37%), 9 Multiple Myeloma (MM) (13%), 6 chronic lymphocytic leukaemia (CLL) (9%) and 28 acute leukaemia or myeloproliferative or myelodisplasic syndromes in acute phase (41%). Disease status at the moment of UCI admission was: 19 (28%) in complete response (CR), 24 (36%) with a newly diagnosis disease and 24 (36%) whit active disease (of those, 18 patients were refractory to disease specific treatment and 6 patients were chemosensitive). 67% of patients were on active oncologic treatment at the moment of UCI admission. Principal ICU diagnosis was sepsis (68%). Median number of organ failures was 2 (IQR: 0-4); 71% presented a respiratory and 70% a hemodynamic failure.70% accurate vasoactive drugs, 64% invasive mechanical ventilation and 34% dialysis. More than one third of patients (36%) presented neutropenia at the moment of UCI admission. Median ICU length of stay was 7 days (IQR: 1-48) and median hospital length of stay was 38 days (IQR: 8-109). ICU mortality was 46% (25% of the deaths occurred the first day of ICU admission, and 75% within the first week). The rates for ICU, 1-month, 6-month and 12 month mortality were 48%, 55% and 66%, respectively. With a median follow-up from the day of UCI admission of 18.5 months (IQR: 8-66), estimated OS at 1, 6 and 12 months were 51%, 44% and 41%, respectively (fig. 1a).Hematologic diagnosis (fig. 1b) status of the disease at the moment of ICU admission (fig. 1c) and the number of organ failure (≤2: 67% *vs* ≥3: 28%, *P*=.009) were the most powerful predictor variables associated with an increased OS.
Summary/Conclusions: OS of our HM patients is not worse than that recently reported from other specialist units.The decision for or against ICU admission of patients with HM should become dependent of the underlying malignant disease and especially with the status of the disease at the moment of admission. Almost 50% of survivors are still alive one year after ICU admission, suggesting that an important subgroup of HM patients benefit from ICU admission. In addition, only 25% of patients died beyond the 1st week of admission, not consuming resources without benefit. A multidisciplinary approach between intensivists and hematologists is essential in these patients.

E1447

THE IMPACT OF HAEMATOLOGICAL MALIGNANCY ON PATIENTS' HEALTH-RELATED QUALITY OF LIFE AND SYMPTOMS: A QUALITATIVE STUDY

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Background: The impact of haematological malignancies (HM) in patients' lives is not well understood. For some there is a possibility of cure, for others treatment aims at prolonging survival or containing symptoms and complications. We present interim results of a cross-sectional qualitative study in patients with HM.
Aims: The aim of this study was to investigate the impact of a wide range of haematological malignancies and their treatments on patients' health-related quality of life (HRQoL) and Symptoms.
Methods: Multicentre Ethics approval was obtained from the NRES South West Bristols, UK. Adult patients with HM as per 2008 WHO classification, capable of reading English and able to give the written informed consent were recruited from inpatient/outpatient clinics of two secondary care hospitals in England and Wales. This qualitative study employed semi-structured face-to-face interviews with open-ended questions related to HRQoL and symptoms. All the interviews were audio recorded and transcribed verbatim and content analysis was carried out using the NVivo 11 qualitative analysis software.

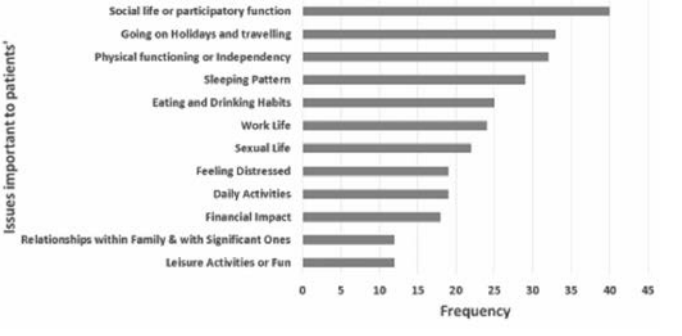

Figure 1. Prevalence of quality of life issues (n=55).

Results: Fifty-five patients (male=27; mean age=60 years; SD=15.2; median age 63 years; and age range =21-83 years) with mean duration of the HM of 3.8 years (SD=4.7; median=2.1 years; and range= 19 days-23 years) were recruited into the study. Diagnoses were Acute Myeloid Leukaemia (11), Acute Lymphoid Leukaemia (3), Chronic Myeloid Leukaemia (6), Chronic Lymphoid Leukaemia (4), Aggressive Non-Hodgkin's Lymphoma (3), Indolent Non-Hodgkin's Lymphoma (7), Hodgkin's Lymphoma (3), Multiple Myeloma (12), Myeloproliferative Neoplasm (3) and Myelodysplastic Syndrome (3). There were overall 44 categories of issues identified which were important from the patient perspective. These issues were then divided into two broad categories: QoL and symptoms. The QoL issues which had the highest impact on patients' lives (Figure1) were: 'social life and participatory function; going on holidays and travelling; physical functioning or independency; sleeping patterns; eating and drinking habits; work life; and sexual life' from high to low prevalence, respectively. Forty-four percent of the patients experienced impairment in different aspects of their work life with the prevalence of sixty-two percent (4 job role function; 2 made redundant; 3 relationship with co-workers; and 24 overall work life). Most of the patients who suffered from financial difficulties because of their HM were from 35-65 age group. With respect to disease related symptoms, 62 issues were identified, the most prevalent being 'tiredness (35%),

feeling unwell (24%), breathlessness (23%), lack of energy (16%), back pain (15%) and weight loss (13%)", from high to low prevalence respectively. Out of 78 treatment related symptoms identified, the most prevalent symptoms were 'tiredness (36%), feeling sick (17%), breathlessness (14%), loss of appetite (9%), neutropenia related symptoms such as infections & slowed healing (8%), nausea (8%) and lack of energy (8%)', from high to low prevalence, respectively.

Summary/Conclusions: The findings of this study clearly indicate that haematological patients' lives are greatly affected by their disease and treatment. This highlights the need for development of a new measure of HRQoL and symptoms, specific for the assessment of patients with HM to evaluate individual intervention strategies in daily clinical practice. This could lead to greater patient engagement in the process of clinical decision-making.

E1448

USE OF PRE-RECORDED AUDIO-VISUAL INFORMATION FOR PATIENTS DIAGNOSED WITH SIX COMMON MALIGNANT HAEMATOLOGICAL DISORDERS WITH AN AIM TO IMPROVE PATIENT EXPERIENCE AND FACILITATE BREAKING BAD NEWS

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Background: The diagnosis of a haematological malignancy can be overwhelming for patients. Shock and fright are common initial patient responses to bad news and may prevent patients from fully understanding important information given to them during the consultation. Use of printed information can be helpful but often these may be too detailed for patients to extract the key information from these in a timely manner. The plan for treatment requires shared decision making with the patient which in turn depends on patient understanding the key facts about the diagnosis and the treatment approaches. Although this key information is provided during consultation, the patient may have difficulty remembering these facts due to reasons noted above. This hurdle can potentially be overcome with the use of pre-recorded audio-visual patient information materials which can help patients to recall and also share key information with their loved ones. We report here on our innovative project to produce pre-recorded audio-visual information for patients about six common haematological malignancies.
Aims: We applied for and secured a grant from a charity for a two year project to develop and produce professionally recorded short films for patient information about six common haematological malignancies. The conditions chosen were: Chronic Lymphocytic Leukaemia, Chronic Myeloid Leukaemia, High grade Lymphoma, Low grade Lymphoma, Myeloma and Hodgkin's disease.
Methods: We started by consulting and seeking guidance from numerous patients with these conditions. We also spoke and took advice from experienced health professionals working with haemato-oncology patients. Based on the wisdom gathered we compiled the key evidence based facts about each of these diseases and their management. Details on specific treatment regimen were not included and the importance of building a partnership with the treating team was emphasized as the source of specific detail about planned therapy. These key evidence based facts were then converted into lay language which became the written scripts for each short film. A 10-15 minute short film was recorded for each of six diseases with the help of a professional team specialising in creating educational films. A clip about the role of specialist nurses was also recorded. We also included additional voice recordings of real patients talking about their experience with the same diagnosis / disease.
Results: The result of this two year project was a professionally produced DVD for each of the six diseases. The patient information films have been recorded in English language with possibility of translation /transliteration as sub-titles. As a proof of concepts each DVD short film has been translated to Polish language subtitles using this method. The copies of the DVDs can be produced at fairly low cost to be given out to patients. However, keeping with current trends, most patients are able to use web based information technology quite easily. In view of this we have expanded the project by developing a dedicated website (www.patientmedianetwork.com) to make these audio-visual materials available online to make it easy to access them without the need for DVD players etc. Also by making it freely available on the web, it shall serve patients beyond our geographic boundaries as anyone can access this patient information resource online now.
Summary/Conclusions: Pre-recorded, purposeful and specific audio-visual information can not only provide key facts about the diagnosis and its management but can also be used to create hope. It can be a resource to improve recall of important information as the patient and family can listen to it again and again after the initial visit to the clinic. And better understanding of their disease helps with patient participation in shared decision making which enhances their subsequent adaptation to the illness and its experience. Most importantly aim of this work is to reduces human suffering associated with diagnosis of these haematological malignancies and help optimize patient adjustment to the journey ahead. Our work also illustrates the importance of informatics and technology to improve patient-centeredness and we are keen to share our innovative work and ideas with the wider professional community.

E1449

SEXUAL DYSFUNCTION AMONG FEMALE LYMPHOMA PATIENTS IN MALAYSIA

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Background: Sexual dysfunction is a recognized long-term consequence among cancer patients who had received cytotoxic chemotherapy. Previous studies on female dysfunction were mainly on patients with breast cancer or gynecological malignancies. This is the first study of sexual dysfunction among lymphoma patients in this region.
Aims: To study the prevalence of sexual dysfunction among lymphoma patients and its association factors.
Methods: This is a cross sectional survey which was conducted among female lymphoma patients who attend haematology clinic at University Malaya Medical Centre, Kuala Lumpur, Malaysia. Patients age 18 years and above were recruited and patients with previous gynecological surgery, other previous malignancy and those who are newly diagnosed were excluded. Demographic data and medical history were obtained from patients and medical notes. Patients were interviewed using the validated Female Sexual Function Index (FSFI), European Organization for Research and Treatment of Cancer (EORTC QLQ C30) and Hospital Anxiety and Depression Scale (HADS). Presence of anxiety and/or depression symptoms were identified with cut off score of 8/21 for each subset. Data were analyzed using SPSS version 21.0. Total sum of FSFI scores were dichotomized into 2 categories; presence and absence of sexual dysfunction based on cut off value of 26.55. Associations between the demographic / clinical factors, HADS groups, and quality of life scores with sexual dysfunction were tested using Pearson Chi-Square or Fischer Exact Test where applicable.
Results: A total of 107 women aged 51.71±17.48 years (18-89) participated, of which 72% (n=77) were not sexually active for more than 4 weeks. The main reasons for non-sexually active were 1) no partner, 63.6% (n=49), followed by 2) problems suffered by partners, 24.7% (n=19) and 3) physical problem suffered by patients, 18.2% (n=14). Among those with partners, 70% (n=21) had sexual dysfunction despite being sexually active. Mean score of FSFI for patients with partner was 16.27 (± 10.28). All of these patients had lack of sexual desire. The sexual dysfunction was not significantly associated with demographic characteristics (age group, ethnicity, religion, education levels) or clinical characteristics (presence of comorbidity, remission status, treatment received, progress of treatment). However it was associated with menopausal state $X^2(1)=7.366$, p=0.007. Presence of anxiety (22.4%) and depression (8.2%) symptoms was not significantly associated with sexual dysfunction. There was no significant association between sexual dysfunction and quality of life.

Table 1.

Table 1. Association of clinical characteristics with sexual dysfunction (FSFI score < 26.55)

Clinical characteristics		Sexual dysfunction,N (%)	p value
Age group	< 56	22 (47.8)	0.301
	≥ 56	24 (52.2)	
Ethnicity	Malay	15 (32.6)	0.604
	Chinese	24 (52.2)	
	Indian	6 (13)	
	Others	1 (2.2)	
Religion	Muslim	15 (32.6)	0.18
	Christian	8 (17.4)	
	Buddhist	18 (39.1)	
	Hindu	5 (10.9)	
Education level	None/ Primary	12 (26.1)	0.243
	Secondary	25 (54.3)	
	College/ University	9 (19.6)	
Menopause status	No	10 (21.7)	0.007*
	Yes	36 (78.3)	
Comorbidity	None	20 (43.5)	0.506
	Yes	26 (56.5)	
Remission status	No	5 (10.9)	0.35
	Yes	41 (89.1)	
Treatment received	Treatment naive	0 (0)	0.742
	Chemotherapy/ immunotherapy	36 (78.3)	
	Chemotherapy + Radiotherapy	9 (19.6)	
	Transplant	1 (2.2)	
Treatment progress	Ongoing treatment	4 (8.7)	0.456
	Completed treatment	41 (89.1)	
	Pretreatment	1 (2.2)	
Current therapy	Nil	42 (91.3)	0.234
	Chemotherapy	4 (8.7)	
Duration from treatment completion	< 1 year	5 (12.2)	0.664
	1 - 5 year	18 (43.9)	
	≥ 5 year	18 (43.9)	
HADA ≥ 8	No	36 (78.3)	0.466
	Yes	10 (21.7)	
HADD ≥ 8	No	42 (91.3)	0.358
	Yes	4 (8.7)	

*p≤ 0.05 is statistically significant: HADA (Anxiety), HADD (Depression)

Summary/Conclusions: The prevalence of sexual dysfunction among lymphoma patients were higher than in general populations and unreported by most of our patients. Further study on association of sexual dysfunction and quality of life is needed. Partners' problem should not be neglected in assessment of female patients with sexual dysfunction.

E1450

FATIGUE, QUALITY OF LIFE AND PHYSICAL FITNESS IN PATIENTS WITH MYELOMA

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Background: Myeloma is a haematological malignancy characterised by periods of active, symptomatic disease that require intensive treatment, followed by periods of stable disease of 'plateau phase'. Complications include anaemia, hypercalcaemia, infections, renal failure, neuropathy and bone destruction which can lead to significant physical disability and impaired quality of life (QOL). Additionally, cancer-related fatigue is a common, chronic and often-debilitating side-effect of treatment. Combined, these factors could potentially lead to very low levels of physical fitness, further accelerating the decline in QOL of patients with myeloma. There are, however, limited data on objectively measured fitness in patients with myeloma, and it is unclear to what extent fitness is related to fatigue and QOL.
Aims: To evaluate levels of physical fitness, cancer-related fatigue and QOL in a myeloma survivorship population, and examine how these variables are related to one another.
Methods: Patients with myeloma were recruited to a survivorship lifestyle cohort study from hospital outpatient clinics. Participants were included if they had stable disease for at least 6 weeks, off treatment or on maintenance treatment, with ECOG 0-2, and were clinically able to carry out exercise testing. Fatigue was measured using the fatigue sub-scale of the Functional Assessment of Chronic Illness Therapy Fatigue Score (FACIT-F). A high score on the FACIT-F represents less fatigue, ranging from 0-52. QOL was assessed using the emotional well-being and functional well-being sub-scales of the Functional Assessment of Cancer Therapy (FACT-G) score. Higher scores in the FACT-G indicate better QOL, ranging between 0-52. VO_2^{peak} was measured during cardiopulmonary exercise testing (CPET) with a cycle ergometer.
Results: 51 patients in plateau phase were included in these analyses. Median age was 64 years (range 42-78), and 59% were male. 5 patients were older than 70. Median time from last treatment was 19 months (range3-139). Mean fatigue (FACIT-F) score was 40.94 (median 45, range 8-52), and 20 patients had a score below the mean reference range of 40.1.Mean quality of life (FACT-G) score was 40.58 (median 42, range 19.4-52), and 21 patients had a score below the mean reference value of 38.4. Mean VO_2^{peak} was 17.59ml/kg/min (SD ±4.94, range 10-28ml/kg/min). FACIT-F scores and VO_2^{peak} were positively correlated r=0.30 p<0.04. FACT-G scores and VO_2^{peak} were positive correlated r=0.31 p<0.03. FACIT-F and FACT-G scores were positively correlated r= 0.60 p<0.001.
Summary/Conclusions: Patients with higher levels of cardiopulmonary fitness scored higher on FACIT-F fatigue and FACT-G QOL measures. This indicates that patients with higher levels of fitness were less fatigued and had higher levels of emotional and functional well-being. A significant proportion of patients had scores below normative means for both fatigue and QOL. This analysis of baseline data in a post treatment myeloma survivorship group provides support for further investigation into the use of PA interventions delivered during and post cancer treatment to improve physical fitness and reduce fatigue so as to enhance QOL in this population.

E1451

PALLIATIVE CHEMOTHERAPY PEPC (PREDNISOLONE, ETOPOSIDE, PROCARBAZINE AND CYCLOPHOSPHAMIDE) IS EFFECTIVE AND TOLERABLE IN PATIENTS WITH RELAPSED/REFRACTORY LYMPHOMAS

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Background: Nowadays there are no universally accepted protocols and specific criteria for carrying out palliative chemotherapy in patients with lymphoproliferative diseases. The main problem of palliative chemotherapy is to choose an optimal treatment strategy for conducting effective therapy with minimization of adverse events. Therefore, there are a lot of needs to organize studies to improve palliative chemotherapy protocols in patients with lymphomas.
Aims: In the department of oncohematology the present trial was aimed to study the efficacy and toxicity of PEPC chemotherapy in research period from August 2011 to November 2015.
Methods: A total of 70 patients were included in study who received PEPC chemotherapy with palliation aim. Indications for palliative treatment were: relapsed or refractory Hodgkin or Non-Hodgkin lymphomas after 2nd and 3rd line treatment without efficacy and contraindications of further treatment, with the aim of nonradical treatment. Patients received therapy every day till leukocytes level <3x10 9/L: prednisone 20mg, cyclophosphamide 50mg, etoposide 50 mg, procarbazine 50 mg. When leukocytes level decreased below (<3 x 10 9/L) therapy was stopped and then renewed when leukocytes were recovered >3x10 9/L in every day regimen or every other day or fractional regimen (5 times a week, then 2 day break). The daily dose was always the same, perhaps it was only a change in the number of days in a week in which to take the drugs. Another goal was to investigate subjective improvement in general condition.
Results: A total of 70 patients were enrolled. Median age of patients was 46,85±4,3 years (from 19 to 74 years old), 38 man, 32 woman. There were included 25 patients with Hodgkin's lymphoma and 45-Non-Hodgkin lymphoma (large B-cell lymphoma-35 pts, MZL-35, CLL/SLL-2, T-lymphoblastic-3, peripheral T-cell lymphoma-1, anaplastic-1). Relapsed lymphoma was diagnosed in 37 patients. The number of relapses ranged from 1 to 5 (median 1,69±0.90). Refractory lymphoma was observed in 43 patients. As a second and third line treatment patients received DHAP, GVP, GEMOX, ICE, MINE chemotherapy. Prior to palliative course they received an average of 2.03+0.62 - treatment lines (from 1 to 5 salvage-regimens). Response rate to therapy was assessed in 46 patients. OR was achieved in 36,95% (n=17), CR - 6,52% (n=3), PR - 30,43% (n=14), SD - 39,13% (n=18). All patients had subjective improvement in general condition. PD on therapy with no apparent positive response was observed in 23.91% patients (n=11). Duration on PEPC therapy ranged from 1 to 15 months, average duration of therapy was 5,15±2,01 months (from 3 to 20 months). Nonhematologic toxicities (II / III grade CTC NCI v.4.02) are seen in 19 patients (27,14%). Infectious complications were observed in 3 patients (8,1%), febrile neutropenia (grade III-IV) - 27 (29,7%) and grade I-II - 12 (17,14%). Grade III anemia occurred in 5 patients (7,14%), grade II anemia-in 23 (32,85%). Thrombocytopenia (grade III) registered in 3 patients (4,28%) without hemorrhagic syndrome.Currently, 22 (31.4%) patients continue treatment. Because of disease progression 48 patients died.
Summary/Conclusions: PEPC chemotherapy is an effective treatment for extremely unfavorable group of patients with relapsed/refractory non-Hodgkin's lymphoma and Hodgkin's lymphoma and has an acceptable toxicity profile.

E1452

OUT-OF-POCKET ECONOMIC BURDEN AMONG COMMERCIALLY INSURED PATIENTS NEWLY DIAGNOSED WITH MULTIPLE MYELOMA IN THE U.S.

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Background: Patient (pt) outcomes have markedly improved with the introduction of novel agents for multiple myeloma (MM), but the economic burden of MM management in the U.S. is high. The increasing burden of out-of-pocket (OOP) costs on cancer pts is of concern especially with a shift in insurance coverage towards more cost-sharing. Annual OOP expenditures have been estimated as $5,054 for lung cancer (2015 US $, Romanus 2008) and $5,472 for solid tumors (Zafar 2013). Data on OOP costs in MM in the era of novel treatments are limited.
Aims: This retrospective cohort study estimated overall healthcare costs (OHC) of MM management and OOP burden among commercially insured U.S. pts newly diagnosed with MM (NDMM) in the U.S.
Methods: Pts<65 years of age with NDMM were identified, based on ICD-9-CM codes, in the MarketScan claims database from 1/1/2008 to 9/30/2013. Pts initiating MM treatments were followed from the date of initial MM treatment (index date). All pts had continuous medical and prescription (Rx) commercial insurance coverage 12 months (mos) before (baseline period) and at least 12 mos after (follow-up) the index date. Pts with claims for transplants or Medicare insurance were excluded. Pt socio-demographic characteristics were evaluated during the 12-mo baseline period. Cumulative OHC (sum of payments to the provider paid by the insurance, the patient and other payers) and OOP payments (sum of copayments, co-insurance and deductibles paid by pts) were determined at 1-, 2-, and 3-years post-index date among pts who had continuous commercial insurance coverage through each corresponding year.
Results: Among 628 commercially insured pts with NDMM, mean age was 56.2 years (SD:6.3); 54.6% were male, 32.8%, 33.4% and 33.8% had a Charlson Comorbidity Index (CCI) score of 0, 1-2, 3+, respectively; 57.2% were enrolled in a preferred provider organization (PPO) plan, 17.7% in a healthcare managed organization (HMO) plan; 38.2% resided in the South, 24.8% in the West, 22.5% in the North Central, and 13.9% in the Northeast regions of the U.S. Mean total healthcare payments per patient in year 1 were $202,593, with $76,595 for outpt medical services, excluding drug and drug administration, $42,681 for inpt services, $35,491 for outpt MM drug and drug administration services, $40,414 for MM drug prescriptions, and $930 for the emergency room (Table). Over time the relative proportions of OHC by service type were consistent. Over the 3 year study period, OOP payments ranged from 2.2% to 2.7% of the total healthcare payments (Table). Total cumulative OOP costs increased from $4,363 in year 1 to $9,909 over the first 3 years after the index date. MM drug related OOP (those administered in outpatient clinics or filled as an Rx) ranged from 1.6% in year 1 and 2 to 1.7% in year 3 of total MM drug treatment expenditures. The main contributor to OOP costs was related to outpatient medical services, $2,200, $3,704 and $5,099 through years 1, 2 and 3, respectively.

Table 1.

Table. Cumulative Payments over 1-, 2-, 3-year Follow-up Time Periods in NDMM.

Service Type	Mean Total Payment ($) (% of total payment)	Mean OOP Payment ($) (% of total OOP)	% OOP of Total Payment by Service Type
Within First Year (n=628)			
Outpatient Medical Services	76,595 (37.8)	2,200 (50.4)	2.9%
Inpatient	42,681 (21.1)	286 (6.6)	0.7%
Emergency Room	930 (0.5)	50 (1.1)	5.4%
Outpatient MM Drug and Administration Services[a]	35,491 (17.5)	622 (14.3)	1.8%
MM Drug Prescriptions[b]	40,414 (19.9)	581 (13.3)	1.4%
Non-MM Drug Prescriptions	6,483 (3.2)	624 (14.3)	9.6%
Total (Inpatient + Outpatient, including Drug Costs)	**202,593 (100)**	**4,363 (100)**	**2.2%**
Within First Two Years (n=310)			
Outpatient Medical Services	107,516 (37.3)	3,704 (49.7)	3.4%
Inpatient	45,391 (15.7)	570 (7.6)	1.3%
Emergency Room	2,246 (0.8)	96 (1.2)	4.3%
Outpatient MM Drug and Administration Services	39,164 (13.6)	731 (9.8)	1.9%
MM Drug Prescriptions	83,590 (29.0)	1,231 (16.5)	1.5%
Non-MM Drug Prescriptions	10,381 (3.6)	1,120 (15.0)	10.8%
Total (Inpatient + Outpatient, including Drug Costs)	**288,289 (100)**	**7,451 (100)**	**2.6%**
Within First Three Years (n=133)			
Outpatient Medical Services	131,736 (36.4)	5,099 (51.5)	3.9%
Inpatient	50,413 (13.9)	408 (4.1)	0.8%
Emergency Room	1,503 (0.4)	108 (1.1)	7.2%
Outpatient MM Drug and Administration Services	47,952 (13.2)	919 (9.3)	1.9%
MM Drug Prescriptions	116,271 (32.1)	1,808 (18.2)	1.6%
Non-MM Drug Prescriptions	14,053 (3.9)	1,567 (15.8)	11.2%
Total (Inpatient + Outpatient, including Drug Costs)	**361,928 (100)**	**9,909 (100)**	**2.7%**

[a]MM drug therapy administered in outpatient clinics; [b]MM drug therapy filled as a prescription in a pharmacy; MM drugs include: immunomodulators (lenalidomide, thalidomide, pomalidomide); proteasome inhibitors (bortezomib, carfilzomib); other: cyclophosphomide, melphalan, vincristine, (liposomal) doxorubicin, interferon, panobinostat, bendamustine, vorinostat

Summary/Conclusions: In this study based claims data from 2009 to 2013, the main cost driver of OHC and OOP among U.S. NDMM pts receiving treatment was accounted for by non-MM drug therapy related services. Mean MM drug therapy and drug administration related OOP costs were less than $100 per month. These results are based on commercial claims and do not reflect reimbursements from copayment assistance programs.

References
1. Romanus *et al. Value in Health* 2008. 11:A482;
2. Zafar *et al. The Oncologist* 2013. 18:381-90.

E1453

HOW PATIENTS PERCEIVE A SWITCH FROM IMATINIB TO NILOTINIB: RESULTS OF A PATIENT-CENTERED ANALYSIS

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Background: Despite impactful result achieved with imatinib, about 40% of patients experience treatment failure and more than 15% the persistence of long-term side effects that impaired quality of life.
Aims: To investigate how a change in TKI therapy has an impact on familiar, social and working setting of CML patients' daily life.
Methods: A structured questionnaire was administered to patients switched to nilotinib second-line after imatinib resistance or intolerance for not less than 6 months and not more than 36 months, regardless type of response. Twenty-five Italian centers participated collecting 142 patients.
Results: There were 61% males and 39% females, median age was 57 years (range 29-91). 65% with superior scholar degree. Median time from diagnosis for the overall cohort was 8 years, with 9% diagnosed less than 4 years, 66% from >4 to<10 years and 25% for ≥10 years. Sixty-one% of patients changed because of loss of efficacy with imatinib, whereas 39% for persistence of intolerance. Median time in imatinib treatment was 5 years, whereas median time with nilotinib after switch was 2 years. Overall, the level of satisfaction was requested to nilotinib and imatinib, respectively, and 93% of nilotinib-treated patients reported high level as compared to 52% of imatinib-treated patients. Dissecting this data, nilotinib-treated population reported in particular high satisfaction for achieved response (64%), for the level of daily quality of life (33%) and for tolerability (32%). Comparing imatinib and nilotinib, the majority of patients reported an impact of therapy on daily activities: 64% with imatinib reported low productivity at work (as compared to 33% with nilotinib) with 57% of them required absences from work (as compared to 35% with nilotinib). Analysing psychological perceptions, 64% of patients treated with imatinib reported the sensation of no longer feel themselves (as compared to 33% with nilotinib), and 36% to feel uncomfortable with their families (as compared to 18% with nilotinib). Overall, after switch, more patients referred a strong improvement of approach in daily living towards disease and therapy. More common emotions requested to patients to represent actual therapy with nilotinib were "trust" (65% *vs* 24% with imatinib), "joy" (52% *vs* 4% with imatinib) and "serenity" (29% *vs* 10% with imatinib); indeed, referred to previous therapy with imatinib, the more common emotions reported were "worry" (52% *vs* 22% with nilotinib), sadness" (30% *vs* 6% with nilotinib). "fear" (27% *vs* 15% with nilotinib), "frustration" (22% *vs* 4% with nilotinib). After switching to nilotinib, it has been asked perceptions about a possible discontinuation: 50% of patients would discontinue due to trust in personal physician, 26% to no longer feel sick and only 18% of patients would not stop for fear to losing all benefits achieved.
Summary/Conclusions: The results of this patient-centered analysis indicating high level of satisfaction in CML patients who switch to nilotinib after previous imatinib therapy, with improvements toward social, working and familiar daily life.

E1454

PSYCHOMETRIC PROPERTIES OF THE GREEK TRANQOL QUESTIONNAIRE FOR MEASURING QUALITY OF LIFE IN GREEK ADULT PATIENTS WITH THALASSEMIA MAJOR

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Background: Greece is a country with a large population of thalassemia patients. To date, there only a few studies measuring quality of life in Greek thalassemia major patients, with the available data mainly derived from generic QoL questionnaires. TranQoL is a novel disease-specific QoL instrument that has been specifically developed to assess QoL in thalassemia major patients.
Aims: This study aimed to evaluate the construct validity, reliability and responsiveness of the Greek translated version of the TranQoL questionnaire.
Methods:
The novel Greek TranQoL and the valid generic Greek SF-36v2 questionnaires were administered in 94 consecutive thalassemia major patients that were followed up at the Adult Thalassemia Unit of "Hippokration" general hospital of Thessaloniki, Greece, one of the largest thalassemia centres in Greece. Both questionnaires were completed anonymously during two subsequent transfusion visits, 2-3 weeks apart. A week after the transfusion visit the participants were asked to complete the TranQol at home, a third time, and describe any change in their quality of life using a 7-point Likert-type scale. Construct validity was assessed using Pearson's correlation between the TranQoL scores and the SF-36v2 scores at the first visit. In addition, the t-test or analyses of variance were used to compare TranQoL scores between patient groups that have known differences in quality of life. Assessment of reliability involved the determination of internal consistency by calculating the Cronbach's α-coefficient. Accordingly, the intra - class correlation coefficient (ICC) between the TranQol scores of the baseline visit and the subsequent visit was calculated in order to assess test-retest reliability. In order evaluate responsiveness, we used the paired t-test method to compare TranQoL scores between the transfusion visit and the home visit, in the group of patients who reported to be at least "a little better" after their last blood transfusion.
Results: There was a moderately strong correlation between the TranQoL summary and both the SF-36 Physical and Mental Component Summaries ($r=0.43$, $p<0.001$ and $r=0.520$, $p<0.001$ respectively). There was also a moderately strong correlation between the Physical Health scale of TranQoL and the relevant SF 36 scales, including Physical Functioning ($r=0.4$, $p<0.001$), role - physical ($r=0.6$, $p<0.001$) and bodily pain ($r=0.5$, $p<0.001$). In terms of reliability, TranQoL exhibited good internal consistency, with a Cronbach alpha coefficient reported of 0.89. Test-retest reliability was excellent (intra-class correlation coefficient, 0.9). Regarding know groups validity, patients with hypothyroidism had significantly lower TranQoL summary scores compared to patients without hypothyroidism (65%, range: 57-83 *vs* 75%, range: 42-98, $p<0.05$). There was also a significant difference in the score of the domain "school and career functioning" for oral (81±17%) *vs* subcutaneous iron chelation (70±29%). In terms of responsiveness, in the patients subgroup that rated their QoL as better in the Likert-type scale, there was a 4.2 point (SD 5.0) improvement in TranQol scores, from their transfusion visit (73.9%) to one week later at the home visit (78.1%), ($p<0.005$).

Summary/Conclusions: In conclusion, the psychometric properties of the Greek version of TranQoL confirmed that it is valid, reliable and responsive to change. The TranQoL can be incorporated into future studies of thalassemia major in Greek patients.

E1455

BIG DATA ANALYTICS & PATHOLOGY SERVICES IN NHS: ACHIEVING STEP CHANGES IN CAPACITY

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Background: A major hospital Trust in London provides healthcare for a large population across Medicine, Surgery, Obstetrics and other specialties. Three laboratories are on sites using automated, high capacity analysers to provide pathology services much of which requires rapid turnaround. The major challenge for laboratories in this situation is centred on the high throughput tests comprising full blood count, assessment of renal/ liver function, and coagulation. To date these services have not been subjected to detailed analysis of demand, efficiency of use of analyser capacity and efficiency of physician requesting.
Aims: First to determine the factors limiting the turnaround times for routine testing throughout periods of varying demand and those factors limiting the optimum use of existing analyser resources. Second, to identify requesting practices that are inefficient or which drive the changes in demand.
Methods: A database consisting 20M data points from 1M pathology test requests, recorded over 1 year was provided including details of requester and site of request with way points defining the passage of the sample from request time through to test reporting. This allowed a map of volume and activity over time and test turnaround times identifying bottlenecks and changes in efficiency. Parameters showing healthcare professional ordering of tests, location of patient and laboratory site were also provided and analysed.
Results: Initial descriptive analysis showed 1 particular laboratory had difficulties achieving its potential capacity utilization rate and target turnaround times. Bottlenecks were clearly identified at sample entry into the laboratory and at validation of results. These resulted in build-up of samples and delays in reporting that were staggered in time: thus although the peak in TAT occurs at 4pm the extra capacity was required prior to this, revealing a negative, delayed impact of test arrival on test turnaround time. This has obvious implications for laboratory management. The fastest 10% of FBC tests were reported in 0.2hr and 80% in 1.5 hrs but the slowest 10% varied from 2-120 hours. Of 200,000 tests processed per year at one lab, 47% were ordered by clinical division Medicine, 34% Surgery & Cancer / Clinical Haematology, 15% Women and Children. Medicine was the leading requester at all 3 sites. Of 2,000 physicians, 87% order fewer than 500 tests per year. Some intensive requesting practices are probably masked by the use of common requesting codes. 61% of patients have only 1 to 2 tests per year, the proportion of patients having 100 tests per year being less than 1%. However at one site >1% of patients had >50 tests per year, identifying an intensively monitored population. Measurement of true machine capacity highlighted a large gap between current performance and maximum potential indicating that better sample profiling and management could produce savings. The maximum capacity for FBC was 420 tests per hour but the average performance was 60/hr and the peak rate of reporting only 95/hr.
Summary/Conclusions: Big data analytics and process mapping identified 2 rate-limiting steps to performance inherent in current test system: test received at lab and validation of results. Close analysis of such a large dataset indicates how we can optimise the laboratory processes and improve utilisation of current installed capacity in NHS pathology services.

E1456

THE EFFECTIVENESS OF CLINICAL TRIAGE: CLOSING THE AUDIT LOOP

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Background: In 2014-2015 there were 85 million outpatient clinic attendances in NHS hospitals across the UK with a cost to secondary care exceeding £15 billion. 25.9 million (30 percent) of these were first appointments. In 2014 we audited a 6 month period of haematology outpatient clinic referrals and established the effectiveness of clinical triage in filtering inappropriate referrals. We established that 74% of all new patient referrals were from primary care in 2014. Of the rejected referrals 96% were from primary care.
Aims: In 2015, we implemented audit guided interventions, consisting of structured consultant led primary care teaching sessions across the North West London region. These sessions were specifically designed to educate and guide primary care physicians regarding haematological symptoms and blood parameters requiring secondary care input. The intervention was made with the aim of both capturing potential haematological malignancies at an early stage and also reducing inappropriate referrals. The proposed secondary outcome of this intervention was cost efficiency saving and improvement in regional haematology care.
Methods: Data was collected retrospectively from a local clinic referral database containing information on all new patient referrals including demographics, referral reason and triage outcome. We compared data from a four month period (June to September) in 2014 with the same four month period a year later in 2015.
Results: Our data shows a 31 percent increase in monthly referrals in 2015. The mean monthly referral number increased from 169 in 2014 to 244 in 2015. Despite this we saw a reduction in the percentage of inappropriate referrals to the service. In 2014 a monthly mean of 22 percent of referrals were rejected with feedback provided to the referring clinician. In 2015 the rejected referral rate was reduced by 6 percent to a monthly mean of just 16 percent for the same time of year period. 37% of referrals were for clotting/ thrombosis, 23% potential haematological malignancy including 2 week waits and the remaining 40% general haematology.
Summary/Conclusions: Our data support the ongoing use of consultant led clinical triage and primary care education in order to reduce the significant cost burden of new patient clinics whilst remaining safe for patient care.

E1457

USE OF COMBINED ORAL ADMINISTRATION OF ANALGESIA AND ANXIOLYSIS FOR PAIN ASSOCIATED WITH BONE MARROW ASPIRATION AND BIOPSY

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Background: Bone marrow aspiration and biopsy (BMB) is central to the diagnosis and management of many haematological disorders and is a safe procedure associated with low morbidity and mortality. For adults, the infiltration of local anesthesia at the biopsy site has been used as the principal form of analgesia for BMBA. Unfortunately pain relief is often incomplete especially during aspiration of the bone marrow. In addition, pain is likely to contribute to the anxiety the patient may already be experiencing.
Aims: In this study we assessed an oral administration of analgesia (fentanil-ACTIQ) and anxiolysis (midazolam). 107 consecutive ambulatory adult patients referred for bone marrow examination were enrolled. Informed consent for the procedure was obtained from all patients.
Methods: All patients received local anaesthesia (LA) with 10 mL of injected 2% lignocaine, but 52 patients received LA alone (group A) and 55 patients LA plus 5 mg midazolam (oral administration) and 200 mcg of Fentanyl transmucoso (group B), 30 min before the procedure. The pain level was assessed with the Numeric Rating Scale which distinguishes ten levels of pain, from 0 to 10 in five times of procedure (baseline, start LA T1, aspiration T2a, biopsy T2b, five minutes after the end of the procedure T3). At the end, all were given a questionnaire about efficacy, satisfaction, comfort with three levels (1/low-2/medium-3/high).
Results: This medium values were found: at time T1 the medium level of pain was 0.87 for the group A *vs* 0.88 of group B, at time T2a 3,63 group A *vs* 3,54 group B, at time T2b 4,63 group A *vs* 4 group B ($p<0,05$), time T3 0,41 group A *vs* 0,16 group B ($p<0.05$). In addition 21 Patients, who have already undergone the procedure without sedoanalgesia, saw to prefer the new medication.
Summary/Conclusions: Our preliminary results seem interesting because underline the different subjective perception of pain in the two groups and especially show a main level of satisfaction and comfortable in our patients undergone medication with sedoanalgesia and a lower level of anxiety in view of a possible repeat of examination.

E1458

THE IMPACT OF AL AMYLOIDOSIS ON ABSENTEEISM, REDUCED PRODUCTIVITY, AND JOB LOSS

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Background: Debilitating chronic conditions and their treatments often negatively impact patient's ability to work, resulting in absenteeism, reduced productivity, and job loss. Light-chain (AL) amyloidosis is a rare disease in which misfolded light chains are deposited in tissues, which may lead to organ failure, disability, and death. Current treatments are known to affect patients' functioning and well-being, but there is little evidence to date on the impact of AL amyloidosis on patients' ability to work.
Aims: To describe the impact of AL amyloidosis on patients' work using data from qualitative and quantitative research.
Methods: Data for these analyses were collected from two phases of a broad research program on the experience of patients with AL amyloidosis. First, qualitative in-depth individual telephone interviews were conducted with 10 patients. Results are presented from coded interview transcripts that were analyzed using a grounded theory approach to identify themes. Second, a quantitative online survey including a battery of patient-reported outcome measures

was conducted in a separate sample of patients (n=341). The data presented are based on the Work Productivity and Activity Impairment (WPAI) questionnaire for the subset of employed patients, including a single-item measure of the number of hours absent from work due to AL amyloidosis and a multi-item scale assessing overall lost productivity. Wilcoxon-Mann-Whitney tests were used to compare mean WPAI scores by time since diagnosis (<12 months ago *vs* ≥12 months ago) among those with cardiac involvement.
Results: In qualitative interviews, 7 of 10 patients reported that AL amyloidosis impacted their ability to work, manifesting as loss of focus or productivity, absenteeism including extended leaves of absence, and job loss. Patients reported underperforming at work and attributed this to symptoms, treatment side effects, and time required for doctor visits. Most felt their employers were supportive of their health needs; however, in some cases, job loss led to financial difficulties for families and frustration at subsequent changes in household roles. In the quantitative study, 115 patients (38.3%) were currently employed. Of these, the mean age was 56.1, 56% were female, and 10% were non-Caucasian. On average, employed patients reported being absent from work 5 hours per week and a 27.6% reduction in overall work productivity due to AL amyloidosis. Patients with cardiac involvement reported significantly higher absenteeism compared to those without cardiac involvement (mean hours absent per week: 15 *vs* 2.5 hours, respectively, $p<0.02$). Within the subgroup with cardiac involvement, overall lost work productivity for those diagnosed within the past year was twice that of patients with cardiac involvement who were diagnosed more than a year ago (54.4% *vs* 25.4%, respectively, $p<0.04$).
Summary/Conclusions: These results indicate that AL amyloidosis has a significant impact on patients' work, causing absenteeism, impaired productivity, and job loss. These results highlight additional costs of AL amyloidosis that are not related to medications or procedures, but are borne by patients, their employers, and their families. Advancements in treatment options for patients with AL amyloidosis and increased attention to patients' functioning and well-being could potentially minimize these hidden costs.
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E1459

HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH MULTIPLE MYELOMA IN RELATION TO LINE OF TREATMENT AND RESPONSE

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Background: Health-related quality of life (HRQoL) in patients with multiple myeloma (MM) has mostly been evaluated in controlled trials composed of largely homogeneous patient populations. There is a paucity of literature examining HRQoL in patients with MM at different treatment stages in a real-world setting.
Aims: To document and compare self-reported HRQoL outcomes in patients with MM across different lines of treatment and response status.
Methods: US patients with self-reported MM were recruited through an online patient-powered network, PatientsLikeMe. Upon completion of informed consent, study participants were asked to provide their MM treatment history and complete the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). HRQoL scores were compared using the Kruskal-Wallis test across the following subgroups: patients who had received stem cell transplant (SCT); patients who had not switched their drug regimens as a result of disease progression; patients who had 1-3 drug regimen switches because of disease progression; and patients who had ≥4 switches because of disease progression. HRQoL domain scores were also compared across subgroups based on response status reported to patients at their last provider visit (complete response, partial response/under control/stable, relapse/recurred or not responsive/progressive disease) irrespective of treatment lines.
Results: A total of 199 patients completed the online survey. Of these, 10 patients were excluded from this analysis because they had not received any MM treatment. Of the remaining 189 patients, the median age was 61 years (range, 37-82 years) and 57.6% were women. 58 patients reported receiving SCT, 66 patients reported not switching treatment regimens because of disease progression, 43 patients reported 1-3 switches because of disease progression, and 22 reported ≥4 switches because of disease progression. Global HRQoL domain scores varied from 58.3 to 68.7 ($P=.279$) as measured by the EORTC QLQ-C30. Comparable HRQoL in other domains were also reported across different treatment lines. Patients reporting their most recent response status as "complete response" had the highest Global HRQoL score (71.7) relative to patients reporting their most recent status as "partial response/under control or stable" (63.0) or "relapsed/recurred or not responsive/progressive disease" (55.6; $P=.012$). Similarly, statistically significant higher HRQoL scores were reported in the Physical Functioning, Social Functioning, and Pain domains in patients who experienced complete responses.
Summary/Conclusions: Domains of Global HRQoL, Physical Functioning, Social Functioning, and Pain in patients with MM treated in usual care settings were associated with their response status.

E1460

POMALIDOMIDE OR CARFILZOMIB USE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA: REAL-WORLD TREATMENT PATTERNS, TIME TO NEXT TREATMENT, AND ECONOMIC OUTCOMES

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Background: Pomalidomide (POM) is an immunomodulatory agent first approved in the United States in February 2013 in combination with dexamethasone (DEX). Carfilzomib (CAR) is a proteasome inhibitor first approved in the United States in July 2012. Both POM and CAR were approved for patients with relapsed multiple myeloma (MM). Real-world data on the use of these agents in the United States are limited.
Aims: The aim of this study was to fill in gaps in real-world data on treatment patterns and outcomes with POM or CAR in patients with relapsed MM.
Methods: Patients with ≥2 MM diagnoses (ICD-9 203.0x) and a POM or CAR claim (index date) from 2/1/2013 to 2/28/2015, assumed to be relapse therapy, were identified in the PharMetrics Plus™ US claims database. Treatment regimens were defined as all MM therapies observed within 60 days of index. Patients with both POM and CAR within 60 days of index were excluded. Time to next treatment (TTNT), a proxy for disease progression, was defined as the addition of a new agent >60 days from index or treatment restart following a >90-day gap in therapy. Plan-paid median monthly apportioned costs were reported for total follow-up time, and Wilcoxon rank-sum tests were used to compare the cohorts.
Results: A total of 454 patients indexed to POM (n=264) or CAR (n=190) were identified. The mean age was 61.6 years (SD=9.3 years), 60.1% were men, and these characteristics were similar between groups. Pre-index stem cell transplant occurred at any time in 34.5% of POM patients and 26.3% of CAR patients ($P=.064$). POM+DEX (47.0%) and POM alone (33.0%) were the most common indexed POM treatments; and CAR alone (45.3%) and CAR+DEX (14.7%) were common indexed CAR treatments. The most frequently observed next-line treatment for POM-indexed patients was addition of or switch to CAR±DEX (29.0%), and for CAR-indexed patients it was switch to POM+DEX (9.3%), and CAR alone (6.7%) or CAR+DEX+cyclophosphamide (6.7%). For patients followed-up to disease progression (POM, n=100; CAR, n=75) the mean TTNT was longer for POM-indexed patients compared with CAR-indexed patients (6.9 *vs* 5.3 months; $P=.0164$). With a mean follow-up time of 8.9 months across both groups, 22.0% were still on index treatment, 17.0% were on subsequent treatment (POM, 17.8%; CAR, 15.8%), 9.7% were not on treatment, 25.3% had died (POM, 25.0%; CAR, 25.8%), and 26.0% had lost eligibility prior to classification. Median monthly costs were lower for POM *vs* CAR patients ($18,298 *vs* $24,734; $P=.001$).
Summary/Conclusions: This retrospective study of POM or CAR treatment in patients with relapsed MM found a significantly longer TTNT with POM-based regimens *vs* CAR-based regimens. POM-based regimens were observed to have lower monthly costs through the end of follow-up compared with CAR-based regimens.

LB2265

INFLUENCE OF EXTENDED CARE ON LIVING QUALITY OF PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM TRANSPLANTATION

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Background: Patient satisfaction with health care has become a widely recognised patient reported outcome of the quality and clinical effectiveness of health services. Assessment of patient satisfaction is essential to ensure that care and services meet the needs of patients because the expectation of quality care can vary between patients and practitioners.
Aims: To evaluate the influence of extended care on living quality of patients after allogeneic hematopoietic stem transplantation.
Methods: A total of 80 patients after allogeneic hematopoietic stem transplantation were enrolled in this study. They were randomly divided into intervention group and control group according to hospital number ending(odd and even numbers).The intervention group cases were given the extended care after discharging from hospital, but no intervention for the control group,and the quality of life was evaluated by using cancer patients quality of life measure scale developed by European Organization for research and treatment of cancer(EORTC) in the two groups at 1 month and 3 months after discharged.
Results: At 1 month after discharge,the cognitive function score and emotion function score in intervention group were better than that of control group; at 3 months after discharge,the scores of physical function,role function,cognitive function,social function,general health status and fatigued,insomnia symptoms in intervention group were better than that of control group.
Summary/Conclusion: Extended care for patients after allogeneic hematopoietic stem transplantation can effectively improve the quality of life of them.

Red blood cells and iron - Biology

E1461

SAFETY IN PROCEDURE OF EVALUATION FOR PRESCRIPTION OF ENDURANCE EXERCISES IN PATIENTS WITH SICKLE CELL DISEASE: A PILOT STUDY

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Background: Physical activity is considered as potentially harmful in patients with sickle cell disease (SCD) and is therefore usually discouraged. Literature reports that during exercise, SCD patients may display gas exchange abnormalities, dyspnea, elevated pulmonary artery systolic pressure, left ventricular diastolic dysfunction, arterial hypoxemia. Vigorous exercise can also induce acidosis, hemorheological alterations, endothelial dysfunction and oxidative stress, which may trigger vaso-occlusive crisis in those patients. However, moderate and regular physical activity seems to be not only safe but also potentially beneficial for SCD patients (Barbeau et al. 2001, Faës et al. 2014, Martin et al. 2015).
Aims: The aim of the present study was i) to propose a submaximal incremental exercise test sufficiently intense to evaluate the physical ability of the patients but not intense enough to induce severe adverse events, and ii) to test the safety of moderate-intensity endurance exercise sessions individualized on basis of data obtained in i).
Methods: Twenty adult patients with homozygous SCD (12 men and 8 women, aged 21-53 years, 78.2±2.0% and 9.8±1.9% of HbS and HbF, respectively) participated in this study approved by the ethics committee (ClinicalTrials.gov NCT02571088). All patients underwent a submaximal incremental exercise (SIE). SIE started at 20 W and 30 W for women and men, respectively. After 2 minutes at this load, and every 2 minutes thereafter, workload increased by 10 W and 15 W for women and men, respectively. During exercise, twelve-lead EKG, heart rate (HR), pulmonary ventilation (VE), oxygen uptake (VO_2), systolic (SBP) and diastolic (DBP) blood pressure, peripheral capillary oxygen saturation (SpO_2), blood lactate concentrations ([Lac^-]) and rate of perceived exertion (RPE) were measured. Exercise was stopped (exercise completion) when a [Lac^-] of 4 mmol·L^{-1} was reached in order to limit acidosis. The first lactate threshold (LT1) was determined as the first inflection point of the [Lac^-] *vs* work rate curve. Besides, eight men and seven women of the group took part in three constant-load submaximal endurance exercise sessions. Each session lasted 40 min and consisted in a 5-min warm-up, 30 min of cycling at the workload corresponding to LT1, and 5 min of active recovery. HR, SpO_2 and [Lac^-] were checked regularly and values at the end of exercise were recorded. Work rate was adjusted between sessions to target a [Lac^-] of ~2.5 mmol·L^{-1}, which is usually used for endurance exercise in healthy sedentary and trained subjects. Descriptive statistics are expressed as mean±standard error (SE).

Table 1.

Table 1: data obtained during incremental and constant load exercises

	Men (n = 12)			Women (n = 8)		
	Rest	LT1	Completion	Rest	LT1	Completion
Mechanical data						
Work rate (W)	na	50±2.8	88.7±5.4	na	32.5±3.1	62.5±3.7
Pulmonary data						
VE (L·min^{-1})	14.6±1.4	31.4±1.0	51.7±2.8	13.1±1.3	25.4±1.6	46.7±3.3
VO_2 (L·min^{-1})	0.30±0.03	0.86±0.05	1.49±0.08	0.30±0.04	0.61±0.06	0.81±0.06
Cardiovascular data						
HR (min^{-1})	83.1±4.2	127±5.4	155±5	87±4	124±4	156±5
%$HR_{max-theor}$	45.3±2.0	69.3±2.6	84.6±2.2	47.1±1.8	67.2±2.4	84.8±2.4
SBP (mmHg)	123±4	144±5	175±4	113±6	125±7	157±5
DBP (mmHg)	74±3	83±2	89±3	72±4	79±3	86±3
SpO_2 (%)	97±0.6	95.8±0.8	95.3±0.8	97.3±0.6	97.1±0.4	96.1±1.1
Metabolic and perceptual data						
[Lac^-] (mmol·L^{-1})	1.7±0.2	2.3±0.2	4.4±0.1	1.6±0.2	1.9±0.1	4.2±0.2
RPE (0-10 scale)	na	1.8±0.7	5.3±1.0	na	2.4±0.8	6.8±0.9
	Men (n = 8)			Women (n = 7)		
	Session 1	Session 2	Session 3	Session 1	Session 2	Session 3
Constant load exercise						
Work rate (W)	50.2±5.7	55.6±4.9	52.9±4.8	37.6±3.0	38.6±3.0	37±4.2
HR (min^{-1})	120±4	126±4	121±6	130±6	132±8	125±5
SpO_2 (%)	94.6±1.1	95.9±0.8	95.6±0.6	96.6±0.7	96.9±0.7	97±0.8
[Lac^-] (mmol·L^{-1})	2.4±0.1	2.9±0.2	2.3±0.2	2.4±0.3	2.7±0.3	2.2±0.2
RPE (a.u.)	1.8±0.4	1.9±0.6	1.8±1	1.7±0.7	2.0±1.1	2.8±1.6

Data are means ± SEM. For symbols see text.

Results: Results are presented in table 1. Although submaximal, the incremental exercise was physiologically stressful since exercise completion intervened at ~85% of maximal theoretical heart rate ($HR_{max-theor}$). SIE allowed estimation of LT1 and related parameters (e.g., workload) for prescription of endurance exercise in SCD patients. HR, SpO_2, [Lac^-] and RPE observed during the constant load exercises were close to the expected values based on SIE. None of the studied patients experienced severe adverse events during or after SIE and endurance exercises.
Summary/Conclusions: We concluded that this procedure of SIE allowed evaluation of physical ability of SCD patients without inducing severe adverse events. Besides, SIE provided useful data (i.e., LT1 and related parameters) allowing prescription of apparently safe endurance exercises in patients with SCD. Armed of this experience, an individualized endurance training program might be proposed to patients with SCD.

E1462

ANXA2 GENE POLYMORPHISM (RS7170178) IS ASSOCIATED WITH OSTEONCROSIS DEVELOPMENT IN SICKLE CELL ANEMIA

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Background: The occurrence of vaso-occlusive crisis (VOC) and chronic hemolysis are clinical complications frequently observed in patients with sickle cell anemia (SCA). Nevertheless, these complications may vary in intensity and frequency within of the disease and several studies support the idea of a genetic component involved in the clinical modulation of these complications. With this in mind, two previously published genetic variants in *ANXA2* gene (*ANXA2**5681 G>A and IVS-14-1046 C>T) were associated with the development of specific clinical complications in SCA. Initially described as an important modulator of the bone mineralization and co-activator of the fibrinolysis process, the imbalance of the *ANXA2* expression may constitute a promising molecular marker for clinical heterogeneity in SCA.
Aims: Here, we evaluated the clinical impact of A*NXA2**5681 G>A and IVS-14-1046 C>T polymorphisms in patients with SCA, diagnosed and followed by a single reference center from northeast Brazil.
Methods: Seven hundred fourteen SS-genotyped patients were enrolled. The median age was 25 years (range: 3-61 years), with 347 males (49%). One hundred ninety four (27%) were younger than 18 years. The main clinical complications described in patients with SCA (osteonecrosis, stroke, acute chest syndrome, priapism, leg ulcer) were obtained from medical records. The last update occurred in January 2016. The A*NXA2**5681 G>A (rs7170178) and IVS-14-1046 C>T (rs7163836) polymorphisms were detected by real-time PCR. For test if both selected polymorphisms could influence the *ANXA2* gene expression, 70 patients with SCA were selected at random and the total RNA was isolated from peripheral blood. The *ANXA2* transcript levels (Hs_00743063_s1) were analyzed by real-time quantitative PCR quantified using the *GAPDH* as endogenous control.
Results: Overall, 144 patients (20%) developed leg ulcer, followed by priapism (97 patients, 14%), osteonecrosis (84 patients, 12%), acute chest syndrome (82 patients, 11%), and stroke (70 patients, 10%). One hundred and thirty-four patients (19%) had none of the main clinical complications aforementioned. According to *ANXA2**5681 genotype, 105/714 patients (15%) carried the GG genotype, while 285/714 (40%), and 314/714 (45%) presented the GA and AA genotypes, respectively. *ANXA2**5681 polymorphism was associated a higher frequency of osteonecrosis (P<0.0001). In fact, univariate analysis demonstrated that AA-genotyped patients had a 3-fold higher risk to develop osteonecrosis (OR: 3.3, 95%CI: 1.9-5.9; P<0.0001). These data were consistent with multivariate analysis (OR: 3.08, 95%CI: 1.6-5.8; P=0.001), considering fetal hemoglobin levels, number of VOC/year, sex, and age as confounders. In addition, the cumulative probability of osteonecrosis was significantly higher in AA-genotyped patients (88%) compared to non AA-genotyped patients (GG+GA patients, 43%; P=0.001). The AA genotype retained its prognostic value in multivariate proportional hazards regression analysis (HR: 2.4, 95%CI: 1.4-3.8; P<0.001). Finally, AA-genotyped patients with osteonecrosis had a lower *ANXA2* transcript levels compared to GG- and GA-genotyped patients (P<0.05). We could find no impact for the IVS-14-1046 polymorphism with none of the variable analyzed.
Summary/Conclusions: *ANXA2**5681 polymorphism in homozygosis may be an independently predictor of osteonecrosis and may impact the *ANXA2* transcript levels, with possible decrease of the encoded protein, in SCA.

E1463

ATP11C IS DOWNREGULATED IN LEUKOCYTES AND RETICULOCYTES IN SICKLE CELL ANEMIA

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Background: In general, the phosphatidylserine (PS) exposure represents the major cause of blood cells adhesion to the vascular endothelium, and may contribute for vaso-occlusive crisis and extravascular hemolysis in beta-hemoglobinopathies, including sickle cell anemia (SCA). Such process may be potentiated by the SS-hemoglobin polymerization and significantly worsened in the absence of physiologic mechanism involved in PS internalization. In this context, members of the P4-type ATPase family constitute an important class of enzymes responsible for phospholipids translocation in plasma membrane, and several evidence suggests that the low expression of the *ATP11C* gene (a specific member of the P4-type ATPase family) is associated with higher rate of PS exposure in peripheral blood cells. Corroborating these data, the evaluation of erythropoiesis in *ATP11C*-deficient mice showed an abnormal shape of erythrocytes, lower rate of PS translocation, and shortened life span of mature erythrocytes, with consequent anemia. Therefore, it is conceivable that lower *ATP11C* transcripts levels may act as an important genetic modifier, increasing the PS exposure and, consequently, enhancing the adhesion of leukocytes, platelets, erythrocytes and vascular endothelial cells in SCA.
Aims: Here, we evaluated the gene expression profile of the *ATP11C* in granulocytes, mononuclear cells and reticulocytes from patients with SCA, beta-thalassemia and healthy donors. We also correlated these findings with the main chronic clinical complications in SCA.
Methods: Sixty-four patients with SCA (median age: 31 years, range, 15-61 years), with 35 male (55%), and fully characterized for β^S-globin gene haplotype (bantu/bantu), and co-inheritance alpha-thalassemia (absence of -3.7kb deletion) were included. Total RNA from granulocytes, mononuclear cells, and reticulocytes was isolated. In addition, 14 patients with beta-thalassemia and 16 healthy donors (hemoglobin profile AA) were enrolled. *ATP11C* transcripts levels (Hs_00937051_m1) were analyzed by real-time quantitative PCR using the median value of *GAPDH* and 18S as endogenous control.
Results: First, we analyzed the *ATP11C* transcript levels in granulocytes, mononuclear cells and reticulocytes from patients with SCA. Reticulocytes presented lower levels of *ATP11C* transcripts ($P<0.05$). We next showed that the SCA reticulocytes had a lower *ATP11C* transcripts levels compared to beta-thalassemia and healthy donors reticulocytes ($P<0.05$). Of interest, *ATP11C* transcript levels were lower in both leukocytes (granulocytes and mononuclear) and reticulocytes from patients with SCA compared to healthy donors ($P=0.023$). According to the chronic clinical complications frequently reported in patients with SCA, 13 patients (20%) had leg ulcer solely, followed by cerebrovascular disease (7 patients; 11%). Twenty-one patients (33%) had more than one clinical complication (including osteonecrosis along with acute chest syndrome, and priapism), and 23 patients (36%) had no documented clinical complication. Patients who presented more than one clinical complication exhibited lower *ATP11C* transcripts levels ($P<0.05$).
Summary/Conclusions: Although limited number of patients and no validation data in an independent cohort constitute the main limitations of our study, our results showed that *ATP11C* transcript levels is decreased in SS-genotyped cells, in particular reticulocytes, and such decrease may be associated with higher rate of clinical complications in SCA.

E1464

A 6 NUCLEOTIDE IN FRAME DUPLICATION IN PIEZO1 GENE IN TWO FAMILIES WITH DEHYDRATED HEREDITARY STOMATOCYTOSIS

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Background: Dehydrated hereditary stomatocytosis (DHSt), also known as hereditary xerocytosis, is an autosomal dominant congenital hemolytic anemia associated with a monovalent cation leak. DHSt consists of an usually compensated hemolysis, associated with moderate splenomegaly. Blood smears show variable numbers of stomatocytes, elevated reticulocyte count and a slightly increased red cell mean corpuscular volume (MCV). DHSt red blood cells (RBCs) exhibit decreased intraerythrocytic K+ content and increased intraerythrocytic Na+ content. The causative gene of this condition was recently identified in the FAM38A gene encoding the mechanosensitive cation channel, PIEZO1 (Andolfo I., et al, 2013; Zarychanski R., et al, 2012). Several functional characterizations of identified PIEZO1 mutations in DHSt families have uniformly demonstrated gain-of-function properties consistent with the increased net ion fluxes leading to DHSt (Andolfo I., et al, 2013; Albuisson J, et al, 2013; Bae C., et al, 2013). Recently, a new causative gene of DHSt was identified in the KCNN4 gene encoding the Gardos channel (Andolfo I., et al, 2015; Raphael R-M, et al, 2015; Glogowska E., et al, 2015). The Gardos channel/KCNN4 is a widely expressed Ca2+-dependent K+ channel that mediates the major K+ conductance of erythrocytes (Begenisich T, et al, 2004).
Aims: In this study we performed osmotic gradient ektacytometry and DNA sequence analysis of PIEZO1/KCNN4 genes in seven patients with clinical suspicion of DHSt from two unrelated families (family 1 and family 2) to assess the clinical diagnosis.
Methods: Deformability of the RBCs of the patients and relative control subjects were evaluated by osmotic gradient ektacytometry using the Laser-assisted Optical Rotational Cell Analyzer (LORCA). Genomic mutational screening was performed by direct sequencing analysis, as previously described (Andolfo I., et al, 2013).
Results: Ektacytometric analyses showed a leftward shift of the bell-shaped curve for all the patients in respect to the healthy controls indicating dehydration of the RBCs. Subsequently, DNA analysis of the patients revealed no mutations in KCNN4 gene and the mutation c.7473_7478dupGGAGCT, p.Glu2492_Leu 2493dup in PIEZO1 gene that cosegregated with the disease phenotypes in both the families. Both Glu2492 and Leu2493 residues are highly conserved in PIEZO1 (http://genome.ucsc.edu/). Interestingly, the duplication is localized in the C-terminal domain of the protein that was recently identified as the pore of the mechanosensitive cation channel. Moreover, the proband of the family 1 showed another de novo PIEZO1 mutation c.5591G>A, p.Arg1864His in cis with the duplication p.Glu2492_Leu2493dup. Of note, the proband presents a more severe clinical phenotype when compared to that observed in his affected father.
Summary/Conclusions: Molecular characterization of seven DHSt patients from two unrelated families revealed a novel in frame duplication and a novel missense mutation in PIEZO1 gene. We are currently performing a functional study to evaluate the causative role of both novel mutations here described. Particularly, we are characterizing the possible modifier role of the de novo mutation, p.Arg1864His, in cis with the inherited duplication on the occurrence of severe phenotype observed in the proband of family 1.

E1465

NON DELETIONAL ALPHA THALASSAEMIA AND STRUCTURAL HAEMOGLOBINOPATHIES OF ALFA CHAIN MOLECULAR CHARACTERIZATION IN SPAIN IN A COHORT OF 1623 PATIENTS

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Background: Thalassaemias are a heterogeneous group of inherited anaemias that are characterized by the reduction or total absence of the synthesis of one or more globin chains. Structural haemoglobinopathies are inherited disorders in which the sequence of one of the globin chains, which form the haemoglobin, is altered.
Aims: To analyze the distribution of demographic variables of patients with α-thalassaemia and structural haemoglobinopathies in our region and to describe the molecular heterogeneity of both diseases and to examine the incidence of non-deletional α-thalassaemia within all cases of α-thalassaemia.
Methods: Studied Subjects: From January 2009 to December 2014, 1,623 individuals were studied from different Spanish regions (both native and immigrant populations). It was an study comprising 1,470 patients with hypochromic and microcytic anaemia, 176 subjects with a peak of abnormal haemoglobin and 23 patients who were studied for both diagnosis. Diagnostic techniques: α-thalassaemia required a conventional haemocytometer study, the reticulocyte count, the determination of Hb A_2 and Hb F by ion exchange HPLC and quantification of the Hb H inclusion bodies. The diagnosis of structural haemoglobinopathies required capillary electrophoresis of haemoglobins and reversed phase or globin chain HPLC. Molecular biology techniques: automatic extraction of the DNA, discarding the most frequent large deletions and point mutations using the α-globin StripAssay®, discarding other large deletions by MLPA, and Sanger sequencing for other point mutations.

Table 1.

Age (Years)	N	Ethnicity	N	Autonomous community	N
0-10	317	Caucasian	642	Madrid	806
10-20	177	Asian	61	Castilla La Mancha	240
20-30	150	Sub-Saharan African	44	Castilla y León	154
30-40	201	Maghrebi	40	Andalusia	128
40-50	155	Latin American	22	Basque Country	91
50-60	104	Hindu	10	Cantabria	71
60-70	79	Gipsy	8	Navarre	37
70-80	57	Jewish	1	Canary Islands	35
80-90	18	Unknown	795	Extremadura	26
90-100	3	Total	1623	Balearic Islands	8
Unknown	362			Murcia	8
Total	1623			Catalonia	7
				Valencia	6
				Asturias	6
				Total	1623

Results: The demographics results are summarised in table 1 (Table 1: Demographics data including age, ethnicity and Autonomous community of residence of the patients). Gender balance was found in this study. Clinical diagnosis: 1

hydrops fetalis, 18 Hb H disease, 1,200 thalassaemias traits and 160 thalassaemia silent carriers were recorded within the α-thalassaemia clinical diagnostics. Regarding structural haemoglobinopathies, there were only 2 cases of haemoglobinopathies with low oxygen affinity and 1 case of haemoglobin M, which represented the only symptomatic patients. Genetic diagnosis: 1,298 carriers of the α-thalassaemia deletion were identified (905 heterozygous, 366 homozygous and 27 double heterozygous). A total of 188 patients with non-deletional α-thalassaemia were found (185 heterozygous and 3 homozygous). A total of 176 patients had structural haemoglobinopathies of the α chain (173 heterozygous and 3 homozygous). Finally, it is important to note that this study described 9 and 5 new alterations responsible for non-deletional α-thalassaemia and structural haemoglobinopathies of the α chain, respectively.
Summary/Conclusions: 1)Most individuals were diagnosed with α-thalassaemia at an early age, particularly in severe cases. Further, the disease occurred predominantly in Caucasians and, to a lesser extent, in Asians. 2)Non-deletional α-thalassaemia represented 12% of all α-thalassaemias in our region, representing a higher value than described to date. 3)The most common deletion in our region was the 3.7Kb deletion that is common in Mediterranean populations, followed by Asian -SEA and -FIL. The alterations responsible for non-deletional α-thalassaemia are most represented by the Hph and Hb Groene Hart and, in the case of structural haemoglobinopathies, Hb Le Lamentin and Hb J-Paris. However, there is a high heterogeneity in the population analysed with the discovery of 36 and 35 different alterations responsible for non-deletional α-thalassaemia and structural haemoglobinopathies of the α chain, respectively.

E1466

OXIDATIVE STRESS IN COMPLEMENT-MEDIATED HEMOLYSIS: THE AMELIORATING EFFECT OF FERMENTED PAPAYA PREPARATION

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Background: The complement (C') and the redox systems play important roles in the physiological functioning of the body, such as the defense system, but they are also involved in various pathological conditions, including hemolytic anemia. Antioxidants ameliorate oxidative stress by preventing generation of free radicals, by scavenging and preventing their accumulation and by correcting their cellular damage.
Aims: Using experimental model systems, we studied the interaction between the C' and the redox systems in C'-mediated hemolysis in Paroxysmal Nocturnal Hemoglobinuria (PNH) and in Auto-Immune Hemolytic Anemia (AIHA), and the ameliorating effect of fermented papaya preparation (FPP), an antioxidant-containing product of yeast fermentation of Carica papaya Linn.
Methods: PNH-like cells were generated by treating normal human red blood cells (RBC) with the sulphydryl compound 2-aminoethyl isothiouronium bromide. AIHA was simulated by treating group-A RBC with anti-blood group A antibodies. Both treated RBC were incubated with fresh (C'-containing) autologous or O-type serum. Reactive oxygen species (ROS), as a parameter of oxidative stress, and the surface C'-protecting CD55 and CD59 antigens were measured by flow cytometry.
Results: The sulphydryl-treated RBC demonstrated a PNH-like phenotype manifested by reduced surface expression of CD55 and CD59. In both systems, treated RBC underwent hemolysis about 40 min following interaction with activated C'. Hemolysis was preceded by an abrupt increase in ROS generation. Inactivation of the C', either by heat (56ºC for 30 min) or by the addition of an anti-C' antibody, effectively reduced the ROS generation and hemolysis, indicating both are mediated by active C'. Addition of FPP (20 mg/ml) during the exposure to activated C' significantly reduced ROS generation and hemolysis.
Summary/Conclusions: The present results suggest that oxidative stress, in conjunction with activated C', may be involved the underlying symptoms of PNH and AIHA such as intra- and extra-vascular hemolysis. Currently, a humanized monoclonal antibody that specifically targets the C' protein C5 is the main treatment modality for PNH and some other hemolytic anemias. The present results raise the possibility that treatment with antioxidants might be considered as a potential therapeutic modality for C'-mediated hemolytic anemias. Since FPP is well tolerated and relatively inexpensive compared to the humanized anti-C' monoclonal antibodies, its use may be considered as an alternative or an adjuvant therapeutic modality in PNH and other C'-mediated hemolytic anemias.

E1467

INEFFECTIVE ERYTHROPOESIS DUE TO AN ALPHA SPECTRIN GENE DEFECT

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Background: Inherited red cell membrane disorders result from genetic changes within membrane and cytoskeletal proteins. When altered these proteins affect the deformability of the red cell and the stability of the membrane, which affects red cell function and results in a reduced red cell life span. Hereditary spherocytosis and elliptocytosis are relatively common with 1 in 2000 North Europeans being affected. Specific alpha spectrin gene variants, such as the low expression allele (LELY) have carrier frequencies of 20%, contributing to the high frequency that hereditary elliptocytosis is seen. The severity of the haemolytic anaemia is variable with some individuals unaware they are affected while others are lethal requiring in-utero transfusion for survival. We have identified two cases of particular interest. Both have a have a thalassaemia like syndrome with marked ineffective erythropoiesis due to mutations in the SPTA1 gene, coding for alpha spectrin. Case 1 presented with neonatal anaemia and liver failure, and is transfusion dependent. Case two presented with fetal anaemia and was treated with intrauterine transfusions; neonatally case two presented with hepatitis, anaemia and relative reticulocytopenia, and is also transfusion dependent.
Aims: To identify pathogenic variants in red cell membrane genes which account for ineffective erythropoiesis observed in the two patients.
Methods: Case 1 used whole exome sequencing of a nuclear family to identify the causative mutation while targeted sequencing using a focused red cell gene panel identified the pathogenic variant in the second case. Bespoke bioinformatics was used to analyse the data and call the variants.
Results: Case 1: Identified to be homozygous for the c. 7132C>T; p.Gln2378 * pathogenic variant in SPTA1 gene. Both parents were carriers and unaffected which is consistent with a recessive inheritance model. Case2. Identified to be homozygous for the c.2659C>T; p.Arg887* pathogenic variant in the SPTA1 gene. The individual was also homozygous for the SPTA1 c.[5572C>G; 6531-12C>T]; p.Leu1858Val low expression allele (Alpha Spectrin LELY). The parents were carriers of the pathogenic variant and also homozygous for the low expression allele. They did not have a clinically significant condition.
Summary/Conclusions: In both cases the affected individual was homozygous for a truncating mutation within SPTA1. The individual in case 1 was also homozygous for alpha spectin LELY and this may have contributed to the more severe phenotype, requiring intrauterine transfusion. We suggest, due to this novel mutation the red cells are too fragile to be released from the marrow, resulting in the phenotype of ineffective erythropoiesis (low reticulocytes) rather than haemolytic anemia, which is typically associated with alpha spectrin mutations.

E1468

IRON DEFICIENCY PREVALENCE AND RISK FACTORS IN CHILDREN YOUNGER THAN 6 YEARS-OLD IN FRANCE: A POPULATION-BASED STUDY

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Background: Iron deficiency (ID) is considered as the most frequent micronutrient deficiency in the world including in well-resourced countries. Iron depletion, the first stage of ID, corresponds to a reduction of body iron stores and is usually diagnosed by serum ferritin (SF) measurement. ID in early years is associated with impaired neurodevelopment both at the short and long terms. Several strategies have been proposed to prevent and/or to screen ID in children. The effectiveness of these strategies can be evaluated by the prevalence of ID. Estimated prevalences of ID in Europe are highly variable, probably because population-based studies are lacking.
Aims: Our objectives were to estimate ID prevalence and to assess ID risk factors among French children younger than 6 years-old in a population-based study.
Methods: We conducted a secondary analysis on the biobank constituted during the national survey "SaturnInf", carried out by the InVS-French Institute for Public Health Surveillance [Etchevers, 2014]. The Saturninf study recruited 3831 French children younger than 6 years-old between 2008 and 2009 according to a two stages probability sample design. For the present ancillary study, we measured SF (electro-chemiluminescence immunoassay) and CRP (immunoturbidimetric assay) on the sera kept frozen at -80°C for children with

the following criteria: parents' written consent, sera aliquot in sufficient quantity, lack of inflammatory syndrome (CRP<10 mg/l), and available socio-demographic data. ID was defined by a SF level<10 or<12 µg/l. Uni- and multivariate analyses were performed to identify ID risk factors and correlations with SF.
Results: The 657 included children (17% of the children included in the Saturninf study) had an average age of 3.1 years (SD 1.6), 58% were boys, 12% had a mother born outside France, and 22% a family with a healthcare coverage type linked to poverty ("CMU"). SF median was 45 µg/l (IQR: 29-75). ID prevalence was 3% (95%CI: 2-5) and 4% (95%CI: 3-6) for a SF threshold of 10 and 12 µg/l, respectively. At a 10 µg/l threshold, ID prevalence was higher (p<0.05) when the mother was born outside France (8%) and when the healthcare expenses were covered by CMU (7%). Significantly (p<0.05) lower SF levels were observed among males (median 42 µg/l *vs* 52), when the mother was born outside France (34 *vs* 46), was unemployed (38 *vs* 46) or had a low educational level (38 *vs* 47), when the father had a low educational level (38 *vs* 48) and when the healthcare expenses of the family were covered by CMU (39 *vs* 47), but not (p>0.4) among youngest children (47 before 2 years-old, 41 between 2 and 3, 46 after 3 years-old) nor when the father was unemployed (39 *vs* 47). After adjustment, a lower SF level was significantly (p<0.01) associated with a male gender and a lower mother's educational level.
Summary/Conclusions: In this first population-based study in France, ID prevalence was much lower (3 to 4% depending on SF threshold) than prevalences retrieved in other French and European studies performed in precarious populations but close to the lower values observed in other population-based studies in Europe. Male gender and low mother's educational level were independent risk factors for ID, as in other studies. Further studies are needed to explore the relation between this low ID prevalence and the national French preventive strategy of ID in children that is mainly based on the recommendation of a universal use of fortified cow's milk formula between 1 and 3 years of age.

E1469

IGG MEDIATED OPSONIZATION MECHANISM ACCORDING TO THE UNDERLYING PROTEIN DEFICIENCY IN HEREDITARY SPHEROCYTOSIS: TESTING A HYPOTHESIS

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Background: Hereditary Spherocytosis (HS) is the most common non-immune congenital hemolytic anemia in individuals of northern European ancestry, ranging from an asymptomatic condition to a severe life-threatening anemia which results from an erythrocyte membrane protein defect. Splenectomy corrects anemia, though the intrinsic erythrocyte membrane defect persists. Reliene *et al*. (1) proposed that the type of protein deficiency underlying HS determines erythrocyte *in vivo* survival since, after splenectomy, the opsonization mechanism mediated by IgG (which involves the binding of natural occurring antibodies to erythrocyte membrane band 3) is distinct for spectrin/ankyrin- and band 3-deficient HS.
Aims: Our study aimed to evaluate the levels of membrane bound IgG and band 3 aggregates, according to the protein deficiency underlying HS, in order to ascertain how our HS population fitted this hypothesis.
Methods: We studied 35 healthy individuals and 125 HS patients [(82 unsplenectomized (unspl) and 43 splenectomized (spl)] previously studied for membrane protein deficiency identification (2). Membrane bound IgG and the high molecular weight aggregates (HMWAg) of band 3 (as percentage of total band 3) were determined by western-blot.
Results: We analyzed our data according to spectrin, ankyrin- and band 3-deficient HS (Figure 1).

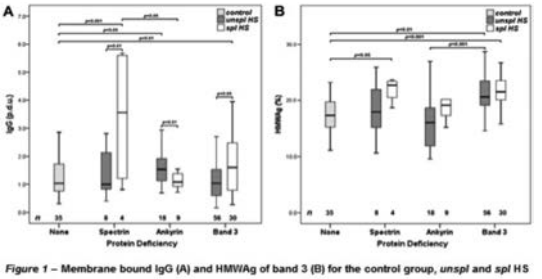

Figure 1 – Membrane bound IgG (A) and HMWAg of band 3 (B) for the control group, *unspl* and *spl* HS patients according to erythrocyte membrane protein deficiency underlying HS. Data presented as median (inter-quartile range). The differences between groups were evaluated using the Mann-Whitney *U* test. The co-variance adjustment test (univariate ANCOVA) was used as these variables were affected by the subject's age. A *p*<0.05 value was considered as statistically significant.

Figure 1.

Summary/Conclusions: For spl band 3-deficient HS, our results were in agreement with the work from Reliene *et al*. (1), since IgG levels were increased, as well as the amount of band 3 aggregates (to which IgG binds), in comparison with either the control or ankyrin deficiency groups, confirming that in these individuals membrane loss through vesiculation does not include band 3 loss, leaving these cells susceptible to IgG mediated opsonization. Since they found that the amount of IgG was not increased in relation to controls, these same authors (1) proposed that for spl spectrin- and ankyrin-deficient HS, the vesiculation of the membrane leads to the loss of band 3 clusters and, consequently, less opsonization occurred, thus explaining the higher deformability of these patients' cells. Our results were slightly different because we analyzed spectrin- and ankyrin-deficient HS separately. For spl ankyrin-deficient HS, our findings corroborate those from Reliene *et al*. (1), as we found lower membrane bound IgG and HMWAg than in band 3 deficiency, and no difference to the control group, meaning that band 3 was lost through vesiculation (hypothesis that is strengthened by HMWAg in unspl ankyrin-deficient being lower than in band 3-deficient HS). However, for spectrin-deficient HS, our results were comparable to those found for band 3 deficiency. This might imply that in spectrin-deficient HS the membrane structural modifications are such that membrane loss does not include band 3, leaving these patients' cells prone to increased IgG linkage. As cell deformability has not been evaluated, we cannot say if the mechanism of opsonization in spl spectrin-deficient HS is comparable to band 3-deficient or to ankyrin-deficient HS, which is an issue that requires further studies.
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Reference
1. Reliene *et al*. Blood 2002,100(6):2208-2215;
2. Rocha *et al*. Br J Haematol 2010, 150(6):679-684.

E1470

GROWTH DIFFERENTIATION FACTOR-15 IS ELEVATED IN PATIENTS WITH COMPOUND HETEROZYGOUS SICKLE CELL AND BETA-THALASSEMIA AND CORRELATES WITH HEMOLYSIS, ENDOTHELIAL DYSFUNCTION AND ANGIOGENESIS

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Background: The clinical manifestations of Sickle Cell Disease (SCD) include episodes of vascular occlusion, chronic hemolytic anemia and frequent infections. SCD is also characterized by the presence of a chronic inflammatory state manifested by leukocytosis and monocytosis and increased circulating levels of pro-inflammatory cyto- and chemokines. Growth Differentiation Factor-15 (GDF-15), also known as macrophage inhibitory cytokine 1 (MIC-1) and non-steroidal anti-inflammatory drug-activated gene (NAG-1) is a member of the transforming growth factor-□ superfamily. Expression of the GDF-15 gene in cardiomyocytes, vascular smooth muscle cells, and endothelial cells is strongly upregulated in response to oxidative stress, inflammation and tissue injury. Increased GDF-15 concentrations have been associated with an adverse prognosis in patients with acute coronary syndromes and chronic heart failure.
Aims: The aim of this study was to evaluate the GDF-15 levels in patients with compound heterozygous SCD and beta-thalassemia (HbS/β^{thal}) and to explore possible associations with disease features, such as hemolysis, inflammation, endothelial dysfunction and angiogenesis.
Methods: Seventy-five adult Caucasian patients with HbS/b^{thal} were included in the study, while 20 healthy individuals served as controls. Patients with HbS/β^{thal} divided in two groups: group A included 36 patients under hydroxycarbamide (HC+) treatment and group B included 39 patients without hydroxycarbamide (HC-) treatment. Along with hematology and blood chemistry parameters determination, measurements of circulating levels of GDF-15, hs-CRP, vWF-antigen, hs-TnT and Placental Growth Factor (PlGF) were measured in patients with HbS/β^{thal} and controls using immunoenzymatic techniques.
Results: The main results of the study showed that: GDF-15 were elevated in patients with HbS/β^{thal} compared to controls (1,980.7±159.8 *vs* 665.4±50.9 pg/mL, p<0.0001). Regarding hydroxycarbamide treatment, GDF-15 levels were elevated in (HC+) patients compared to (HC-) patients (2,478±222.6 *vs* 1,520±204.7 pg/mL, p=0.002), or 30/36 *vs* 21/39 patients with elevated GDF-15 levels (c^2=0.002). GDF-15 levels correlated significantly with markers of erythropoiesis, such as Hb, HbF, ferritin and reticulocytes (r=-0.424, p<0.01; r=0.562, p<0.001; r=0.423, p<0.001 and r=0.510, p<0.001, respectively), with markers of hemolysis, such as LDH and uric acid (r=0.412, p<0.001 and

r=0.321, p=0.005, respectively), and with markers of endothelial dysfunction and angiogenesis such as vWF-antigen and PlGF (r=0.238, p<0.05 and r=0.461, p<0.001, respectively). Surprisingly, no correlation was found between GDF-15 and hs-CRP levels (p>0.65).
Summary/Conclusions: These findings suggest a multifactorial role of GDF-15 in patients with HbS/β^{thal} as it correlates with hemolysis, endothelial dysfunction and angiogenesis. The higher GDF-15 levels measured in patients treated with hydroxycarbamide may reflect a possible drug reaction. More studies are needed to clarify the exact role of GDF-15 in HbS/β^{thal}.

E1471

MOLECULAR CHARACTERISATION OF 85 INDIVIDUALS WITH PYRUVATE KINASE DEFICIENCY
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Background: Pyruvate kinase (PK) deficiency is the most common glycolytic cause of non-spherocytic haemolytic anaemia. It is inherited as an autosomal recessive condition with 50 individuals per million being affected in the Caucasian population. The *PKLR* gene codes for Pyruvate Kinase, a red cell specific isoenzyme that converts phosphoenolpyruvate to pyruvate which releases ATP. ATP is essential for red cell function and without it the half-life of the red cell is reduced, being cleared by the spleen and liver. There are 200 known mutations which can cause PK deficiency, which is diagnosed using an enzyme assay. KLF1 is a transcription factor known to bind to the promoter of the *PKLR* gene and drives expression. Variants in the *KLF1* gene have previously been shown to produce a PK deficient phenotype.
Aims: We screened for genetic variants in individuals with PK enzyme activity<11 IU/g Hb to determine if a genetic cause could be identified in every case.
Methods: Individuals with reduced PK enzyme activity underwent *PKLR* gene sequencing. Cases without an affected genotype were screened for indels in the *PKLR* gene followed by sequencing of the *KLF1* gene.
Results: Of the 85 cases, 21 were identified with genotypes consistent with PK deficiency with 27 cases left having identified only a single pathogenic variant. 35 Cases were left without a pathogenic variant (although 38% of samples were heterozygous for the c.-248delT polymorphism), all falling within the range of 8-11 IU/g Hb. This is consistent with other labs that use lower normal range. The sequencing analysis identified ten variants not previously described (c.319 A>G, c.508-57 G>A, c.508-2 A>G, c.602 G>T, c.627 G>C, c.822 C>G, c.959 T>C, c.965+2 T>C, c.1063 A>G, c.1299 C>A) of which seven are likely to be highly pathogenic. One individual had a deletion encompassing exon 11 in combination with a missense variant. One unknown variant (c.881 C>A) was identified in the *KLF1* gene and in silico tools predicted this to be damaging. There were no *PKLR* variants found in this patient, yet they had a PK activity of 3.5 IU/g Hb.
Summary/Conclusions: Genetic investigations into the PKLR gene is not diagnostically informative in individuals with a PK enzyme activity of >8 IU/g Hb. Of the 39 individuals with an enzyme activity of<8 IU/g Hb, 46% had two affected alleles, 44% had one affected allele, and in 10% of the cases no pathogenic variants were identified. The enzyme activity range of the 10% group (n=3) was 7-7.9 IU/g Hb excluding the individual with a variant in *KLF1*. This indicates that the more severe the PK enzyme deficiency the more likely the assay is to identify a genetic cause. In those cases with a genetic cause most are non-synonymous base changes probably resulting in protein instability. Two splice site variants were identified affecting the core consensus motif and also a single case with a deletion, all predicted to be severe alleles. It is unknown if the KLF1 gene variant is influencing PKLR gene expression, and further work is necessary to understand this.

E1472

STRUCTURAL HAEMOGLOBINOPATHY CARRIERS CAN BE IDENTIFIED BY LABORATORY PARAMETERS PROVIDED BY ADVIA 2120 ANALYSER
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Background: Hemoglobinopathies have spread owing to human migrations, and the number of people needing diagnosis and management of these conditions is increasing. More than 1500 variant Hb have been described to date. In more than 95% of variant Hb, the structural alteration is a point mutation that produces a single amino acid substitution in a globin chain. Carriers are asymptomatic, and clinicians need to accurately identify them and provide adequate genetic counselling to them in order to prevent the occurrence of homozygous or compound heterozygous offspring.
Aims: The aim of our study was to identify red blood cell (RBC) laboratory parameters that discriminate between structural hemoglobinopathy carriers and healthy subjects
Methods: Samples of 500 variant Hb carriers (355 HbAS, 104 HbAC, 19 HbAD, 7 HbAE, 7 HbAO-Arab, 4 alpha-chain variants and 4 Hb Lepore) and 251 normal controls were run on an Advia 2120 analyser (Siemens Medical Solutions Diagnostics, Tarrytown, New York, USA). Hb variants were detected by HPLC in the HA-8160 analyzer (Menarini Diagnostics, Florence, Italy) and further characterised according to their alkaline and acid electrophoretic pattern using cellulose acetate electrophoresis. Classic hematological parameters and RBC populations of the complete blood count (CBC) were assessed in all subjects. A multivariable binary logistic regression model was created to predict the probability of a subject carrying any structural hemoglobinopathy.
Results: Variant Hb carriers presented significantly higher RBC count (4.96×1012/L *vs* 4.84×1012/L, p=0.031), lower MCV (85.39 fL *vs* 92.81 fL, p<0.001), lower MCH (28.97 pg *vs* 30.18 pg, p<0.001), higher MCHC (34.29 g/dL *vs* 32.71 g/dL, p<0.001), higher RDW (14.10% *vs* 12.94%, p<0.001), lower%MACRO (0.67% *vs* 1.63%, p<0.001), higher%MICRO (2.31% *vs* 0.42%, p<0.001), higher%HYPER (1.99% *vs* 0.45%, p<0.001), lower%HYPO (1.51% *vs* 2.47%, p<0.001) and higher M/H (9.31% *vs* 0.67%, p<0.001). A clinical prediction rule was developed by assigning one point to each of the most efficient variables: mean corpuscular volume (MCV)<88.4 fL, RBC distribution width >13.4%, percentage of microcytic RBCs (%MICRO) >0.7% and the ratio of microcytic RBCs to hypochromic RBCs >0.8. A score of 0, 1, 2, 3 or 4, resulted in a probability of 9.6%, 36.3%, 66.7%, 85.2% or 98.3%, respectively.
Summary/Conclusions: Although molecular analysis (gold standard) or another laboratory technique such as electrophoresis and/or HPLC is mandatory for the diagnosis of structural hemoglobinopathies, a CBC is a quick, cheap and handy test that provides useful information for a presumptive diagnosis of these entities. RBCs of variant Hb carriers are smaller, denser and show a higher degree of anisocytosis in comparison to RBcs of normal controls. Structural hemoglobinopathy investigation should be performed if a score of 3 or 4 is seen in subjects belonging to ethnic groups with a high prevalence of variant Hb. Our model is not structured as a complex mathematical formula, and in our opinion, this facilitates its clinical use. Although differences in haematological parameters have been reported by many authors, none evaluated the value of the CBC as a screening tool to identify variant Hb carriers.

E1473

HEMOGLOBINOPATHIES WITH LEVELS HBA2 DECREASED. DELTA GENE MUTATIONS FOUND IN A SPANISH CENTER
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Background: HbA_2 reaches its final value (2.5% -3.5%) in the first year of life. Due to this low expression and the lack of significant pathology, HbA_2 is not considered relevant, but mutations in the δ gene can cause δ-thalassemia and Hb variants of δ chain are interesting because they interfere in HbA_2 quantification. HbA_2 consists of chains of α globin and δ; therefore, alterations in any of these genes may influence HbA_2 levels.
Aims: We present structural hemoglobinopathies and thalassemias that present with decreased levels of HbA_2, as well as the first compilation of mutations in the gene δ globin, found in Spain.
Methods: The 208 patients that were analyzed presented HbA_2 values below 2.5% and microcytic and hypochromic or normocytic and normochromic without iron deficiency anemia. HbA_2 and HbF levels, as well as the different hemoglobins, were measured and analyzed by ion-exchange HPLC (VARIANT™ II). Hemoglobins were studied by capillary zone electrophoresis (CE) (Sebia) and globin chains by reverse-phase HPLC. Genetic analysis of the α and δ genes was carried out by automatic sequencing and, in the case of α genes, by multiplex PCR.
Results: In α-thalassemia (n=94), HbA_2 levels ranged from 1.39 to 2.43%; in δ-thalassemia (n=5), HbA_2 levels researched in δ^+-thalassemia 1.77% and for δ^0-thalassemiain 1.70%; in δβ-thalassemia (n=13), homozygosity resulted in undetectable HbA_2 levels. Structural hemoglobinopathies (n=96), all heterozygous, in the α chain (n=84) and δ chain (n=12) had HbA_2 rates of 1.76% and 1.75%, respectively.
Summary/Conclusions: 208 samples, with HbA_2<2.5%, showed α gene or δ globin anomalies of quantitative (thalassemia) or qualitative (structural hemoglobinopathies). In the α-thalassemia, the greatest loss of genes corresponds to the lowest levels of HbA_2, since α genes are preferably combined to form the β HbA. The heterozygous δ^+-thalassemia had higher levels than δ^0-thalassemia. It should be suspected of a δ-thalassemia when, in normal and without iron deficiency, HbA_2 levels are lower than the 2%., in these cases the direct sequencing of the gene δ, it will reveal a point mutation in homozygous or heterozygous state. In the δβ-thalassemia, homozygosity no levels of HbA2. The decrease in levels of HbA_2 in α chain hemoglobinopathies is owing to a new α globin chain (α^X) because its variant corresponding HbA_2 ($\alpha_2{}^X\delta_2$) is formed. The greater decrease corresponds to homozygosity, because there is less α^A globin chain synthesis, regardless of the mutated gene (α1 or α2). In

this work we have identified five variants of δ chain because they are relatively common and generally easy to detect not only because halved the HbA_2 value, but because they often show a second fraction of HbA_2 almost the same expression, whether prior or after normal HbA_2. The variant HbA_2-Madrid was first described in the literature. HbA_2 is an essential parameter in the differential diagnosis of thalassemia. Using the HPLC and EC provide not only the quantification of HbA2 but also information other hemoglobins, so the same analysis helps identify hemoglobin variants of either strand and detect thalassemias not otherwise It has been possible diagnosis, avoiding that risk couples may be advised incorrectly.

LB2266

ACTIVIN B PRODUCED BY SINUSOIDAL ENDOTHELIAL/KUPFFER CELLS IN RESPONSE TO INFLAMMATORY STIMULI INDUCES HEPCIDIN EXPRESSION THROUGH UNCANONICAL SIGNALING IN HEPATOCYTES

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Background: Hepcidin, a liver-derived peptide hormone, is involved in the pathogenesis of anemia under inflammation. Activins and BMPs bind to corresponding type I and type II receptors and phosphorylate Smad2/3 and Smad1/5/8, respectively, as canonical signaling pathway. BMPs are well known to up-regulate hepcidin expression through the canonical signaling pathway when hepatocytes accumulate excess iron. On the other hand, activin B is suggested as a key molecule responsible for the up-regulation of hepcidin in inflammation based on the following two evidences: (1) the elevation of activin B mRNA level in the livers of LPS-injected mice, and (2) the stimulation of hepcidin expression by activin B in hepatocytes. Furthermore, activin B has been shown to activate uncanonical Smad1/5/8 signaling as well as canonical Smad2/3 signaling in hepatocytes.
Aims: We examine the following three points in the present study: (1) which cell-types in the liver are responsible for the up-regulation of activin B by inflammatory stimuli, (2) which receptor combination is responsible for the induction of Smad1/5/8 phosphorylation and hepcidin expression by activin B, and (3) whether activin B induces uncanonical Smad1/5/8 signaling in extrahepatic cells.
Methods: A male Sprague-Dawley rat was intraperitoneally injected with LPS (5 mg/kg). After 2.5 h of injection, hepatocytes and non-parenchymal cells were collected from the liver by collagenase-perfusion. Sinusoidal endothelial and Kupffer cell-rich fraction was isolated from non-parenchymal cells by a two-step density gradient centrifugation. The expression of activin B was measured by RT-qPCR. Rat primary hepatocytes, HepG2 human hepatoma cells, C2C12 myoblasts, 3T3-L1 pre-adipocytes, and RAW264.7 murine macrophage-like cells were stimulated with 2 nM of activin B. Phosphorylation of Smad1/5/8 and mRNA level of hepcidin or Id1 were examined. HepG2 cells were transfected with siRNAs of type I or type II receptors and luciferase reporter of murine hepcidin promoter. After transfection, cells were treated with activin B. Transcriptional activity of hepcidin was assayed.
Results: Significant expression of activin B was detected in sinusoidal endothelial and Kupffer cell-enriched fraction isolated from the liver of a rat injected with LPS, while expression of activin B in hepatocyte-enriched fraction was almost negligible, suggesting that sinusoidal endothelial cells or Kupffer cells are the major sources of hepatic activin B under inflammation. Knockdown of the BMP type I receptor, ALK2 or ALK3, indicated that ALK2, but not ALK3, is involved in the responsiveness of the hepcidin promoter to activin B through the activation of Smad1/5/8 signaling in HepG2 cells. Knockdown of the type II receptor ActRIIA inhibited activin B-mediated Smad1/5/8 phosphorylation and hepcidin transcription. These results suggest that upon the binding of activin B, the receptor complex, ALK2 and ActRIIA, stimulates Smad1/5/8 signaling, followed by induction of hepcidin. Unexpectedly, the induction of Smad1/5/8 phosphorylation and hepcidin expression by activin B was resistant to LDN-193189, an inhibitor of ALK2 and ALK3, whereas those actions of BMP2 was sensitive to the inhibitor. These results implicate that unknown mechanisms distinct from BMP signaling are involved in the modulation of Smad1/5/8 signaling by activin B. We also showed that activin B increased phosphorylation of Smad1/5/8 and mRNA level of Id1, a representative BMP response gene, in C2C12 cells, 3T3-L1 cells and RAW264.7 cells, implicating that activin B-induced uncanonical Smad1/5/8 signaling is independent of cell-type.
Summary/Conclusion: Upon inflammatory stimuli, sinusoidal endothelial cells and/or Kupffer cells up-regulate activin B expression in the liver, followed by induction of Smad1/5/8 phosphorylation and hepcidin expression in hepatocytes via the receptor complex of ALK2 and ActRIIA. In addition, activin B-induced Smad1/5/8 signaling is not limited to hepatocytes. The present study provide novel insights into the molecular mechanisms underlying induction of hepcidin by inflammation and the possible roles of activin B-mediated Smad1/5/8 signaling in several tissues.

Red blood cells and iron - Clinical

E1474

TREATMENT WITH CYCLOSPORIN IN AUTO-IMMUNE CYTOPENIAS IN CHILDREN: THE EXPERIENCE FROM THE FRENCH COHORT OBS' CEREVANCE

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Background: Auto-immune cytopenias are rare in children, and there is no recommendation about treatments in case of resistance or dependence to steroids.
Aims: Since 2004, the cohort OBS'CEREVANCE has been recording the cases of auto-immune cytopenias in children treated in the 30 French pediatric hematology units. This study reports the treatment with cyclosporin for all the patients of the cohort treated with cyclosporin for auto-immune hemolytic anemia (AIHA), Immune throbopenic Purpura (ITP) and Evans Syndrome (ES).
Methods: We analyzed the data of 15 AIHA, 7 ITP and 12 ES. Median age was 4.5 years for AIHA and ES, 7.2 for ITP. Ten patients had underlying immunodeficiency and 5 had auto-immune disease.
Results: The best response was a complete remission (CR) for 7 AIHA, 1 ITP and 6 ES, maintained in median 8.6 months for AIHA and 25.5 months for ES. The treatment allowed steroids decrease in 9 cases of AIHA and 9 ES. Three patients treated for AIHA maintained CR after discontinuation of treatment, whereas all the patients with ES relapsed during or after treatment. Toxicity was acceptable. Median residual level was 96.5 mg/L (31-369) for patients achieving CR or PR and 117 mg/L (23-956) for patients with no response.

Table 1.

		AIHA (N=15)	ITP (N=7)	ES (N=12)
Median duration of treatment (months)		15 (2-76,8)	3,3 (1-8,1)	20 (0,9-86,5)
Best response	CR	7	1	6
	PR	1		1
	NR	7	6	5
	Decrease/stop steroids	9	3	9
Delay initiation of treatment-CR (months)		1,5 (0,4-12,9)	3	2,3 (0,5-4,8)
Overall response	6 months CR+PR	7	1	6
	12 months CR under treatment	5	0	4
	2 years CR (in which under treatment)	4 (3)	0	3 (2)
	3 years CR (in which under treatment)	4 (1)	0	1 (0)
Duration of response (months, median)		**8,6** (4,4-51)	3	**25,5** (3-52)
Need for subsequent treatment		12	7	12
Subsequent relapse (after CR)		3	7	12
Reason of discontinuation	CR	4	0	2
	Failure	11	7	6
	Adverse effect	0	1	3
	death	0	0	2
Median follow-up time (months)		86,5 (0,5-151)	9 (1-12,9)	81 (22-168)
Status at last follow-up	SCR (in which after cyclosporin alone)	13 (3)	3 (0)	5 (0)
	NR	1	2	3
	Dead of toxicity of other treatments	0	1	1
	Dead of disease	1	0	3

Summary/Conclusions: Given our results, we propose to use cyclosporin for AIHA (excluding in context of immunodeficiency) and ES, after failure of corticosteroids, or in case of dependence to steroids. We propose to base the prescription on aplastic anemia treatment recommendations with a blood level residual target between 50 and 100 mg/L: treatment at least three months before concluding to failure, treatment at least a year at full dose in case of response, very slow decrease over several months, return to previous dose in case of relapse during the decrease, or to full doses in case of relapse at treatment discontinuation, monitoring of residuel level, in case of success (CR), toxicity or failure.

E1475

INTERNATIONAL SENTINEL SITE SURVEILLANCE OF PATIENTS WITH TRANSFUSIONAL HEMOSIDEROSIS TREATED WITH DEFERASIROX IN ACTUAL PRACTICE SETTING

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Background: Long-term iron chelation therapy (ICT) is required in regularly transfused patients (pts) to reduce the chronic iron overload (IOL) which is a

major complication causing morbidity and mortality. The oral iron chelator deferasirox (DFX) is indicated for the treatment of chronic IOL due to blood transfusions in adult and pediatric pts aged ≥2 years (yrs). Multiple clinical studies have established the efficacy and safety of DFX in transfusion-dependent pts with IOL. The present study reports the results of a postmarketing active surveillance program for DFX.

Aims: To evaluate the long-term safety and clinical management of DFX in adult and pediatric pts aged ≥2 yrs with chronic transfusional IOL in the actual practice setting.

Methods: Pts aged ≥2 yrs treated with DFX for transfusional hemosiderosis according to the local prescription information were enrolled in this non-interventional study. Data were collected for 3 yrs from initiation of treatment with DFX, and retrospective data were collected in pts who had treatment with DFX for up to 1 yr prior enrollment. The primary endpoints were as follows: (a) the proportion of pts with at least 1 notable increase in serum creatinine (SrCr), defined as >33% above baseline (BL) and the age adjusted upper limit of normal (ULN) in at least 2 consecutive measurements (≥7 days apart), and (b) notable increase in alanine aminotransferase (ALT), defined as >5×ULN in at least 2 consecutive measurements (≥7 days apart).

Results: Of the 120 pts enrolled, majority were diagnosed with β thalassemia (n=49), sickle cell disease (SCD, n=31), and myelodysplastic syndrome (MDS, n=21) (Table 1). The mean age (±SD) was 7.5±4.2 yrs in pts with<18 yrs (n=69) and 57.9±22.1 yrs in pts with ≥18 yrs (n=51) of age. Median duration of DFX exposure was 29.9 months and average actual dose was 23.2±8.2 mg/kg/d. Overall, 42.5% (<18 yrs, n=45; ≥18 yrs, n=6) of pts completed the study. The most common reasons for study discontinuation (>10%) were subjects who no longer required study drug (19.2%), adverse events (AEs, 12.5%), and consent withdrawal (10.8%). Dose reductions and interruptions occurred in 39.3% and 23.9% of pts, respectively. Of the 46 pts who had dose reductions, 5 pts reported increase in SrCr (n=3) and ALT (n=2). Of the 28 pts who had dose interruptions, 3 pts reported increase in SrCr (n=2) and ALT (n=1). Notable increase in SrCr was observed in 12% (14 of 117; 95% CI, 7.1-19.2) of pts with MDS (n=3), SCD (n=7) and other anemias (n=4). Notable increase in ALT was observed in 1 pt (0.9%; 95% CI, 0.0-5.2) with β thalassemia (BL ALT missing). Overall, 34.2% (40 of 117) of pts had AEs suspected to be related to DFX, including AEs (>5%) such as diarrhea (9.4%), increase in SrCr (7.7%), and vomiting (6.0%). A total of 4.3% (5 of 117) of pts had serious AEs (SAEs), suspected to be related to DFX, and SAE >1% was gastrointestinal hemorrhage (1.7%). The AEs leading to the discontinuation of DFX occurred in 18.8% of pts (MDS, n=11; β-thalassemia, n=2; SCD, n=2; other anemias, n=7) regardless of the relationship with DFX, including AEs (>2%) such as increase in SrCr (3.4%) and diarrhea (2.6%). None of the pts discontinued DFX due to the increase in ALT. Eight (6.8%; MDS, n=4, other anemias, n=4) on-treatment deaths were reported, all in pts ≥18 yrs of age, majorly due to gastrointestinal disorders (n=2) and neoplasms (n=2). Of these 8 deaths, 7 were not suspected to be related to DFX (data unknown, n=1).

Table 1.

Characteristics	<18 years (n=69)	≥18 years (n=51)	All Patients (N=120)
Mean age±SD, years	7.5±4.2	57.9±22.1	28.9±29.0
Female:Male, n	35:34	21:30	56:64
Race, n (%)			
Caucasian	37 (53.6)	44 (86.3)	81 (67.5)
Black	21 (30.4)	6 (11.8)	27 (22.5)
Asian	7 (10.1)	0 (0.0)	7 (5.8)
Native American	0 (0.0)	1 (2.0)	1 (0.8)
Other	4 (5.8)	0 (0.0)	4 (3.3)
Disease, n (%)			
β thalassemia major	34 (49.3)	7 (13.7)	41 (34.2)
β thalassemia intermedia	7 (10.1)	1 (2.0)	8 (6.7)
Refractory anemia	0 (0.0)	2 (3.9)	2 (1.7)
Sideroblastic anemia	0 (0.0)	3 (5.9)	3 (2.5)
Myelodysplastic syndrome	0 (0.0)	21 (41.2)	21 (17.5)
Diamond-Blackfan anemia	2 (2.9)	0 (0.0)	2 (1.7)
Hemolytic anemia	0 (0.0)	1 (2.0)	1 (0.8)
Sickle cell disease	24 (34.8)	7 (13.7)	31 (25.8)
Other	2 (2.9)	9 (17.6)	11 (9.2)

Summary/Conclusions: The results demonstrated a safety profile for DFX which is consistent with previously published data. The data demonstrates that changes in SrCr can be managed with dose adjustments or interruptions and occasionally led to permanent discontinuation. DFX has been associated with rare hepatic toxicity. Only 1 pt had a notable increase in ALT. The overall incidence of AEs that lead to DFX discontinuation was higher in pts with MDS during the study.

E1476

EVALUATION OF THE FOOD INTAKE, NUTRITIONAL STATUS AND NUTRITIONAL DEFICIENCY IN CHILDREN WITH SICKLE CELL DISEASE: PRELIMINARY RESULTS OF A CROSS - SECTIONAL STUDY

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Background: Children with Sickle Cell Disease (SCD) have poor growth compared to their peers; they also present a higher percentage of cognitive impairment compared to age matched controls. Clinical practice shows that the presence of nutritional deficiencies with important clinical implications (anemia, abnormal bone metabolism..) is frequent in these patients and that it can have an adverse impact on disease severity. Currently there are no European studies that correlate specific nutritional deficiency to the nutritional intake; demonstrating the correlation between reduced or inappropriate dietary intake and selective nutritional deficiencies could lead to targeted nutritional interventions aimed at improving the nutritional status and the altered hematological parameters.

Aims: 1) To identify any nutritional deficiencies in patients with SCD and correct them promptly. 2) To identify, if possible, the correlations between nutritional status, dietary intakes and deficiency states, and between states of deficiency and clinical expression of the disease, including patient's performance assessed through ad hoc questionnaires.

Methods: Children with SCD of the clinic of Pediatric Hematology-Oncology in Padua were included in this prospective study. An ad hoc questionnaire (on food frequency, quantity and eating habits) was administered; the data were processed with GEDIP 4 software to derive the minimum, average amounts, maximum and standard deviations of various nutrients and to compare them with the recommended requirements in the Recommended intake levels (RDAs, 2014 edition). Nutritional status was assessed by weight, height, Body Mass Index and arm circumference that were used for drawing the percentiles using CDC growth curves. The following hematochemical parameters were evaluated at Time 0: Complete Blood Count, PTH, Vitamins A, D, E, ferritin, serum iron, zinc, copper, calcium, phosphate, folic acid, methylmalonic acid, homocysteine, blood gas analysis. These parameters will be re-assessed at Time 1 (6 months) and 2 (12 months) to evaluate the effectiveness of any additional therapy.

Results: 72 consecutive pediatric patients with SCD were included in our study (SS 62/72, 8/72 SC, 2/72 Sß), 31 F and 41 M, mean age 9.6 years (range: 8 months-23 years), from 1st March 2015 to 31st August 2015. The main nutritional deficiencies were: folic acid (9/72); Vitamin D (62/72) correlated with increased PTH (27/72); Vitamin A (43/72); Vitamin E (0/72); serum iron (23/72); zinc (18/72). In some cases high levels of methylmalonic acid (10/72) and homocysteine (4/71) have been reported, showing lack of Vitamin B12. The following supplementations were given: folic acid (55/72), vitamin D (57/72), iron (15/72), zinc (1/72); in the remaining cases with slight deficits were provided appropriate dietary advices. At the moment 37/72 patients underwent the nutritional assessment questionnaire, which shows a nutritional intake of 69% of energy intake established by the RDAs, and values between 25° and 50°P for weight, height, BMI and arm circumference for age. Their protein intake is on average 150% from the RDAs, while recording contributions of about 50% for calcium, iron and folic acid and 8% for Vitamin D. Even if most of the patients are African first generation immigrants with a specific but balanced dietary habits; a significant part of them belongs to low-income families and feeds with "junk food", which could be the cause of their nutritional deficits.

Summary/Conclusions: Preliminary data of our study does not show an adequate intake of macro and micro nutrients for SCD children; calcium, iron, folic acid and especially Vitamin D are the most deficient nutrients and determine suboptimal growth state. The continuation of the study, with a larger number of subjects, will help us to highlight the possible correlation of nutritional status with the progress and severity of the disease.

E1477

CLINICAL SAFETY AND EFFICACY OF FERRIC CARBOXYMALTOSE IN THE TREATMENT OF IRON DEFICIENCY: META-ANALYSIS EVALUATING INDIVIDUAL PATIENT DATA OF 18 RANDOMIZED TRIALS

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Background: Iron deficiency and iron deficiency anemia are highly prevalent in both industrialized and developing countries. Patients and special populations with a wide variety of underlying conditions are affected and may experience debilitating symptoms. Intravenous iron therapy is indicated when oral iron supplementation is not tolerated or iron absorption is ineffective. Ferric carboxymaltose (FCM) is an intravenous iron formulation for administration of single doses up to 1000 mg iron during a short administration time (≥15 minutes).

Aims: The aim of this analysis was to describe the safety and efficacy of FCM across studies with different patient populations.

Methods: Individual patient data collected at European trial sites of 18 randomized controlled clinical trials performed by Vifor Pharma were analyzed. Study populations included patients with chronic kidney disease, inflammatory bowel disease, chronic heart failure, cancer, and women of childbearing age. FCM treated patients were compared to a pooled comparator group, including other intravenous iron formulations, oral iron, placebo or standard medical care. All treatment emergent adverse events (TEAEs) and related TEAEs were

investigated, with focus on special adverse events of interest, including changes in serum phosphorus levels. Furthermore, therapeutic efficacy parameters (hemoglobin, ferritin and transferrin saturation) were analyzed over time. Changes from baseline in laboratory parameters are statistically presented as least-squares means (LS means) and standard errors (SE).
Results: Data from 3,331 patients with iron deficiency were analyzed. Of these, 1,969 patients had received FCM. The mean cumulative dose of FCM over the first 6 weeks was 1,124±417 mg iron, with single doses of 1,000 mg iron in 36% of the patients. Overall, the most frequent related TEAEs in the FCM group were serum ferritin increased (1.4%), headache (1.4%), and nausea (1.0%). In the pooled comparator group, the most frequent related TEAEs were constipation (2.5%), diarrhea (1.8%) and nausea (1.0%). Among patients with available serum phosphorus values over time (FCM n=1,370; comparator n=1,194), 27.4% of subjects in the FCM group and 2.0% in the comparator group had a value below 2.0 mg/dL at any time during the first 8 weeks of follow-up. Signs and symptoms that could possibly be related to transient decrease in phosphate level (using the broad SMQ-definition) were reported in 9.6% of FCM-treated subjects and in 11.3% in the comparator group. In the FCM group, hemoglobin steadily increased from the first post-treatment assessment at week 1 or 2 (n=1,404; mean changes from baseline: 0.411 [0.098] g/dL) to week 4 (n=1,619; 1.176 [0.168] g/dL) and week 8 (n=1,398; 1.466 [0.190] g/dL). Ferritin and transferrin saturation were significantly increased in the FCM group compared with comparator pool, at all time points (p<.0001; week 1 or 2, 4, 8), and remained stable throughout the 8-week period (mean change from baseline at week 8: +227.7 [53.7] *vs* 105.7 [53.8] ng/mL and +13.4 [1.2] *vs* 8.5 [1.2]%, respectively; p<.0001), reflecting repletion of iron stores.

Table 1.

Table 1. Incidence of treatment emergent adverse events.

	FCM <1,000 mg n=1,267	FCM ≥1,000 mg n=702	FCM total n=1,969	Control n=1,463
Any TEAE	54.9%	61.8%	57.4%	55.0%
Treatment-related	12.5%	17.7%	14.3%	13.0%
Severe TEAE	6.4%	5.6%	6.1%	7.1%
Treatment-related	0.5%	1.0%	0.7%	0.8%
Any serious TEAE	9.7%	11.3%	10.3%	11.8%
Treatment-related	0.2%	0.3%	0.2%	0.1%
TEAE leading to study drug withdrawal	3.3%	2.7%	3.1%	6.1%
Treatment-related	1.3%	1.0%	1.2%	2.3%
TEAE with outcome of death	1.0%	2.1%	1.4%	1.8%
Treatment-related	0.0%	0.0%	0.0%	0.0%

FCM = ferric carboxymaltose, TEAE = treatment emergent adverse event.

Summary/Conclusions: This large-scale analysis is based on individual patient data and includes a broad variety of different patient populations. The results confirm that FCM is effective and has a similar tolerability to the pooled comparator. FCM causes a transient decrease in phosphate levels but this does not appear to increase the reported frequency of associated signs and symptoms.

E1478

CHANGES IN GAMMA GLOBIN MRNA LEVELS DO NOT CORRELATE WITH TOTAL HEMOGLOBIN OR FETAL HEMOGLOBIN LEVELS IN BETA THALASSEMIA INTERMEDIA PATIENTS ON HYDROXYUREA

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Background: Hydroxyurea is a hemoglobin-F inducing drug used to increase hemoglobin levels in sickle cell disease and β-thalassemia intermedia. The response to hydroxyurea is variable among individuals and its mechanism of action is less well understood. This prospective study on β-thalassemia intermedia patients aimed at analysing the effects of hydroxyurea on γ-globin mRNA levels and correlate its dynamics with changes in total hemoglobin and fetal hemoglobin levels to better understand its mechanism of action. The underlying β thalassemia mutations, α thalassemia deletions and *Xmn-1^{G}*γ (C-T) polymorphism were characterised and correlated with changes in γ-globin mRNA levels.
Aims: To study the changes in γ-globin mRNA levels in β-thalassemia intermedia patients on hydroxyurea compared to baseline levels and correlate them with changes in total hemoglobin and fetal hemoglobin levels.
Methods: Eleven newly diagnosed individuals with β-thalassemia intermedia were included in the study. Changes in γ-globin mRNA levels were compared at baseline and after 6 months of hydroxyurea therapy. These were correlated with changes in hemoglobin, hemoglobin-F, hemoglobin-A2 levels and red cell parameters. The quantitation of γ-globin mRNA levels was done by real time PCR (Roche Lightcycler 480®). ARMS-PCR was used to study the β thalassemia mutations and GAP-PCR was employed to study the α globin gene deletions. *Xmn-1^{G}*γ (C-T) polymorphism was studied by PCR-RFLP.
Results: The study group included nine pediatric cases and two adult patients with age at enrolment ranging from 2 to 30 years. The male to female ratio was 1.2: 1. Four mild, five moderate and two severe β-thalassemia intermedia cases were enrolled. Milder β-thalassemia mutations were responsible in majority of cases for the thalassemia intermedia phenotype. The most frequent β-thalassemia mutation in our study group was -88 (C-T) followed by Cap+1 (A-C). All the eleven patients were negative for the 8 α-globin gene deletions tested by GAP-PCR. Seven patients were *Xmn-1^{G}γ* (-/-), two patients *Xmn-1-^{G}γ* (+/+) and two were of *Xmn-1^{G}*γ (+/-) genotype. Seven patients (63.6%) were good responders, two (18.2%) were partial and two (18.2%) were non-responders to hydroxyurea therapy. The mean increase in Hb post-HU therapy was 1 g/dl. The mean HbF increased by 1.5 g/dl in the post-HU group. Six (54.5%) patients had increased relative expression of γ-globin mRNA after HU therapy, 4 (36.4%) patients had decreased relative expression and one patient (9%) did not show any change measured by qRT-PCR. Four patients who had a decrease in relative expression of γ-globin mRNA after hydroxyurea therapy did not show a drop in Hb; rather all had a rise in Hb ranging from 0.8 to 2.6 g/dl. The relative expression of γ-globin mRNA levels did not correlate with rise in hemoglobin. *Xmn-1^{G}γ* (C-T) polymorphism was associated with high fetal hemoglobin levels at baseline and also post hydroxyurea. Hydroxyurea showed good response in majority of the patients. These results indicate that the actions of hydroxyurea might extend beyond γ-globin gene regulation. The mean hemoglobin A2 levels were decreased post hydroxyurea compared to baseline. This indicates hydroxyurea might affect regulation of other globin genes apart from the γ-globin gene. In addition there was improvement in red cell parameters (red cell indices, nucleated red cell count and reticulocyte count), bilirubin and lactate dehydrogenase levels indicating that hydroxyurea has a role in improving red cell rheology and survival.

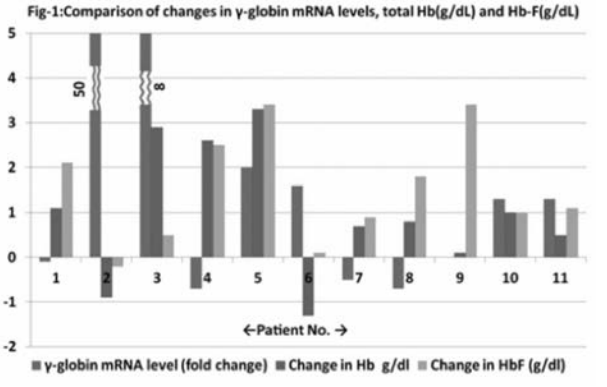

Figure 1.

Summary/Conclusions: In our study, the changes in relative expression of γ-globin mRNA levels (both increased and decreased levels) as measured by qRT-PCR did not directly correlate with changes in either of total hemoglobin or fetal hemoglobin levels. The increase in hemoglobin levels even in patients with decreased relative expression of γ-globin mRNA levels suggests that alternate mechanisms of action of hydroxyurea exist to increase the fetal hemoglobin and total hemoglobin in β-thalassemia intermedia patients. *Xmn-1^{G}γ* (C-T) polymorphism is associated with better response to hydroxyurea.

E1479

FERROPORTIN DISEASE: A CASE SERIES AT THE REGIONAL REFERRAL CENTER FOR IRON DISORDERS IN VERONA

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Background: Ferroportin Disease (FD), due to mutations in the *SLC40A1* gene encoding for the hepcidin receptor and cell membrane iron exporter ferroportin, is deemed as the most frequent form of "atypical" or "non-HFE" Hereditary Hemochromatosis (HH) (Wallace DF, Genet Med 2015). To date, knowledge on FD is mainly based on individual case reports, with heterogeneous clinical and biochemical features.
Aims: The aim of the work is to describe our experience as Regional Referral Center for Iron Disorders, after the recent implementation of a next generation sequencing (NGS)-based 2nd level genetic test for the molecular diagnosis of non-HFE HH.
Methods: We used our recently validated molecular test (Badar S, Am J Hematol 2016) combining capture and enrichment of the five HH genes (HFE, HFE2, TFR2, SLC40A1/ferroportin, and HAMP/hepcidin) through HaloPlex™ Technology and NGS using the IlluminaHiSeq 1000 platform (Illumina, San Diego CA).
Results: New cases of FD have been detected, so that we are now following 9 FD patients from 7 families. The 3bp-deletion p.Val162del, the most frequent FD-associated variant reported in literature was found in 3 families. We recently detected a new variant (p.Leu233Gln) in a patient showing mixed biochemical/pathological features between Type 4A/4B HH, *i.e.* marked hyper-

ferritinemia (6,923 ng/ml), elevated transferrin saturation (85% at diagnosis), iron deposition in both liver and spleen at MRI, and iron overload in both Kupffer cells and hepatocytes at liver biopsy. Noteworthy, the mutation affected the same residue involving a different mutation (p.Leu233Pro) previously described in a patient with a quite similar phenotype (Girelli D, J Hepatol 2008). All 4 patients with p.Val162del (all females, age range 30-69) had marked hyperferritinemia (range 1,101-3,020 ug/L) and normal transferrin saturation. Despite in some cases hyperferritinemia dated back several years without treatment before the correct diagnosis, all patients were clinically asymptomatic, with no overt biochemical signs of liver disease. Hepcidin levels in FD patients were high-normal at diagnosis, but tended to decrease with iron depletion through phlebotomies.
Summary/Conclusions: Our single center experience confirm FD disease as the most frequent atypical HH, and call for multicentre registries to better define the clinical features of this heterogeneous condition.

E1480

LONG-TERM SAFETY AND EFFICACY OF DEFERASIROX IN PEDIATRIC PATIENTS WITH TRANSFUSIONAL HEMOSIDEROSIS: RESULTS FROM A 5-YEAR OBSERVATIONAL STUDY (ENTRUST)

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Background: Young patients with red blood cell disorders, such as β thalassemia major (βTM), sickle-cell disease (SCD) and Diamond–Blackfan anemia (DBA), receive regular transfusions from an early age and consequently accumulate iron. Complications of iron overload include organ damage and delayed growth/development, therefore optimal management of iron is required. Deferasirox, a once-daily oral iron chelator, has demonstrated safety and efficacy in adult and pediatric patients with transfusional hemosiderosis. As requirements for therapy may be lifelong, continual assessment of long-term outcomes is valuable, as conducted in the 5-year multinational, observational ENTRUST study.
Aims: Evaluate the long-term safety and efficacy of deferasirox in younger transfusion-dependent children with chronic iron overload in clinical practice.
Methods: Patients aged 2–<6 years at enrolment were prescribed deferasirox according to local labels, with dose adjusted based on serum ferritin, therapeutic goals, tolerability and changes in patient weight. Parents/guardians provided written, informed consent. The primary objective was to evaluate safety, specifically renal and hepatic function, assessed by ≥2 consecutive post-baseline measurements ≥7 days apart of serum creatinine (SCr) >age-adjusted upper limit of normal (ULN) and >33% from baseline and alanine aminotransferase (ALT) >5xULN. Secondary objectives included analysis of serum ferritin, investigator-reported adverse events (AEs) and growth.

Table 1.

Table. Most common investigator-assessed drug-related AEs (occurring in ≥4 patients)

	All patients (Safety set* N=261)
Patients with any drug-related AE, n (%)	102 (39.1)
AE by preferred term, n (%)	
Investigations	
Alanine aminotransferase increased	55 (21.1)
Aspartate aminotransferase increased	31 (11.9)
Serum creatinine increased	10 (3.8)
Serum bilirubin increased	4 (1.5)
Gastrointestinal disorders	
Vomiting	14 (5.4)
Abdominal pain	8 (3.1)
Diarrhea	5 (1.9)
Skin and subcutaneous tissue disorders	
Rash	13 (5.0)
Hepatobiliary disorders	
Hepatocellular injury	4 (1.5)
Infections and Infestations	
Gastroenteritis	4 (1.5)
Renal and urinary disorders	
Proteinuria	4 (1.5)

*All patients who received ≥1 dose of deferasirox over the analyzed period and ≥1 post-baseline safety assessment; AE, adverse event

Results: In total, 267 patients (mean age 3.2 years: 61.4%<4 years, 38.6% ≥4 years) with βTM (n=176, 65.9%), SCD (n=52, 19.5%), DBA (n=12, 4.5%) and other anemias (n=27, 10.1%) were enrolled and received ≥1 deferasirox dose. 122 (45.7%) patients discontinued, most commonly (>5% of patients) due to 'Other' reasons (n=55; 20.6%), loss to follow-up (n=19, 7.1%) and AEs (n=18, 6.7%; most commonly increased ALT and increased AST, n=7 each). Mean±SD deferasirox exposure and dose were 44.1±21.2 (range 1.2–65.6) months and 25.8±6.5 mg/kg/day, respectively. Most patients (n=172; 64.4%) received an average blood intake >7 mL/kg/month. Median serum ferritin decreased from 1702 (range 334–9577) ng/mL at baseline (n=243) to 1127 (range 38–6428) ng/mL at 5 years (n=84). Eight (3.1%) patients had two consecutive SCr increases >age-adjusted ULN and >33% from baseline; two received deferasirox dose adjustments to resolve the observed SCr increase. Overall, 11 (4.2%) patients had two consecutive ALT increases >5 or >10xULN, three of whom received deferasirox dose interruptions. There were no unexpected adverse events (AEs) or laboratory abnormalities. The most frequently observed AEs with suspected relationship to study drug (Table) were increased ALT (21.1%), increased AST (11.9%), vomiting (5.4%) and rash (5.0%). A steady increase in weight was observed.
Summary/Conclusions: This long-term, observational study of deferasirox in pediatric patients supports previous trials indicating favorable safety and efficacy. Over half the patients completed the 5-year study, with limited discontinuations due to AEs. Few patients experienced notable increases in renal or hepatic assessments and continued deferasirox therapy at the same or reduced dose. In this pre-pubertal study population, growth was normal. Thus, with regular monitoring and dose adjustments, effective long-term chelation therapy with deferasirox is manageable in the majority of pediatric patients in clinical practice.

E1481

CONCURRENT TREATMENT OF APLASTIC ANEMIA (AA) WITH IMMUNOSUPPRESSIVE THERAPY AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) WITH ECULIZUMAB

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Background: At least 50% of aplastic anaemia (AA) patients have a PNH clone. Many have a small proportion of PNH cells requiring monitoring alone whereas others may present with both significant aplasia as well as PNH. Concurrent treatment for AA and PNH may therefore be required.
Aims: This retrospective analysis assess's the outcome of patients with concurrent PNH and aplastic anaemia who require complement blockade with eculizumab alongside immunosuppressive therapy (IST) for AA
Methods: The PNH National Service database (Leeds) was reviewed retrospectively. Patients who commenced eculizumab within a year of their aplastic anaemia treatment, or those treated for aplasia who were already established on eculizumab were selected.
Results: Fourteen patients were treated with eculizumab and immunosuppressive therapy concurrently. Median age was 38.5 years (range 7-76). AA treatment varied as per national guidelines dependant on the patient's age, patient choice and co-morbidities. Seven out of fourteen received ATG and ciclosporin (median follow-up 20 months post ATG), six received ciclosporin monotherapy (median follow-up from commencement of ciclosporin 97 months). 6 of the 7 patients receiving ATG+Ciclosporin responded, two achieved complete response (CR) and four a partial response (PR), one of whom required tacrolimus and subsequently achieved a CR with androgen therapy and one who achieved PR with oxymetholone. One did not respond and achieved a CR with a bone marrow transplant (BMT). Median granulocyte count reduced from 88% to 50% post ATG treatment however there was no change in the monocyte PNH clone. Of the six patients treated with ciclosporin monotherapy, one had a CR with concurrent androgen therapy, two had a PR, one had a CR then relapsed and is now in PR on recommencement of ciclosporin, two had no response. There was no change in the granulocyte PNH clone following ciclosporin treatment. One patient proceeded to first line bone marrow transplantation (mean follow-up from presentation 7 months). Both BMT recipients stopped eculizumab post transplant due to resolution of PNH. Two patients died, one from infection following a PR with ATG and ciclosporin, and one from unknown causes who had not responded to treatment. One patient had spontaneous remission of their PNH and stopped eculizumab; the remaining nine patients continue on eculizumab. Fourteen age matched controls not on eculizumab received similar therapies, nine of whom received ATG and ciclosporin (median follow-up from ATG commencement 37 months). Four had a CR, one had a CR then relapsed with no response to re-introduction of ciclosporin, one had a PR and two had no response, one of whom had a BMT with CR and one achieved CR with oxymetholone. One ATG patient is awaiting response (four months post ATG). Five patients were treated with ciclosporin monotherapy (median follow-up from ciclosporin commencement 115 months). Two had a CR, one had a PR then relapsed, one had no response and achieved a PR with oxymetholone and one is awaiting response (three months post ciclosporin commencement).
Summary/Conclusions: Concurrent treatment of AA with IST and PNH with eculizumab has not previously been reported. The response rates for IST in patients on eculizumab compared with age matched controls were similar, with similar numbers of patients achieving CR or PR with IST. Eculizumab therapy does not appear to affect response to IST for aplastic anaemia patients.
This strategy may be required especially with improved life expectancy for PNH patients receiving complement inhibition therapy. The patients in this study were treated in accordance with current aplastic anaemia guidelines and the presence PNH should not influence this.

E1482

4 YEAR COST-ANALYSIS OF AUTOMATED RED CELL EXCHANGE TRANSFUSION FOR MANAGEMENT OF RECURRENT PAINFUL CRISES IN ADULT PATIENTS WITH SICKLE CELL DISEASE

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Background: Automated red cell exchange transfusion (ARCET) is a promising modality in treating chronic complications in sickle cell disease (SCD). However, concerns around the cost of the procedure may limit its adoption in some institutions.
Aims: To assess the cost implications / financial gains of regular ARCET for SCD patients who have been on the programme for 4 years for recurrent pain in our institution.
Methods: We adopted ARCET in our institution in June 2011 and 8 patients have been on the programme for 4 consecutive years for management of recurrent painful crises. Data were collected retrospectively and the hospital attendance of these patients one year prior to the intervention (as a comparator) and every year after starting ARCET were analysed. Prices for different aspects of the service were provided by the divisional operational manager of the department and the haematology laboratory manager and were as follows: Haematology day unit unplanned attendance: £1022, emergency department (ED) attendance: £235, in-patient stay up to 8 days: £1540 and in-patient stays in excess of 8 days: £1540+£294 / every day in excess of 8. The total cost of blood over these 4 years was £309,200, the cost of using the day unit for the procedures £218,400 and the cost of the consumables used £36,839 giving a total cost of £564,439 for the 4 years. Cost of hospital attendance without the intervention was projected over 4 years based on the hospital attendance the year prior to commencing the intervention.
Results: 1) Total day unit unplanned attendance (without admission): 222 days one year prior to intervention, projected 888 over 4 years with projected cost £907,536. Attendance after intervention was 103, 142, 78 and 37 days 1, 2, 3 and 4 years post intervention respectively with a total of 360 days in 4 years and an actual cost of £367,920 and a 4 yr projected cost reduction of £539,616. 2) Total ED attendance (without admission): 173 days one year prior to intervention, projected 692 over 4 years with projected cost £162,620. Attendance after intervention was 130, 86, 72 and 53 days 1, 2, 3 and 4 years post intervention respectively with a total of 341 days in 4 years and an actual cost of £80,135 and a 4 yr projected cost reduction of £82,485. 3) Total in-patient episodes up to 8 days: 42 one year prior to intervention, projected 168 over 4 years with projected cost £258,720. Episodes after intervention were 48, 31, 37 and 32 1, 2, 3 and 4 years post intervention respectively with a total of 148 episodes in 4 years and an actual cost of £227,920 and a 4 yr projected cost reduction of £30,800. 4) Total in-patient episodes in excess of 8 days: 26 one year prior to intervention, projected 104 over 4 years with projected cost £160,160. Episodes after intervention were 11, 13, 10 and 11 1, 2, 3 and 4 years post intervention respectively with a total of 45 episodes in 4 years and an actual cost of £69,300 and a 4 yr projected cost reduction of £90,860. 5) In-patients days in excess of 8: 485 days one year prior to intervention, projected 1940 over 4 years with projected cost £570,360. In-patient days in excess of 8 after intervention were 73, 138, 57 and 86 days 1, 2, 3 and 4 years post intervention respectively with a total of 354 days in 4 years and an actual cost of £104,076 and a 4 yr projected cost reduction of £466,284. Overall, cost was £564,439 with projected savings of 1,210,045 and a 4y projected cost benefit of £645,606.
Summary/Conclusions: ARCET appears cost-effective for management of recurrent pain in SCD in the medium-long term.

E1483

PREDICTIVE ROLE OF MYOCARDIAL FIBROSIS IN THALASSEMIA INTERMEDIA PATIENTS

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Background: Myocardial fibrosis detected by late gadolinium enhancement (LGE) cardiac magnetic resonance (CMR) is currently used to predict adverse cardiovascular events in different cardiomyopathies. However, its clinical implications in thalassemia intermedia (TI) have not been studied.
Aims: The aim of this study was to assess the distribution and the predictive value of myocardial fibrosis for future events in TI.
Methods: We considered 218 white TI patients (37.82±11.00 years, 113 females) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network and free of cardiac complications concomitant to CMR. LGE images were acquired to detect myocardial fibrosis and the extent of LGE areas was quantified using a semiautomatic, previously validated software.
Results: Myocardial fibrosis was detected in 46 patients (21.1%). LGE followed a subendocardial ischemic pattern typical of coronary artery disease in two patients. One patient showed both an ischemic and non ischemic pattern. Thirty-two patients had a single focus while 14 had at least two foci. The mean number of LGE segments per patient was 2.70±1.52. LGE involved the septal region in 76.1% of patients. The extent of LGE areas was 2.09±1.77% of the total left myocardial mass. Twenthy-two (47.8%) patients showed fibrosis in the infero- or anteroseptal junction, 18 (39.1%) had a non ischemic no-junctional pattern (intra or subepicardial) and 6 (13.0) had both a junctional and no-junctional pattern. Mean follow-up was 56.76±23.17 months. We recorded 13 cardiac complications: 1 heart failure, 7 arrhythmias and 5 pulmonary hypertension (PH). Myocardial fibrosis was a significant prognosticator for arrhythmias, PH and cardiac complications globally considered (see Table). Number of segments with LGE and extent of myocardial fibrosis were comparable in patients who developed cardiac complications *versus* the patients who did not.

Table 1.

	N(%) in Group	N(%) with Arrhythmias	Univariate analysis HR (95%CI)	P
Myocardial fibrosis				
no	172 (78.9)	3 (1.7)	Reference	0.039
yes	46 (21.1)	4 (8.7)	4.84 (1.08-21.63)	
	N(%) in Group	N(%) with PH	Univariate analysis HR (95%CI)	P
Myocardial fibrosis				
no	172 (78.9)	1 (0.6)	Reference	0.017
yes	46 (21.1)	4 (8.7)	14.53 (1.62-130.12)	
	N(%) in Group	N(%) with cardiac complications	Univariate analysis HR (95%CI)	P
Myocardial fibrosis				
no	172 (78.9)	4 (2.3)	Reference	<0.0001
yes	46 (21.1)	9 (19.6)	8.14 (2.51-26.44)	

Summary/Conclusions: In TI patients, myocardial fibrosis is a predictor of adverse outcome.

E1484

ORAL HIGH DOSE LIPOSOMIAL IRON *VS* INTRAVENOUS IRON IN SIDEROPENIC ANEMIA PATIENTS INTOLERANT/REFRACTORY TO IRON SULPHATE. MULTICENTRIC RANDOMIZED STUDY

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Background: In iron deficiency anemia support with intravenous iron allows a faster anaemia correction and a faster ferritin increase than iron sulfate. Frequently iron sulfate and intravenous iron generate adverse events as hypotension, urticarioyd reactions, shock, epygastralgia, constipation or diarrhea. High doses of oral iron frequently are poorly tolerated because of adverse events.
Aims: Aim of this study is to verify if high doses of oral liposomial iron are safe, cost-effective and well tolerated as standard doses of intravenous ferrigluconate in patients with iron deficiency anemia intolerant/refractory to iron sulphate.
Methods: We considered two group of patients(RANDOMIZED 1:1) with iron deficiency anemia without other relevant comorbidities. In group A M/F was 2/3, 15 patients had haemorragic gastritis, 8 hemorragic enteric bleeding angiodysplasia, 22 hypermenorrhaea, median level of hemoglobin (Hb) was 8.5 g/dl(R 6.5-10), median ferritin level was 5 ng/ml (R 3-21), with a normal level of CRP or ESR, and received liposomial iron 30 mg 4 tablet/day. In group B M/F was 2/3, 18 patients had haemorragic gastritis, 6 hemorragic enteric bleeding angiodysplasia, 21 hypermenorrhaea, median level of Hb was 8.2 g/dl(R 7.5-9.5), median ferritin level was 7 ng/ml (R 2-19), with a normal level of CRP or ESR, and received iv sodium ferrigluconate 62.5 mg iv in NS 100 ml in 3 h/day. The median treatment costs in each group were calculated considering the monthly global treatment cost for each patients in the treatment period. This provided an estimate of the costs, independent of the precise cost of the drug, but tied to the final outcome (efficacy) of the therapeutic strategy used during the observation period.
Results: In group A,1g Hb increase was observed after a median of 9 days (R 7-15), a target Hb level of 12g/dl was achieved in a median time of 4 weeks (R 2-5) with a median cost of € 120/months (R 95-180), 12(26%) patients showed adverse events (7 epigastralgia, 5 diarrhoea). In group B,1 g Hb increase was observed after a median of 7 days (R 6-11), a target Hb level of 12g/dl was achieved in a median time of 3 weeks (R 1.5-4) with a median cost of €300/months (R 250-380), 10(22%) patients showed adverse events (2 hypotension, 2urticaria and headhace).
Summary/Conclusions: Oral high dose liposomial iron support is safe, fast, well tolerated and cost-effective as intravenous iron in sideropenic anemia. This study needs confirmation on a larger cohort of patients.

E1485

NEXT GENERATION OSMOTIC GRADIENT EKTACYTOMETRY FOR THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS: METHOD VALIDATION AND FIRST DIAGNOSTIC EXPERIENCE OF TWO CENTERS

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Background: Osmotic gradient ektacytometry (Osmoscan) is part of the laboratory diagnosis process of hereditary spherocytosis (HS) and other red blood cell membrane disorders. A new generation ektacytometer, the LoRRca MaxSis, has recently become commercially available. We here present for the first time the experience of two independent institutions with this analyzer in HS diagnostic settings.
Aims: The objectives were (a) to validate the LoRRca MaxSis analytically independently in two centers, (b) to estimate the diagnostic accuracy of the various parameters available from the Osmoscan curve and their clinical application for HS diagnosis, (c) to establish a standardization of result reporting and cut-off values of Osmoscan parameters for HS diagnosis, and (d) to introduce the next generation osmotic gradient ektacytometry in the diagnostic work-flow of RBC membrane disorders.
Methods: Inter- and intra-assay variability and sample stability for different storage (4°C and 20°C) and anticoagulant conditions (*K2*-EDTA, *Li*-Heparin and acid-citrate-dextrose) were studied. In addition, we performed Osmoscan on samples from patients with HS (N=40), probable HS (N=21), auto-immune hemolytic anemia, AIHA (7), and patients with other pathologies (N=37). Daily control samples (n=54 in Erasme Hospital and n=80 in University Medical Center Utrecht) were run in parallel with patient samples. Laser-assisted measurements of RBC deformability under changing osmotic gradient and constant sheer stress were expressed and recorded as elongation index (EI). An Osmoscan curve was created with the following parameters: EI *min* (minimal elongation index), O *min* (the osmolality at EI *min*), EI *max* (the maximal elongation index), O *max* (the osmolality at EI *max*), O *hyper* (the osmolality in the hypertonic region at 50% of the EI *max*), EI *hyper* (the EI at O *hyper*), and *area under the curve* (AUC). Patient results for the various Osmoscan parameters were expressed as percent of change (increase or decrease) compared to the mean of controls.
Results: Analytical performance of the LoRRca MaxSis ektacytometer showed an inter-assay variability between 0.2% and 3%. Samples were stable for 48-72 hours depending on temperature storage and anticoagulant used. No difference was observed for any of the Osmoscan parameters between the "HS" group, "probable HS" and "AIHA" group. However, a significant difference was observed between the "HS" group and the "other pathologies" group for O *min*, EI *max*, EI *hyper*, and the AUC. The following diagnostic cut-offs were established for HS: an increase of more than 21.5% for the osmolality point at the minimal elongation index (O *min*), a decrease of more than 8.5% for the maximal elongation index (EI *max*), and a decreased area under the curve (AUC) of more than 18.5% compared to the mean of controls.
Summary/Conclusions: The next generation ektacytometer is an efficient tool for the laboratory diagnosis of HS. Samples are stable, thereby enabling long-distance shipping to specialized laboratories. Additionally, the proposed standardized reporting of results allows inter-laboratory exchange and comparison. Different parameters of the Osmoscan curve were found useful for HS diagnosis but particularly the AUC, the O *min* and the EI *max* parameters. Finally, we considered the introduction of the next generation ektacytometry Osmoscan in the diagnostic work-flow of RBC membrane disorders at two levels: (1) as a potent screening method in a general laboratory, and (2) as an effective intermediate step between the screening laboratory methods and the confirmatory protein deficiency tests or DNA-based methods in a reference laboratory.

E1486

NEURO-PSYCHIATRIC INVOLVEMENT IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Background: PNH is a rare disorder due to a deficiency in GPI–anchored proteins, characterized by haemolytic anaemia, marrow failure and thrombosis. Thrombotic events in PNH generally occur in unusual sites, such as hepatic, portal, mesenteric, splenic, and renal veins. Case reports of cerebral venous sinus thrombosis and arterial ischemic strokes are described in PNH. However, no systematic studies have been reported in asymptomatic patients
Aims: to investigate neuro-psychiatric involvement and neuroradiological findings in PNH patients
Methods: 17 PNH patients underwent non-enhanced cerebral magnetic resonance imaging (MRI), and intracranial arterial and venous angio-MRI. The following variables were evaluated: chronic ischemic small vessel disease, quantified according to ARWMC scale (Wahlund LO, Stroke 2001); focal alterations consistent with silent ischemic strokes; active or previous bleeding or micro-haemorrhages; atrophy. Moreover, abnormalities of the circle of Willis and its branches, and of cerebral venous sinus were evaluated. Psychiatric evaluation included the Brief Psychiatric Rating Scale, the Structured Clinical Interview 1 for DSM IV Axis Disorders (SCID-1), the Short Form (36) Health Survey, and the Trail making test
Results: Clinical and haematological parameters of patients are shown in table; 15/17 patients were classic haemolytic (60% transfusion dependent), and 2 PNH in the context of aplastic anemia (both transfusion-dependent until treatment with ATG-Cya). Abdominal pain was present in 41% (N. 1, 2, 4, 10, 11, 14, 15), and abdominal thrombosis in 18% (N. 2, 7, 10). Eculizumab was administered in 53% of cases for transfusion dependence, abdominal pain and/or thrombosis. On MRI, 10 subjects showed white matter (WM) abnormalities related to chronic ischemic small vessel disease. In particular, 5 subjects displayed periventricular WM vascular degeneration, and 6 deep WM focal chronic ischemic lesions. In one subject (N.10) a focal abnormality >5 mm was detected. The evaluation of WM and basal ganglia lesions (ARWMC scale) gave a score of 2 in 2 subjects (N.7, 17), a score of 4 in 3 subjects (N. 2, 10, 11), and a score of 5 in 1 subject (N. 9). No subject displayed active or previous bleeding. Two patients (80 and 81 yrs) showed atrophy of the cerebral hemispheres. Regarding vascular abnormalities, one subject (N.1) had hypoplastic left transverse sinus with irregularities in the sinus wall, suspected for prior partial venous thrombosis. Intracranial artery stenosis or aneurysm, and Moya–Moya like alterations were not observed. Finally, cerebral MRI was unremarkable in 7/17 subjects. Neurological clinical examination was normal in all patients. Psychiatric evaluation did not reveal any psychotic behaviour (except a doubt case in which emerged ideas of delusional thoughts), and test scores for visual attention and task switching resulted appropriate to age. SCID-1 highlighted the presence of a case of generalized anxiety disorder and the suspect of a case of bipolar disorder type 2. The SF36 results evidenced a better health perception than the norm, and even a perception of increased quality of life.

Table 1.

Patient N.	Year of diagnosis	PNH type	Data at diagnosis				Therapy with eculizumab, starting date	Data at last follow-up (2015/2016)			
			Age (yrs)	Hb (g/dL)	LDH (t>ULN)	PMN (% GPI neg)		Age (yrs)	Hb (g/dL)	LDH (t> ULN)	PMN (% GPI neg)
1	1996	haemolytic	48	12,0	9,5	48	yes, 2005	68	11,9	1,2	33
2	1997	haemolytic	31	8,2	4,3	63	yes, 2009	50	14,2	0,8	58
3	1997	haemolytic	31	11,4	na	87	yes, 2009	50	10,2	1,1	99
4	1999	haemolytic	24	9,9	1,5	33	no	41	8,6	6,7	80
5	2002	haemolytic	20	8,4	6,8	80	yes, 2008	34	8,4	0,9	97
6	2004	haemolytic	68	9,2	9,1	60	no	80	13,1	5,2	6
7	2005	haemolytic	43	6,7	5,4	42	no	54	11,1	2,2	76
8	2006	haemolytic	25	11,5	2,5	75	yes, 2007	35	12,7	1,6	nd
9	2006	haemolytic	63	8,1	4,0	67	yes, 2009	73	9	1	99
10	2006	haemolytic	50	10,8	7,8	20	no	60	13,9	1	13
11	2007	haemolytic	46	8,7	16,3	90	yes, 2008	55	11,2	1	92
12	2007	aplastic	31	9,6	1,2	14	no	40	10,6	4,4	75
13	2007	aplastic	49	7,9	1,9	56	no	58	12,1	4,7	89
14	2011	haemolytic	31	10,5	8,6	81	yes, 2011	36	10,5	1	94
15	2013	haemolytic	17	9,8	3,4	69	yes, 2014	20	12,5	1,1	66
16	2014	haemolytic	50	10,9	2,6	64	no	52	11,1	4,9	74
17	2014	haemolytic	80	8,1	3,1	93	no	82	11,1	4,0	nd

t>ULN: times over the upper limit of normal, nd: not done, na: not available

Summary/Conclusions: Although largely related to age, unexpected chronic ischemic white matter involvement was present in PNH, and particularly important in two subjects with severe haemolytic disease (N. 9 and 10), of whom one not in eculizumab. White matter lesion burden in asymptomatic patients may help the decision to start therapy, which is not always easy on the clinical/haematological basis.

E1487

SLEEP OBSTRUCTIVE APNEA SYNDROME (SOAS) IS NOT ASSOCIATED WITH MARKED ERYTHROCYTOSIS. A STUDY ON 337 PATIENTS

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Background: Normal values of hemoglobin concentration (Hb) and hematocrit (Ht) depend on gender only.
Aims: To check the role of age and frequent disorders that may influence erythropoiesis (renal function, iron metabolism and oxygenation abnormality).
Methods: This prospective study enrolled 337 patients (pts) aged 16 to 88 years (median 61, M/F=1.51) referred to the sleep unit of Hospital of Lens. All patients had arterial blood gas, blood cell count and routine chemistry. Apnea hypopnea index (AHI) was available in 250 pts.
Results: Severe SOAS (AHI>30/hour) was found in 121 pts (48% of evaluated pts). Median HB and HT was 13.3 g/dL (range from 8.5 to 17.2 g/dL) and 40.7% (range 18,1 to 51,4%) respectively. Only 5 pts presented with increased HB (>17.5 g/dL in male, >15.5 g/dL in female) and HT (>50% in male and >47% in female). Conversely, anemia (HB<13.5 g/dL in male,<12.5 g/dL in female or HT<40% in male,<37% in female) was present in 118 pts (35%): Renal failure (MDRD creatinine clearance<60 ml/min), iron deficiency (ferritin<30 µg/L) or

features of anemia of chronic disease (CRP>10 mg/L or ferritin >400 µg/L) were observed in 84 (25%), 58 (17%) and 72 (21%) pts respectively. Finally, 173 pts presented none of these disorders (no cause of anemia) and 219 pts presented without anemia according to HB and HT criteria (non-anemic pts). Female pts had lower HB and HT than male pts (median 12.6 g/dL and 38.5% *vs* 13.9 g/dL and 42.4%,respectively, p<0.0001). HB and HT were mainly influenced by age and also MDRD creatinine clearance (that takes age and gender into account) (table 1). The relationships between age and HB and HT were confirmed in linear models (R2=0.133 and 0.118, respectively, p<0.0001) and these inverse relationships were validated in an independent seris of 3892 patients referred in the medicine units of the Centre Hospitalier Universitaire of Amiens. Testing the lack of fit showed that the linear model was inadequate in the subgroup of male pts, and smoothing spline analysis showed an increase in the inverse relationship between HB and age in elderly male pts older than 60 years. AHI was the only respiratory parameter that influenced only HB (r=0.13085, p=0.047). There was no significant correlation between AHI and age, no significant difference in the distribution of AHI in male and female. Nevertheless, significant correlations were observed between AHI and HB and HT when the analyses were performed in the non anemic pts (r=0.20676, p=0.0067 and r=0.18340, p=0.0163 respectively) and in pts without cause of anemia (r=0.20092, p=0.0224 and r=0.16335 p=0.0644 respectively).

Table 1.

Characteristics	HB		HT	
Age	r= -0.34650	p<.0001	r= -0.32086	p <.0001
Creatinine clearance (MDRD)	r=0.23686	p<.0001	r=0.21106	p=0.0003
Ferritin	r=0.30928	p<.0001	r=0.13743	p=0.016
CRP	r=-0.12961	p=0.0227	r=-0.10795	p=0.058
AHI	r=0.13085	p=0.047	r=0.11799	p=0.0735

Summary/Conclusions: This prospective study showed an inverse relationship between age and HB and HT. We confirmed the rarity of marked erythrocytosis in pts with SOAS and the weak correlation between AHI and HB and HT. Our data suggested that SOAS pts also frequently present with characteristics that may play a part in reducing HB and HT. Thus, checking the presence or the absence of associated comorbidity should be considered in SOAS pts with erythrocytosis.

E1488

"SEPARATING THE WHEAT FROM THE CHAFF" CONGENITAL HEMOLYTIC ANEMIA STUDY WITH A TARGETED NEXT GENERATION SEQUENCING PANEL

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Background: Diagnosing a Coombs negative hemolytic anemia (HA) can be a true challenge, specially in the absence of family history or when the peripheral blood smear is not very informative. A few years ago, we had to sequence gene by gene by Sanger methodology, which was time costly. Nowadays, with Next Generation Sequencing (NGS) panels it is much faster, nevertheless we may be drowned in a large number of putative pathological mutations. The concept of one mutation (or two for the autossomal recessive disorders) one disease, have changed and now we have to deal with pathological variations or not, or modulating factors. As a reference laboratory dedicated to the study of benign red blood cell disorders (RBC) we have many of HAs with an identified molecular lesion, but also several cases, that even though thoroughly screened for hemoglobinopathies, enzymes and membrane defects remained without identification of the pathological mutations. This has implications at prognostic and therapeutic level, and makes the correct genetic counseling impossible.
Aims: Identify the molecular causes in a group of 14 patients with congenital HA, after screening for the most common hemoglobinopathies, enzymopathies and membranopathies.
Methods: Informed consent was signed for all the individuals in the study. We have used a targeted NGS panel encompassing the exonic regions of 24 genes (del Orbe et al, 2015) and sequenced 14 samples from patients with a congenital HA. In all the samples the common screening tests for RBC disorders were preformed, like high performance liquid chromatography (HPLC) for Hb variants, enzymatic quantification and membrane disorders screening tests as cryohemolysis test and eosin-5'-maleimide. The variants found were classified using *in sillico* analysis and the presence of pathological and likely pathogenic variants were confirmed by Sanger sequencing and, when available, familial studies were performed.
Results: All the samples showed a great number of variants, the majority were benign or likely benign (a media of 320/sample) or of unknown significance or likely pathogenic (media of 25/sample). Only 8 samples showed pathologic variants that can justify the clinical presentation. Most of them are associated with membrane disorders (Table). In 5 of the samples no pathological mutations were found, in one sample (sample 11) we found a *NT5C3A* missense mutation but in the heterozygous state (Sanger sequencing of all gene also didn't reveal any other mutation). Three samples are compound heterozygous for mutations in the *SPTA1* gene, three have a combination of mutations in different genes affecting membrane structure, one is a compound heterozygous for two missense *CDAN1* mutations not described before. Sample 5 is form a sickle cell disease patient with an uncommon presentation in the newborn period, a *PIEZO1* mutation inherited from the mother and a *EPB41* mutation inherited from the father are likely to be the causes of the aggravated phenotype.

Table 1. Results of the NGS sequencing-pathological variants.

Sample ID	Gene	Mutation
Sample 1	*SPTA1*	p.Gln2146Arg / p.Tyr653Cys
Sample 2	*SPTA1*	p.Ala1945Pro / p.Glu671Stop/ p.Leu1485Phe
Sample 3	no mutations	
Sample 4	*SPTA1*	p.Ser1163Ala;
	ANK1	p.Gly460Glu
	SPTB	p.Asn1151Asp
	CDAN1	p.Ala448Gly
Sample 5	*HBB*	HbS/ HBD
	PIEZO1	p.Arg2476Cys
	EPB41	p.Arg409His
Sample 6	no mutations	
Sample 7	*SPTA1*	p.Gln2146Arg / p.Tyr653Cys
	ANK1	p.Val1318Met
Sample 8	no mutations	
Sample 9	*SPTA1*	p. Leu1485Phe / p. Leu1858Val
Sample 10	*SPTA1*	p. Leu1858Val/ p. Leu1858Val / p.Ala970Asp
	ANK1	p.Asn251Lys
Sample 11	*NT5C3A*	p.Asp283His
Sample 12	no mutations	
Sample 13	no mutations	
Sample 14	CDAN1	p.Trp1184Ter / p.Tyr713Ser

Summary/Conclusions: After *in sillico* analysis of the variants found by NGS (almost 350 per sample) and confirmation by Sanger sequencing of the pathological variants, we have identified a molecular cause for the HA in 8 of the 14 samples studied. This study confirms that target NGS is the most complete and cheaper method for sequencing the genes associated with AH but, for the cases that remain without mutations, whole exome sequencing or whole genome sequencing need to be done, in order to identify new genes that can later be added to the AH panel.

E1489

SERUM HEPCIDIN-25 IN COMPARISON WITH BIOCHEMICAL MARKERS AND HAEMATOLOGICAL INDICES FOR THE DIFFERENTIATION OF ANAEMIA STATES AND CORRELATION WITH RESPONSE TO ORAL IRON

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Background: Hepcidin is a key regulator of iron metabolism and may be a useful analyte in the differentiation of iron states in anaemia. Hepcidin is down regulated in the presence of iron deficiency to promote iron absorption and is up regulated when iron stores are in excess or in the setting of inflammation via IL-6. In combination with hypochromia indices and other markers of iron deficiency such as reticulocyte haemoglobin (Ret-He), a raised hepcidin level may provide evidence of both reduced iron absorption and iron restricted erythropoiesis. Conversely, a low or normal hepcidin level may be suggestive of true iron deficiency. In recent years immunoassays have become more readily available, allowing hepcidin to complement existing routine biochemical indices to differentiate anaemia states.
Aims: The primary aim of this study was to assess whether there was a correlation between hepcidin and markers of iron status in patients referred via the iron deficiency clinic at Plymouth Hospitals NHS Trust. The second objective was to investigate the association of baseline hepcidin-25 with response to oral iron.
Methods: Patients referred to the iron deficiency anaemia clinic by their GP were prospectively recruited. A clinical history, examination, blood tests including haematological indices and biochemical markers, appropriate investigations and oral iron supplementation were prescribed where appropriate. To determine the response to oral iron, the full blood count was repeated at 1 and 3 months. For the measurement of hepcidin-25, serum was frozen at -70C and then determined by batch analysis using a commercial ELISA kit (DRG Instruments, Marburg, Germany). Patients were classified as having iron deficiency anaemia (IDA), anaemia of chronic disease (ACD) or the combined state of iron restricted erythropoiesis (IRE) with ACD (ACD/IRE) using clinical findings and standard biochemical markers and haematological indices.
Results: The Hepcidin-25 immunoassay performed well, with a low within- and between- assay coefficient of variation. The table shows the numbers and characteristics of the patients allocated to the 3 types of anaemia. Numbers given

as median and interquartile range. Hepcidin concentrations correlated significantly with ferritin concentrations. Serum hepcidin was less than 1 ng/mL in 80 of 85 patients with IDA. This is consistent with previous data. There were no linear associations seen between hepcidin and other indices of anaemia (Total Hb, MCV, MCH, Ret-He, Transferrin, Transferrin saturation, Iron and CRP). 96 patients had a trial of oral iron, with 65 responding (increase of >10g/l by 12 weeks) and 31 failing to achieve an adequate rise in haemoglobin. There was no correlation between response to oral iron and hepcidin.

Table 1.

Variables	IDA	ACD	ACD/IRE
Number	85	30	15
Age	72 (61-80)	76 (65-79)	79 (64-79)
Haemoglobin (g/L)	109 (99-120)	112 (98-121)	111 (106-117)
MCV (fL)	80.4 (76.2-84.9)	86.7 (83.8-90.7)	82.5 (77.5-83.8)
MCH (pg)	25.1 (23.6-27.1)	28.1 (26.6-29.8)	26.1 (24.7-27.7)
Ferritin (ug/L)	12 (9-16)	119 (63-256)	40 (34-72)
CRP (mg/L)	2 (1-5)	9 (2-56)	4 (2-14)
Transferrin saturation (%)	7.29 (5.67-11.13)	16.45 (9.87-21.93)	13.72 (11.41-18.51)
Ret-He (pg)	27.6 (24.9-30.5)	31.9 (28.5- 35.6)	31.6 (30.1-32.9)
Hepcidin (ng/mL)	0 (0 - 0)	5.4 (0 - 75.4)	0 (0 - 1.268)

Summary/Conclusions: A positive correlation exists between Hepcidin-25 and serum ferritin. The majority of patients in this study had iron deficiency anaemia and correspondingly low hepcidin concentrations. A low hepcidin did not correlate with subsequent response to oral iron therapy. Further investigation, however, of hepcidin in anaemic patients with normal to high ferritin levels is warranted to further evaluate the role of this hormone in the management of these patients.

E1490

EFFECT OF ZINC SUPPLEMENTATION ON GLUCOSE METABOLISM, OXIDATIVE STRESS AND IRON TRAFFICKING PROTEINS IN TRANSFUSION DEPENDENT PATIENTS WITH THALASSEMIA

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Background: Thalassemia (Thal) is a genetic anemia of incomplete erythropoiesis causing iron overload and roughly 25% of patients to be marginally zinc (Zn) deficient. Nearly 30% of adult subjects with Thal will develop diabetes, thought to be related to transfusional iron overload, but the diabetogenic effects of altered Zn status are not well known. It is hypothesized that a functional Zn deficiency in Thal may affect insulin secretion, glucose homeostasis and/or oxidative stress. Oxidative stress can be manifested by labile plasma iron (LPI), a component of non-transferrin bound iron that is often found in Thal suffering from iron overload. LPI is both redox-active and chelatable, and is the likely culprit of distributing iron to peripheral tissues inducing tissue iron overload. LPI can catalyze lipid peroxidation and release malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) from cells, which are lipid peroxide damage-associated molecular patterns that indicate tissue damage and induce inflammation. To monitor the proteins that may traffic this free iron we used an advanced proteomics technique, measuring soluble transferrin receptor (sTfR), transferrin, haptoglobin, hemopexin and inflammatory proteins C-reactive protein (CRP) and Immunoglobulin G (TgG).
Aims: The purpose of this pilot project is to determine Zn supplementation effects on 1) circulating levels of oxidative stress and iron trafficking proteins and 2) glucose homeostasis and insulin secretion in Thal.
Methods: 39 subjects with informed consent were enrolled (9 Thal with diabetes (DM), 20 Thal without DM, 10 controls). Thal on intervention (60% Female, 28.4±11.0y) had a 2 hr OGTT at 3 time points (0, 3 and 6 mos) and took 25 mg Zn/d between 3 and 6 mos. 30% of Thal and 44% of DM had low serum Zn at baseline. LPI was measured using fluorogenic dihydrorhodamine 123 (measures reactive radicals) with desferrioxamine as a chelator. Both MDA and MDA+4-HNE was measured using N-methyl-2-phenylindole. Iron trafficking proteins were measured by MRM mass spectrometry.
Results: C-peptide levels were significantly reduced in DM compared to controls (p=0.002) as were lipase levels compared to controls (33 *vs* 21 IU/L, p=0.04). Serum Zn was related to beta cell function by the Homeostatic Model Assessment (HOMA) (r=0.45, p=0.049) and in subjects with low serum Zn supplementation improved insulin response to the glucose challenge (p=0.04). LPI, MDA, and 4-HNE were significantly elevated in Thal *vs* Control (4.95µM±3.12 *vs* -1.81±2.45 p=0.0004; 4.8 *vs* 3.0 µM, p=0.005; 9.37±2.94 *vs* 13.9±5.84 µM, p=0.024). MDA patients with low iron levels at baseline were found to have an increase in MDA over time *vs* those with high iron (p=0.005). LPI, MDA, and 4-HNE comparisons between all three time points of Zn therapy in Thal patients–with results of both Control and DM patients–did not show significant differences. However, significant differences were noted in Thal patients *vs* Controls for the following iron trafficking related proteins: sTfR, transferrin, haptoglobin, hemopexin and TgG (p<0.05).
Summary/Conclusions: These preliminary data suggest that in patients with Thal, Zn status is related to insulin resistance and beta cell function, possibly related to circulating LPI, oxidative stress and some iron trafficking proteins such as sTfR or hemopexin. More results are needed to explore how non-invasive therapy may improve glucose tolerance in Thal patients.

E1491

AN SPECKLE TRACKING IMAGING REVEAL MYOCARDIAL IRON OVERLOAD IN THALASSEMIA MAJOR? A COMBINED ECHOCARDIOGRAPHY AND CARDIAC MAGNETIC RESONANCE STUDY

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Background: Cardiac complications related to myocardial iron overload (MIO) remain the main cause of morbidity and mortality in thalassemia major (TM). Cardiac magnetic resonance (CMR) is a unique non-invasive technique to quantify MIO and the multislice T2* technique allows the identification of different patterns of MIO. Unfortunately, the availability for CMR scans in validated centers is still limited in many countries. On the other hand, echocardiography is a widely diffused, non-expensive and feasible technique, with elevated accuracy in the evaluation of cardiac function and morphology. Nevertheless, standard echocardiographic examination fails in detecting MIO until a substantial reduction of the left ventricular ejection fraction (LVEF) occurs. The evaluation of myocardial deformation by two-dimensional speckle tracking imaging (2DSTI) demonstrated a great accuracy in detecting subtle myocardial dysfunction in many different pathologic conditions.
Aims: We aimed to investigate the role of 2DSTI in the detection of MIO in patients affected by TM, comparing the data of myocardial deformation with T2* values derived by CMR.
Methods: We recruited 31 TM patients [15 males (48,4%); mean age: 37.87±9.64 years] consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. All patients underwent to CMR and to echocardiography in the same day. CMR was performed with a 1.5-T scanner and segmental and global T2* values were measured with a previously validated software (HIPPO MIOT®). Values of GLS were derived from the three apical views, while radial and circumferential strain were obtained from the three parasternal short axis views. All the echocardiographic examinations were performed with a commercial ultrasound system (Mylab-alpha, Esaote) equipped with a 1-4 Mhz phased array probe.
Results: Mean global heart T2* was 35.91±12.69 ms (range:5.63-47.13 ms). Six patients (19.4%) showed pathologic T2* values (<20 ms) indicative of MIO. GLS showed a significant correlation with T2* values (R=-0.401; P=0.025), moreover, the percentage of patients with altered GLS (lower or equal -19) was significantly higher in the group with a significant MIO than in the group with no significant MIO (global heart T2* ≥20 ms) (83% *vs* 28%, P<0.05). Logistic regression demonstrated that patients with impaired GLS had a significant higher risk of showing pathological T2* values (Odds-ratio-OR=12.86, 95%CI=1.27-130.54; P=0.031). No relation was observed between GLS, age and sex, and between T2* values, LVEF, radial strain and circumferential strain.
Summary/Conclusions: Left ventricular GLS can be useful in detecting subtle myocardial dysfunction due to MIO in TM patients, demonstrating a significant correlation with MIO detected by CMR.

E1492

THE IMPACT OF CARDIAC AND HEPATIC MRI ASSESSMENT ON THE CLINICAL MANAGEMENT OF AUSTRALIAN PATIENTS WITH TRANSFUSION DEPENDENT ANEMIAS OR NON-TRANSFUSION-DEPENDENT THALASSEMIA IN THE TIMES STUDY

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Background: Iron overload can lead to impaired organ function and is associated with significant morbidity and mortality, the risks of which can be reduced by effective long-term iron chelation therapy (ICT) and control of iron loading. Magnetic resonance imaging (MRI) allows accurate, reproducible assessment of iron load and its use may affect clinical management decisions, leading to improved patient care. The epidemiological TIMES study used MRI to assess prevalence and severity of cardiac and hepatic siderosis in a large population of Australian patients with transfusion-dependent anemia or non-transfusion-

dependent thalassemia (NTDT). In this analysis of the TIMES study, we report the impact of MRI results on investigator treatment decisions.
Aims: To determine the prevalence of iron overload by MRI and its impact on the clinical management of iron overload in a population of patients with transfusion-dependent anemia or NTDT.
Methods: Patients with thalassemia major (TM), NTDT (β thalassemia intermedia, β thalassemia/Hb E, Hb H disease), myelodysplastic syndromes (MDS) or other chronic anemias were enrolled. Patients with NTDT had serum ferritin (SF) >300 ng/mL; others had a lifetime history of ≥20 units red blood cell (RBC) transfusions and SF >500 ng/mL. Past medical history was collected (including red blood cell (RBC) transfusion, ICT and hematologic data). Prospective MRI (FerriScan) was used to determine R2 liver iron concentration (LIC) and myocardial T2* (mT2*). Treatment decisions were assessed and recorded using an investigator questionnaire after evaluation of patient MRI results.
Results: Of the 243 enrolled patients, 10 and 48% had cardiac and hepatic siderosis, respectively. In all disease groups, mean LIC was above the target range (3-7mg Fe/g dw), while mean mT2* was normal (≥20ms). 65.8% of patients received ICT for ≥1 month before or during the study. During the 12-month period prior to the study, 55.9% received deferasirox, 11.9% received deferoxamine and 2.5% received deferiprone. All patients with TM had received ICT; among patients with MDS, NTDT or other anemia types, some patients were chelation naïve (CN)/minimally chelated (MC, chelation<1 month in lifetime; Table). MRI assessment led to a change in management in 105 (45.9) of all evaluable patients (n=229) and in ~60% of CN/MC patients with MDS (17/27 [63.0%]) and other anemias (22/37 [59.5%]; Table). Across all diseases, the predominant changes were increasing chelator dose (43/75 [57.3%]) and starting chelation (27/75 [36.0%]; Table). In patients who had received ICT for >1 month, the most frequent change was increasing chelator dose (TM, 20/23 [87.0%]; MDS, 11/16 [68.8%]; NTDT, 4/7 [57.1%]; other anemias, 5/6 [83.3%]). In CN/MC patients, starting ICT was the predominant change (MDS, 10/13 [76.9%]; NTDT, 2/2 [100.0%]; other anemias, 8/8 [100.0%]). Cardiac MRI assessment (mT2*) was the key driver for ICT decisions in patients with TM (63.8%; ranked as the most important factor in the treatment decision); however, LIC was the key determinant in patients with MDS (42.0), NTDT (44.4%) and other anemias (56.3%).

Table 1.

Table. Summary of MRI-based treatment decisions

Change to ICT	Overall	TM	MDS		NTDT		Other anemias	
Any change, n (%)	n=229	ICT n=77	CN/MC n=27	ICT n=42	CN/MC n=11	ICT n=7	CN/MC n=37	ICT n=28
Yes	105 (45.9)	26 (33.8)	17 (63.0)	19 (45.2)	3 (27.3)	7 (100.0)	22 (59.5)	11 (39.3)
No	124 (54.1)	51 (66.2)	10 (37.0)	23 (54.8)	8 (72.7)	0 (0.0)	15 (40.5)	17 (60.7)
Type of change, n (%)	**n=75**	**ICT n=23**	**CN/MC n=13**	**ICT n=16**	**CN/MC n=2**	**ICT n=7**	**CN/MC n=8**	**ICT n=6**
Start ICT therapy	27 (36.0)	0 (0.0)	10 (76.9)	4 (25.0)	2 (100.0)	3 (42.9)	8 (100.0)	0 (0.0)
Stop ICT therapy	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Increase ICT dose	43 (57.3)	20 (87.0)	3 (23.1)	11 (68.8)	0 (0.0)	4 (57.1)	0 (0.0)	5 (83.3)
Decrease ICT dose	2 (2.7)	1 (4.3)	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Switch to other ICT	2 (2.7)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)
Change ICT delivery	1 (1.3)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Patient numbers based on evaluable patients. CN, chelation naïve; ICT, iron chelation therapy; MDS, myelodysplastic syndromes; MC, minimally chelated; MRI, magnetic resonance imaging; NTDT, non-transfusion dependent thalassemia; TM, thalassemia major

Summary/Conclusions: These data provide real-life insight into the impact of MRI on treatment decisions in a large population of patients with heterogeneous causes of chronic anemia receiving RBC transfusions. A change in ICT management due to MRI analysis occurred in nearly half of all patients. This emphasizes the importance of accurate monitoring of iron load in patients with transfusion-dependent anemias or NTDT to allow for informed clinical decision making.

E1493

HOME-BASED CONTINUOUS 24-HOUR DEFEROXAMINE VIA A PERIPHERALLY INSERTED CENTRAL CATHETER IN SEVERE AND REFRACTORY IRON OVERLOAD: A CONVINIENT AND EFFECTIVE APPROACH IN THALASSEMIA MAJOR PATIENTS

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Background: Severe iron overload leads to significant morbidity and mortality in β-thalassemia major (TM) patients. Intensive chelation is required in these patients to reduce iron to lower risk thresholds and avoid the development or worsening of end-organ dysfunction. Continuous intravenous deferoxamine (DFO) infusion in the inpatient setting remains the only management option in patients who fail to respond to high chelator doses, combination therapy, or those with symptomatic dysfunction requiring rescue therapy. The approach, however, may be associated with inconvenience and time lost in prolonged hospital admissions.
Aims: To evaluate the efficacy and safety of home-based continuous 24-hour DFO infusion via a peripherally inserted central catheter (PICC) in TM patients with severe and refractory iron overload.
Methods: This was a retrospective cohort study of TM patients attending our center between October 2013 and August 2015 who had received home-based continuous 24-hour DFO infusion via PICC. To receive such therapy, patients had to have no contraindication to PICC line insertion of DFO. They also had to have severe iron overload with evidence of no response to mono or combined therapy with subcutaneous DFO, defeiprone, or deferasirox. Severe iron overload was defined as a liver iron concentration (LIC) >15 mg/g, cardiac T2*<8 msec, or cardiac T2* 8-15 msec with any of the following: a left-ventricular ejection fraction (LVEF)<45% per echocardiogarphy, cardiac arrhythmia, compensated or decompensated clinical heart failure, or two or more endocrinopathies. The dose of DFO was 40 mg/kg titrated based on the index (<0.025) by Porter el al, administered with or without concomitant oral chelation.
Results: A total of 41 patients received home-based continuous 24-hour DFO infusion via PICC during the observation period (mean age 28.4±5.6 years, 51% male). Among these, 9 (22.0%) discontinued therapy, 7 (17.1%) only had<6 months follow up, while 25 (61.0%) patients had >6 months follow up and were included in efficacy analysis. Seventeen (42%) received concomitant deferasirox and 21 (51%) received deferiprone. Reported adverse events in the 41 patients included: local reaction (n=17), renal tubular acidosis (n=6), arthritis (n=4), line thrombosis (n=4), line displacement (n=4), local infection (n=4), systemic infection (n=2), retinopathy (n-1). None of the patients required hospitalization, developed new endocrine complications or progressed to advanced cardiac disease and no treatment related mortality was observed.
In the efficacy cohort (n=25, mean age 28.2±5.0 years, 36% male), patients had receive treatment for an average of 431±137 days. There was a significant and remarkable decrease in mean serum ferritin level (10848±5872 to 4095±4116 ng/mL, p=0.0001) and LIC (37.5±9.9 to 12.6±11.0, p=0.001) and a significant increase in cardiac T2* (8.1±3.5 msec to 12.4±6.3 msec, p=0.002). Heart failure was reversed in 2 out of 3 patients and LVEF improved from a mean of 40% to 60%. Eight out of 9 insulin-dependent diabetic patients had insulin dose reduction (mean reduction 34.3±0.2 Units), while one patient shifted to an oral hypoglycemic. All of the eight patients on oral hypoglycemic agents prior to treatment manged to stop it successfully.
Summary/Conclusions: Home-based continuous 24-hour DFO infusion via PICC is an effective treatment option in TM patients with severe iron overload that could not be controlled otherwise by other chelation regimens. The convenience of home-based therapy further promotes the role of this therapy in such high risk patients, although data from prospective clinical trials remain of merit.

E1494

A STUDY OF BONE AND JOINT INVOLVEMENT IN THALASSEMIA MAJOR

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Background: Thalassemia major is an inherited hemoglobinopathy causing imbalance in globin chain synthesis, ineffective erythropoiesis and increased peripheral haemolysis. Thalassemia patients have a variety of bone and joint disorders like bone pain, deformity, growth failure, rickets, scoliosis, spinal deformity, nerve compression, pathological fractures, osteopenia and osteoporosis. There is a paucity of data about the extent of bone and joint disorders in thalassemia in developing countries.
Aims: 1. To determine bone and joint involvement in thalassemia major. 2. To evaluate serum biochemical parameters of bone formation and resorption and their relation with bone and joint formation. 3. To evaluate DEXA scan scores of Thalassemia patients and find a correlation, if any, with daily physical activity.
Methods: The study was carried out on 40 thalassemia patients between 5-18 years of age under regular follow up at our centre. All patients were interviewed as per a well-structured proforma for symptoms relating to bone and joint disease as described by the patient/parents in past one year and physical activity by QAPACE questionnaire. Venous blood samples were drawn under aseptic conditions and used for estimation of biochemical parameters (calcium, phosphorus, alkaline phosphatase, vitamin D, parathyroid hormone, thyroid hormone and serum ferritin). Enrolled subjects were scanned for bone mineral density (BMD) at femur neck, trochanter and Ward's angle using dual energy X ray absorptiometry.
Results: A total of 40 patients were enrolled in the study (M:F=80:20). Half the patients (n=20) had symptoms pertaining to bone and joint disease. The most common symptom was leg pain (42.50%), followed by backache (22.50%), generalized weakness (20%) and joint pains (20%). Asymptomatic patients had greater mean serum vitamin D as compared to the symptomatic patients (22.2 *vs* 15.08 ng/ml)(p= 0.225). Five patients were detected to have osteopenia, all above 10 years of age. The mean BMD in symptomatic patients at femur neck, trochanter and ward's angle was 0.781, 0.639 and 0.735 g/cm^2 respectively as compared to 0.754, 0.635 and 0.722 g/cm^2 in asymptomatic patients. The difference was not statistically significant. Patients with low BMD had lower mean pretransfusion Hb (<9 g/dl) (p= 0.01).Eighty percent of osteopenic patients had sedentary lifestyle as compared to 44% of patients with normal BMD. Bone mineral density did not have any correlation with vitamin D levels, serum ferritin and type of chelation therapy.

Summary/Conclusions: Pre transfusion hemoglobin of more than 9 g/dL has a protective role in maintaining good bone health and bone mineral density and helps to prevent osteopenia. Engaging thalassemia patients in physical activity has a positive effect on bone mineral density. Low vitamin D levels contribute to symptoms of bone and joint involvement in thalassemia and the same needs to be determined and supplemented.

E1495

EVALUATION OF WHILE MATTER INTEGRITY BY DIFFUSION TENSOR MRI AND NEUROCOGNITIVE FUNCTIONS IN NON-STROKE CHILDREN WITH SICKLE CELL DISEASE

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Background: Cerebral vasoocclusion may result in overt or silent infarctions, whether in absence of infarction, it may induce subtle ischemic changes need to be determined. Conventional brain MRI cannot precisely delineate microstructural changes and the white matter tracts of the brain. Diffusion-tensor imaging (DTI) can detect and quantify microstructural brain changes.
Aims: We aimed to evaluate the ability of multiple diffusion measures to detect subtle changes in the white matter integrity in patients with SCD compared with age and sex matched healthy control subjects; and to correlate the MRI findings with neurocognitive functions.
Methods: We performed a cross-sectional case control study including twenty one patients with SS and Sβ0 with age between 5-15 years. All patients were free from any neurological symptoms with no history of stroke. They were subjected to history taking and revision of hospital records for details of diagnosis including age, hemoglobin electrophoresis, transfusion, hydroxyurea, hospital admission and SCD complications. Diffusion tensor MRI (DTI) were done for all patients and ten age matched healthy control subject. Fractional anisotropy (FA) and apparent diffusion coefficients (ADCs) were calculated in regions of interest selected in various brain areas (superior and inferior frontal, parietal, occipital, and temporal white matter areas), centrum semiovale, basal ganglia (lentiform nucleus), pons, cerebellar white matter areas. Transcranial doppler was done for the patients with assessment of flow velocities in different brain regions. Cognitive assessment was done using the following tests, Wechsler intelligence scale for children for measuring IQ, the Benton visual retention test for visual memory and Wisconsin card sorting test is used as a measure of 'executive' or higher-order cognitive functions.
Results: Patients group included 7females (33.3%) and 14 males (66.7%), with mean age of 11.2±3.2 years, weight for age SDS was (-0.029±1.6), height for age SDS (-0.243±1.4) and median age at diagnosis was 18 months. As regards admission etiologies over the last year, 19 patients had vaso-occlusive crises (90.5%), 3 patients had acute chest syndrome(14.3%). Twelve patients were receiving frequent transfusions (57.1%). Patients with SCD had mean full scale IQ, verbal and performance IQ below 90 and there is no significant difference between patients and control group in full scale IQ (P= 0.892), verbal IQ (P= 0.759) and performance IQ (P =1). Concerning executive functions tested by WCST there was no significant difference in all parameters of the tests between patients and control group except for (non preservative errors) (P= 0.045). Regarding MRI DTI there was no significant difference between patients and control group in both FA and ADC in superior and inferior frontal, temporal, parietal, occipital, basal ganglia and cerebellum.No significant difference in FA and ADC results in patients with CBF velocity less and above 70cm/sec. There was positive correlation between difference in errors in BVRT and ADC of right frontal lobe (r=0.458) and parietal lobe (r=0.455). While there was negative correlation between ADC of right temporal lobe and FSIQ (r=-0.452), VIQ (r=- 0.518) and positive correlation between FSIQ and FA of left temporal lobe (r=0.475).
Summary/Conclusions: In absence of clinical evidence of cerebral infarction, white matter integrity by MRI DTI and neurocognitive functions in children and adolescents with SCD were preserved compared with healthy control group.

E1496

PROHEPCIDIN CONCENTRATIONS IN GAUCHER DISEASE AND FABRY DISEASE: INVESTIGATING THE ROLE OF HEPCIDIN IN ABNORMAL IRON HOMEOSTASIS AND ANAEMIA

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Background: Hepcidin is a 25-amino acid peptide first identified in 2001. It is recognised to have a key role in the regulation of iron stores, acting on duodenal enterocytes, macrophages and hepatocytes. Hepcidin levels have been found to respond to inflammatory cytokines (IL-6), iron status, erythropoetic activity and oxygen tension. Its precursor, prohepcidin, is synthesised by the liver and can be measured by a commercially available ELISA. Gaucher disease (GD) and Anderson-Fabry disease (AFD) are lysosomal storage disorders, a heterogenous group of diseases resulting from an inherited defect in a specific lysosomal enzyme or associated proteins. Haematological abnormalities including anaemia and disordered iron homestasis are recognised in GD. AFD is more commonly associated with organ dysfunction, but anaemia has also been described.
Aims: The Lysosomal Storage Disorders Unit at the Royal Free Hospital, London, has a unique cohort of patients with GD and AFD. By measuring serum prohepcidin levels, along with haematological indices and levels of circulating inflammatory cytokines, we sought to investigate the role of hepcidin/prohepcidin in GD and AFD. We looked specifically at whether it may have a role in the pathophysiology of the disordered iron profile and anaemia associated with GD.
Methods: Consecutive samples from patients with GD (n=33) or AFD (n=41) and normal control subjects (n=10) were obtained. All patients gave informed consent. Commercial ELISA kits were used to measure soluble transferrin receptor (Biovendor Medicine Inc) and IL-6 (BD Biosciences). A commercial ELISA for prohepcidin (DRG International Inc) was used.
Results: A total of 33 patients with GD were included, with 27 receiving enzyme replacement therapy (ERT). A total of N= 41 patients with AFD were included, of which 26 were receiving ERT. All groups had mean haemoglobin (Hb) within normal reference ranges. Mean soluble transferrin receptor (sTfR) levels were below lab reference ranges (1.8-4.6mg/L) for both ERT and non-ERT receiving patients in GD and AFD groups (GD on ERT mean 1.51mg/L, no ERT 1.47mg.L, AFD on ERT mean 1.04 mg/L, no ERT 0.93mg/L). GD patients had mean ferritin levels elevated above the upper limit of normal reference range (Non-ERT group mean=453 µg/L, ERT group mean 398 µg/L). For AFD patients, mean ferritin was within in the normal range for both ERT (88 µg/L) and non-ERT (54 µg/L) receiving groups. In all groups, there was a wide range of serum prohepcidin levels (figure 1). Mean prohepcidin was lower in both AFD and GD groups *vs* controls, but this did not reach statistical significance in any group. There was no correlation between prohepcidin and Hb, serum IL-6 or serum sTfR in any group.

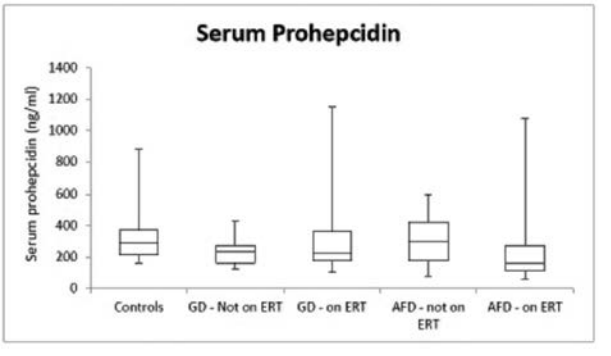

Figure 1.

Summary/Conclusions: Our study confirms previous results that GD is associated with a hyperferritinaemic state which is not found in AFD. As sTfR levels in GD and AFD patients were low, this appears to be secondary to inflammation rather than iron status. As in previous studies, we found IL-6 levels to be higher in GD patients then controls. Anaemia was not prevalent in any patient groups, although this may be masked by treatment and appropriate transfusion in a carefully monitored patient group. There was no significant difference between prohepcidin levels in AFD or GD patients and controls, and no correlation between Hb, IL-6, or iron status with prohepcidin in any group. The extent to which prohepcidin reflects hepcidin itself is controversial, but there is a growing body of evidence that prohepcidin levels are affected by inflammatory conditions, iron deficiency anaemia and changes in EPO levels, amongst other things. Further studies, with a higher number of patients, and using an ELISA targeted against hepcidin itself are required to further explore what role it may play in AFD/GD patients.

LB2267

L-ARGININE SUPPLEMENTATION DECREASES HEMOLYSIS AND PULMONARY ARTERIAL HYPERTENSION IN SICKLE CELL DISEASE PATIENTS: A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED TRIAL

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Background: Low nitric oxide (NO) bioavailability contributes to vasculopathy in sickle cell disease (SCD), and is associated with increased morbimortality. Since the obligate substrate for NO production is arginine and enhanced NO bioavailability markedly decreased hemolysis and oxidative stress in SCD, we

hypothesized that oral L-arginine (LA) supplementation could be beneficial for SCD outcomes and a reduction the in degree of hemolysis.
Aims: We assessed the impact of 6 months of LA supplementation on SCD clinical outcomes: leg ulcers, priapism, pulmonary arterial hypertension (PAH), and vaso-occlusive pain episodes, and the degree of hemolysis rate.
Methods: Sixty-eight patients with SCD were randomized for this double-blinded placebo-controlled trial. Patients received LA (0.1g/kg/day) or placebo for 6 months.
Results: The LA group presented more pronounced decrease in lactate dehydrogenase (DHL) (1065±495 U/L to 784±267 U/L vs. 952±383 U/L to 907±395 U/L; p=0.03) and a tendency of PAH reduction. Ten patients of the study group had PAH (6 in the LA group and 4 in the placebo group). The tricuspid regurgitant jet velocity (TRV) in the 6 patients receiving LA decreased from 2.83±0.36 m/s to 2.46±0.36 m/s, while the TRV in the 4 patients receiving placebo increased from 2.75±0.35 m/s to 2.80±0.18 m/s (p=0.13), representing a tendency of PAH reduction in the LA group. Non drug-related severe adverse events were perceived.
Summary/Conclusion: In conclusion, supplementation of LA was associated with a significant reduction in hemolytic activity with and a tendency towards reduced PAH. Weighing outlays and benefits, these preliminary results provide a strong rationale for therapeutic use of LA in SCD disease and other chronic hemolytic diseases, although an extended multi-center trial with larger number of SCD individuals is warranted to confirm these findings.

LB2268

HOW SHOULD WE SELECT THE MOST VULNERABLE THALASSEMIC PATIENTS FOR IRON OVERLOAD EVALUATION BY MRI IN A RESOURCE LIMITED COUNTRY?

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Background: Serum ferritin (SF) can be used for determination of iron overload (IO), however, it does not perfectly correlate with tissue iron status in transfusion-dependent thalassemia (TDT) and even worst in non-transfusion-dependent thalassemia (NTDT). The magnetic resonance imaging (MRI) for cardiac T2* and liver iron concentration (LIC) is now considered as a gold standard for IO evaluation in thalassemia worldwide. Nevertheless, this tool was not widely available and expensive in Thailand.
Aims: To evaluate the SF cut-off level for diagnosis of severe IO in real-life situation and determine the utility of SF in predicting IO in thalassemic patients and select the most vulnerable patients for further MRI evaluation.
Methods: In this retrospective cross-sectional study, total 1,034 standard MRI (LIC and cardiac T2*) evaluations performed at Siriraj hospital during 2009-2014 and paired clinical data including SF were collected. Regardless of globin genotypes, all 350 thalassemia patients were divided into NTDT (N=108, 162 LIC and 89 cardiac T2* results) and TDT (N=242, 696 LIC and 692 cardiac T2* results). Different SF cut-off values were tested at 100 or 250 ug/l intervals and receiver operating characteristic (ROC) analysis was performed.
Results: Using SF >800 ug/l to predict LIC ≥5 mgFe/g dw, 86.4% of NTDT would be correctly started on iron chelation. However, 42% of patients (42/100) with LIC ≥5 mgFe/g dw had SF between 301-800 ug/l and 62.7% (42/67) of those with SF 301-800 ug/l had LIC ≥5 mgFe/g dw. Using threshold of SF >300ug/l, we could detect IO in 92% (92/100) of those with LIC ≥5 mgFe/g dw. When α-NTDT patients were excluded, ROC analysis found that SF >400 ug/l correctly predict LIC ≥5 mgFe/g dw in 90% of β-NTDT with lower risk of delayed treatment than using SF threshold >800 ug/l (40.6% vs. 61.9%, according to NPV). Of 89 cardiac T2* results in NTDT (SF range of 24-11,668), none had T2* <20ms. For TDT, ROC analysis suggested SF >3,500 ug/l was the best predictive threshold for T2* <20ms (AUC=0.785), while SF >2,500 ug/l was an appropriate cut-off level for LIC ≥15mgFe/g dw (AUC=0.861).
Summary/Conclusion: In a resource limited setting for MRI, SF could be used as a predictive marker for selecting severe IO in both TDT and NTDT. In NTDT, MRI for cardiac T2* could be omitted and, once for diagnosis, MRI for LIC should be done in those with SF >300 ug/l. For TDT, the SF cut off of >2,500 and >3,500 ug/l are useful to predict patients with severe LIC and cardiac siderosis, respectively.

Stem cell transplantation - Clinical

E1497

ASSESSING NPM1 MUTATIONS TYPE A AS MINIMAL RESIDUAL DISEASE MARKER BY DIGITAL DROPLET PCR BEFORE STEM CELL TRANSPLANTATION IS A STRONG PROGNOSTIC FACTOR IN PATIENTS WITH AML

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Background: Acute myeloid leukemia (AML) patients (pts) who relapse after hematopoietic stem cell transplantation (HCT) have a dismal prognosis. Identifying AML pts with high risk of hematological relapse after HCT is crucial for treatment guidance. Mutations in the *NPM1* gene (*NPM1*mut) occur early in leukemogenesis & are relatively stable during disease course, representing suitable Minimal residual disease (MRD) markers. Recently, MRD assessment, based *e.g.* on the quantitative detection of *NPM1*mut, has been shown to be of clinical relevance. However, data in pts receiving HCT is limited.
Aims: We tested the feasibility of *NPM1*mut type A (*NPM1*mutA) - representing up to 80% of all *NPM1*mut-as pre-HCT MRD marker using digital droplet PCR (ddPCR) for sensitive & specific absolute quantification without the use of standard curves.
Methods: We identified 46 AML pts with available bone marrow (BM) pre-HCT (within 30 days before HCT) & *NPM1*mutA who were in complete remission with (CR; n=42; 91.3%) or without complete hematological recovery (CRi; n=4; 8.7%). Median age was 64.5 (range 33.1-74.1) years (y). Pts received HCT after myeloablative (Cyclophosphamide 2x60mg/kg+12Gy total body irradiation [TBI], n=11) or non-myeloablative (Fludarabine 3x30mg/m²+2Gy TBI, n=35) conditioning at our institution between 2000 & 2015. Median follow-up was 2.1y. *NPM1*mutA & *CEBPA* mutation status were assessed by Sanger sequencing & presence of an internal tandem duplication in *FLT3* by fragment analysis in diagnostic BM. *NPM1*mutA copy numbers were assessed by ddPCR in pre-HCT BM. Results were normalized to *ABL1* copy numbers. Samples were measured in triplicates & those with mutation burden ≤0.01% or<3 positive droplets were defined as negative according to the manufacturer's recommendations.
Results: At diagnosis 40 pts (87.0%) had a normal karyotype. According to European LeukemiaNet (ELN) classification 26 (56.5%) had favorable, 14 (30.4%) intermediate-I, 5 (10.9%) intermediate-II & 1 (2.2%) adverse genetic risk. 18 pts harbored a *FLT3*-ITD & 4 were *CEBPA* mut. We found 13 (28.3%) of the 46 pts to be MRD+ pre-HCT (*NPM1*mutA/*ABL1* range 0.014% - 612.88%). Except for a trend for lower peripheral blast count at diagnosis ($_{dx}$) in the MRD+ group (*P*=.07) no differences were detected between MRD+ & MRD- pts in other clinical or molecular characteristics (i.e. age_{dx}, sex, ELN group distribution, Hb_{dx}, $Platelets_{dx}$, white blood $count_{dx}$, BM $blasts_{dx}$, CR1 *vs* CR2 *vs* CRi, *CEBPA*mut, *FLT3*-ITD). The number of chemotherapy cycles pre-HCT was not different between the MRD+ & MRD- group. Eleven pts relapsed of which 9 (81.8%) were MRD+ pre-HCT. Four patients did not experience relapse but were MRD+, 2 of them died within 100 days after HCT due to treatment-related complications. With respect to the remission status, only 2 (50%) of the 4 pts transplanted in CRi suffered relapse & both were MRD+. MRD+ pre-HCT was associated with a significantly higher cumulative incidence of relapse (*P*<0.001, Figure 1A) & shorter overall survival (OS, *P*=0.004, Figure 1B).

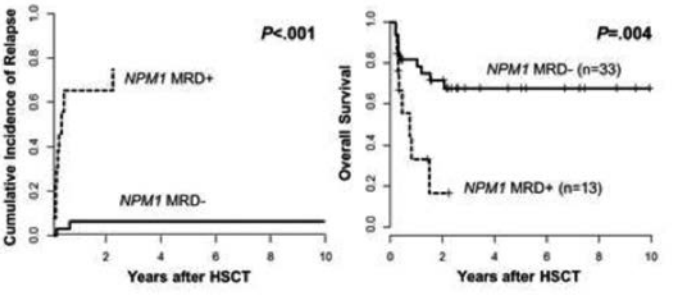

Figure 1.

Summary/Conclusions: Our study demonstrated that the novel ddPCR is a feasible method to determine *NPM1*mutA MRD burden by absolute quantification. Pre-HCT MRD+ identified pts with a significantly higher relapse rate & subsequently shorter OS independently of the clinical, cytogenetical & molecular context. MRD monitoring by *NPM1*mut detection before HCT should be incorporated in clinical trials to validate these data & guide therapeutic decisions for AML pts.

E1498

HLA-DRB1*04 CONFERS BETTER OVERALL SURVIVAL TO MALE PATIENTS WITH LYMPHOID MALIGNANCIES FOLLOWING ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Several studies reported associations of individual human leukocyte antigen (HLA) genes and outcome of allogeneic haematopoietic stem cell transplantation (allo-HSCT). Similarly, HLA genes were found to predispose to certain lymphoid malignancies. HLA-DRB1*04 is among the documented genetic risk factors for lymphoid malignancies, and in some studies the risk was identified to be male-restricted.
Aims: In this study we looked into the effect of HLA-A, -B and -DRB1 allele groups on overall survival (OS) following allo-HSCT in patients with lymphoid malignancies.
Methods: Data of 186 consecutive adult patients, who underwent first allo-HSCT for a lymphoid malignancy at our single centre in Hungary between 2007 and 2013, were retrospectively analysed. The median follow-up time for surviving patients was 44 months. In the cohort five patients (3%) received a graft, mismatched for HLA-A, and none for HLA-B or HLA-DRB1 at the antigen level. There were slightly more patients undergoing transplantation from a sibling donor (106/186, 57%).
Results: Examining the role of HLA status on survival, analyses showed that carriers and non-carriers displayed no survival difference for any of the HLA-A or -B allele groups. However, OS of HLA-DRB1*04 carriers was significantly better compared with non-carriers (24-month OS 66±8% [n= 36] *vs* 50±4% [n=150], p=0.01). As baseline transplantation characteristics were uneven in the two groups, we conducted multivariate analyses, which showed that the association remained independent (p=0.05, odds ratio [OR] 0.55, 95% confidence interval [CI]: 0.3-1). In view of previous reports of sex-specific features of HLA-DRB1*04, we performed separate analyses for male (n=114) and female (n=72) patients. Interestingly, the survival benefit was confined to males (p=0.01), whereas in female individuals no difference was detected (p=0.41). The baseline transplantation characteristics were comparable in female and male patients. Furthermore, we found that the donor gender also affected the outcome. The survival of male patients varied significantly according to recipient/donor gender pairs and HLA-DRB1*04 carriership (p=0.04). Surprisingly, in male recipients the best survival was achieved in HLA-DRB1*04 carriers who received a graft from a female donor, followed by those HLA-DRB1*04 carriers for whom the donor was male. HLA-DRB1*04 negative patients displayed worse survival compared with carriers, and among them the survival was worse for those whose donor was female. No clear explanation was found to explain the survival benefit, although there was a tendency for less CMV reactivation/disease among HLA-DRB1*04 carriers (3/36, 8% *vs* 31/150, 21%, p=0.097).
Summary/Conclusions: In summary, we observed in a cohort of 186 patients with lymphoid malignancies that HLA-DRB1*04 male carriers had significantly better overall survival following allo-HSCT compared to non-carriers. Our findings support that HLA-DRB1*04 carriership might result in male-specific consequences in the lymphoid malignancy patient group. Our results suggest that the prognosis of patients with sex-specific disease risks might be modified by transplantation from the opposite sex. Our findings should be confirmed in larger patient populations, and similar data could affect donor selection preferences for HLA-DRB1*04 carrier patients in the future.

E1499

IMPACT OF T CELL DOSE ON OUTCOMES OF NON-MYELOABLATIVE ALLOGENEIC TRANSPLANTS IN MULTIPLE MYELOMA

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Background: Donor T cells are responsible for graft *versus* host disease (GVHD) following allogeneic stem cell transplantation (alloSCT). Studies have shown a reduced incidence of GVHD but increased relapse risk with T cell depleted transplants. T cell replete stem cells are generally used for non-myeloablative (NMA) alloSCT to prevent graft failure and to avail the benefit of a potential graft *versus* tumor effect which is considered the major tool for disease control in this type of transplant. T cell doses in individual T cell replete transplants vary and to the best of our knowledge no previous studies have looked at the impact of T cell dose on the outcome of NMA alloSCT. We routinely perform tandem autologous (ASCT)-NMA alloSCT for high risk as well as relapsed multiple myeloma (MM) patients at our centre. High risk MM patients are defined by the presence of at least 2 out of 5 risk features including International Staging System score 3, adverse cytogenetics [t(4;14),17p- on FISH and/or complex karyotype], elevated lactate dehydrogenase, plasma cell leukemia (all at diagnosis) and induction failure (<PR) with bortezomib or IMID-based induction therapy. These patients receive 'upfront' tandem ASCT-NMA SCT and the patients relapsing following conventional treatment receive 'deferred' tandem ASCT-NMA SCT. All patients receive high dose Melphalan conditioned ASCT and within 2-3 months, receive an outpatient-based Fludarabine (48mg/m2 X 3days) and TBI (2Gy X1day) conditioned NMA SCT from matched sibling or unrelated donors. We use Cyclosporine and Mycophenolate mofetil as GVHD prophylaxis.
Aims: To find out whether the outcomes after NMA SCT for MM were influenced by the T cell dose received.
Methods: After obtaining informed consent, we undertook a retrospective analysis of patients who underwent tandem ASCT-NMA SCT from May 2008 to June 2015 for MM. Primary end points were progression free survival (PFS) and overall survival (OS). Secondary end points were cumulative incidences of acute GVHD, chronic GVHD and relapse, treatment related mortality and achievement of donor chimerism.
Results: Out of 59, 19 patients had received T cell dose of ≥3x10^8/kg. This high dose cohort (Hi-T) was compared to 40 patients who got <3x10^8/kg T cells (Lo-T). Median age was 59yrs (range 22-66yrs) for Hi-T and 54yrs (range 38-67yrs) for Lo-T (p=0.43). 52.5% of patients received upfront transplants in Hi-T against 42% in Lo-T (p=0.23). 68% of patients had unrelated donors in Hi-T *versus* 57.5% in Lo-T (p=0.22). After a median follow up of 45.7 months, PFS and OS were significantly inferior in Hi-T group (median PFS 366 days *vs* 1067 days, p=0.05, median OS 752 days *vs* not reached, p=0.002). Cumulative incidences of grade 2-4 acute GVHD and extensive chronic GVHD were higher in Hi-T (47% *vs* 17.5%; p=0.03 and 80% *vs* 50%; p=0.04 respectively). There were 5 treatment related deaths (26.3%) in Hi-T (all due to GVHD) against 2 (5%) in Lo-T (both due to infections); (p=0.009). Cumulative incidence of relapse was not different between the arms (48% *vs* 49% respectively at 4yrs.). Achievement of donor CD3 chimerism at different time points (days 30, 60, 90 and 180) was not different between the arms. All patients who were alive at 1yr achieved full donor chimerism. On multivariate analysis, Hi-T was independently associated with inferior OS.

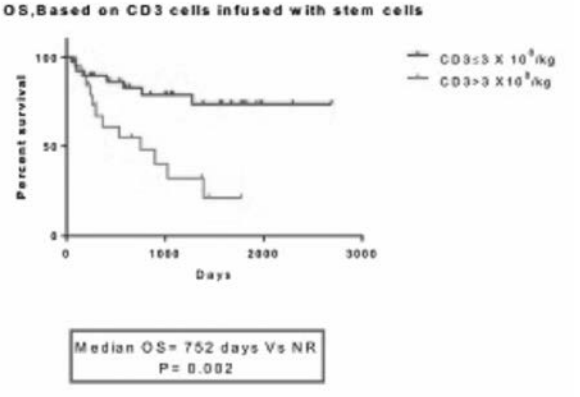

Figure 1.

Summary/Conclusions: To conclude, in NMA alloSCT for MM, the T cell dose infused has a significant influence on outcomes. Limiting the T cell dose to <3x108/kg can improve the OS and reduce TRM and GVHD. Larger randomised trials are required to confirm this observation.

E1500

LOW DOSE TOTAL MARROW/TOTAL LYMPHOID IRRADIATION (TMI/TLI) INSTEAD OF TOTAL BODY IRRADIATION (TBI) INDUCE FULL DONOR CHIMERISM BEFORE T-REPLETE HAPLOIDENTICAL TRANSPLANTATION

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Background: T cell replete haploidentical stem cell transplantation (haploSCT) with post-transplantation cyclophosphamide (Cy) (PT-Cy) has been increasingly applied in high-risk hematologic patients. In the original, nonmyeloablative conditioning regimen (NMAC) included low dose total body irradiation (TBI).
Aims: The aims of the study were to evaluate the engraftment, chimerism, and tolerance of NMAC regimen with low dose total marrow and lymphoid irradiation (TMI/TLI) instead of TBI, in patients with advanced disease.

Methods: Between April 2009 and August 2015, 98 patients received a haploSCT at our institution. NMAC was used in 60 patients. For 20 patients (33%), TMI/TLI (2 Gy) was used instead of TBI. 12 of 20 (60%) had bone and/or bone marrow involvement. The median age was 49 years (range 20-68). NMAC consisted of fludarabine (30mg/m^2/day) on day -6 to -2, Cy (14.5mg/kg/day) on days -6 and -5, TMI/TLI (200 cGy in a single fraction) on day -1, followed by bone marrow or peripheral blood stem cell graft. Graft *versus* host disease/host *versus* graft prophylaxis consisted of Cy (50mg/kg/day) on days +3 and +4, and from day +5 mycophenolate mofetil until day +35 days, and tacrolimus or cyclosporine A.
Results: Patient characteristics are shown in Table 1. The median follow-up time was 15 months (range 6-48). Immediate tolerance was good and no patients developed nausea or vomiting nor parotid hyperplasia. The median time to ANC >500/μL, and platelet recovery >20,000/μL was 22 days (range 16-28) and 27 days (range 12-41), respectively. At day +30, full donor chimerism was obtained in all evaluable patients (19/20). The cumulative incidence of acute grade ≥2 was 20% (95% CI: 6.0-39.9). No severe/moderate cGVHD was observed. The 1-yr cumulative incidence of relapse, overall survival and non-relapse mortality were 23% (95% CI: 6.1-45.8), 60% (95% CI: 38.4-81.6) and 30% (95% CI: 11.8-50.8), respectively. Causes of death were: disease progression (n=2), septic shock (n=2), fungal pneumonia (n=2), renal failure (n=1) and JCV encephalopathy (n=1). Engraftment, chimerism, aGVHD incidence were not different to patients receiving conventional TBI.

Table 1. Patient characteristics.

	n=20
Median age (range), yr	49 (20-68)
Gender	
Male	14 (70%)
Female	6 (30%)
Diagnosis	
Hodgkin lymphoma	10 (50%)
Non-Hodgkin lymphoma	6 (30%)
Chronic Lymphoid Leukemia	3 (15%)
Multiple Myeloma	1 (5%)
Disease status at haploSCT	
Complete remission	9 (45%)
Partial remission	6 (30%)
Stable/progressive disease	5 (25%)
Graft source	
Bone marrow	11 (55%)
Peripheral blood stem cell	9 (45%)
Median CD34+ infused cell dose (range)	4.8 x10^6/kg (2.1-8.0)

Summary/Conclusions: This retrospective analysis suggests that TMI/TLI could substitute conventional low dose TBI, with a sufficient degree of immunosuppression of recipient, allowing engraftment and full donor chimerism. Moreover, the 100 days TRM, the relapse incidence, aGVHD and cGVHD were comparable to that observed in the TBI group.

E1501

POSITIVITY OF WHOLE BODY POSITRON EMISSION TOMOGRAPHY (PET) BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION HAS NEGATIVE PROGNOSTIC IMPACT BUT IT DIFFERS ACCORDING TO LYMPHOMA SUBTYPE AND LINE OF ASCT

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Background: Pre-transplant PET is considered as an important prognostic variable. Positive PET is usually linked with worse prognosis. There is however limited information if there are any differences in PET impact according to line in which is ASCT performed as well as different lymphoma subtypes.
Aims: To analyze the impact of the pre-transplant PET on post-transplant outcome of patients with lymphoma according to lymphoma subtypes and the line of treatment.
Methods: Retrospective analyzes of patients who underwent ASCT between 2010 and 2014. Only pts. with pre-transplant PET were eligible for the analysis. PET had to be performed after the last cycle of chemotherapy before ASCT in interval less than 60 days. Patients who were PET negative and received one more cycle of chemotherapy due to logistic reasons were eligible. Visual PET evaluation was used in comparison to mediastinum and liver accumullation. All pts. signed the informed consent and agreed with data analysis.
Results: There were 350 pts. transplanted for lymphoma between 2005 and 2014. Pre-transplant PET was performed in 253 pts. Patients who were PET positive (PET+), but received additional chemotherapy before ASCT without PET reevaluation (n=20) were excluded from the study. In two patients PET findings were inconclusive and were excluded as well. Altogether 231 patients were evaluated, age at ASCT 53y (21-71), male/female 134/97, DLBCL 108/43% (68 in 1. line, 40 at relapse) FL 33/14% (2 in 1. line, 31 at relapse), MCL 43/19% (41 in 1. line, 2 at relapse), HL 21/9% (1 primary progressive in 1. line, 20 at relapse) and others 26/11% (13 in 1. line, 13 at relapse). Altogether 79 (34.2%) patients were PET positive (PET+) and 152 were PET negative (PET-). PET+ pts. had significantly higher death or relaps risk (HR 1.75, p 0.04). There was however only borderline impact of PET in 1st line ASCT (p 0.06) and significant in at relapse ASCT (p 0.002). It was mainly due to DLBCL, where was found significant risk for PET+ in the whole group (HR 2.35, p 0.02). No difference was observed in the 1st line ASCT (p ns) with PET negative predictive value (NPV)=0.91 and PET positive predictive value (PPV)=0.13. Patients with DLBCL and ASCT at relapse had significantly worse outcome (HR 2.42, p 0.037) with NPV 0.63 and PPV 0.47. The majority of MCL pts (41) underwent 1st line ASCT. There was significanty worse outcome for PET+(HR 157.3, p<0,0001) with NPV 0.88 and PPV 0.43. There was limited number of FL (31) and HL (20) patients resp. transplanted for relapse and there was found only trend for worse outcome of PET+ pts for HL (HR 3.7, p 0.11, NPV 0.76, PPV 0.42) and no difference in FL subgroup (HR 0.64, p 0.49, NPV 0.75, PPV 0.14).
Summary/Conclusions: We have confirmed prognostic value of pretransplant PET. We have however found that there are differences in lymphoma subtypes (1st line ASCT for DLBCL *vs* MCL), and differences according to the line of ASCT (1st line ASCT *vs* at relapse ASCT DLBCL) with differences in NPV (high in 1s line ASCT DLBCL and MCL *vs* low at relapse ASCT for all subtypes) and PPV (low in 1st line ASCT for DLBCL). Lymphoma subtype as well as line of treatment should be considered in the PET finding interpretation before possible treatment change based on pretransplant PET.

E1502

WHAT IS THE OUTCOME OF PATIENTS WITH ACUTE LEUKEMIA WHO SURVIVE SEVERE ACUTE GRAFT-VERSUS-HOST DISEASE?

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Background: Acute graft-*versus*-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation (HSCT). With new promising therapies, survival may improve in patients with severe acute GVHD.
Aims: We wanted to analyze the long-term outcome among patients who survive severe acute GVHD.
Methods: This is a landmark analysis of 23,567 patients with acute leukemia who survived >6 months after HSCT during 2002-2014. Patients with severe acute GVHD (n=1,738) were compared to controls.
Results: Patients with severe acute GVHD had a higher non-relapse mortality (NRM) and chronic GVHD compared to controls ($p<10^{-5}$). Extensive chronic GVHD was 26.9% before 6 months and 27.2% after 6 months in the severe acute GVHD group ($p<10^{-5}$). The probability of relapse was significantly lower in the severe acute GVHD group, and leukemia-free survival (LFS) and survival was significantly lower than for the controls ($p<10^{-5}$). Five-year LFS in patients who survived severe acute GVHD was 49% as opposed to 61% in controls with no or mild, and 59% in patients with moderate GVHD.
Summary/Conclusions: HSCT patients who survive severe acute GVHD have a high risk of extensive chronic GVHD, a higher NRM, a lower relapse probability and lower LFS, compared to other HSCT patients.

E1503

SCLERO-CORNEAL LENSES SAFE AND EFFICIENT FOR THE TREATMENT OF KERATOCONJUNCTIVITIS SICCA IN PATIENTS WITH REFRACTORY OCULAR GVHD: A STUDY ON BEHALF OF THE SFGM-TC

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Background: Keratoconjunctivitis sicca syndrome (KCS) due to chronic GvHD (cGvHD) is responsible for major alteration in quality of life of patients undergoing allogeneic stem cell transplantation (allo-CST). The conjunctival fibrosis secondary to inflammation is often slow and subtle and is responsible for impaired corneal and conjunctival epithelial surfaces potentiated by tear quantitative and qualitative deficiency. Treatment of KCS remains disappointing; variable success in the correction of the conjunctival dryness has been obtained with variety of topical treatments with or without systemic immunosuppressive agents. These treatments, though, do not seem to have any effect on sicca symptoms and patients' quality of life. Sclero-corneal lenses bring a valid therapeutic option by creating a pre-corneal reservoir of tears allowing permanent lubrication of the epithelium, the protection of the corneal surface against the eyelid and ciliary mechanical friction and against environmental stresses and the creation of a uniform refractive surface to be taken into optimum optical load and stable visual acuity.

Aims: Scleral lenses bring a valid therapeutic option by creating a pre-corneal reservoir of tears allowing permanent lubrication of the epithelium, the protection of the corneal surface against the eyelid and ciliary mechanical friction and against environmental stresses and the creation of a uniform refractive surface to be taken into optimum optical load and stable visual acuity. In this multicenter retrospective study we wanted to assess the incidence of scleral lenses on sixty one patients concerning by refractory ocular GVHD.
Methods: We describe the safety and efficacy of Sclero-corneal lenses in a retrospective analysis of 62 patients with KCS due to cGvHD following allo-CST. All patients had superficial punctate keratitis refractory to standard treatments. Evaluation of patients was carried out by two ophthalmologists. cGVHD was recorded according standard criteria. Ocular surface disease index (OSDI) and Oxford score were used to evaluate ocular symptoms. The scale of "Monoyer" was used and converted into visual acuity "LOG MAR" for comparative purposes of the study.
Results: All patients but two agreed to hold the lenses. The 62 patients were equipped with sclero-corneal lenses. With a median follow-up of 22 months (IQR 8-43) from the treatment with lenses, all patients have experienced an improvement in their quality of life with a clear improvement of dry-eye symptoms. This quality of life is also improved by decreasing the frequency of eye-drop instillation and the attenuation of the discomfort and post-instillation visual fluctuation. At two months, all patients but one experienced improvement in OSDI score with a median of 92 points (range, 40-100) before lenses to a median of 25 points (range, 3-90). We also observed improvement or stability of visual acuity with median gain of -0.2 (range, -1 to +0.1) LOG MAR acuity and improvement or stability of the Oxford score score with a median of 3 points (range, 0-5) before lenses to a median of 1 points (range, 0-4) after lenses. with a median gain of 2 points in almost all patients.
Summary/Conclusions: Treatment of KCS with sclero-corneal lenses is promising. Whenever possible, this approach should be considered in patients experiencing KCS due to cGVHD.

E1504

UNMANIPULATED HAPLOIDENTICAL STEM CELL TRANSPLANTATION FOLLOWING BUSULFAN BASED REDUCED INTENSITY CONDITIONING AND POST-TRANSPLANTATION CYCLOPHOSPHAMIDE FOR ADVANCED HODGKIN´S LYMPHOMA

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Background: Hodgkin´s Lymphoma (HL) is a potentially "curable malignant disease" with chemotherapy, radiotherapy or, eventually, high-dose chemotherapy followed by autologous stem cell transplantation (SCT). For patients relapsing after auto-SCT or those with advance refractory lymphoma, allogeneic SCT is a valid option. In this setting, our institution has opted for haploidentical donors as source of stem cells for allo-SCT in advanced HL patients.
Aims: Patients with advanced HL who received haploidentical SCT (haplo-SCT) were retrospectively analyzed in terms of overall survival (OS), progression-free survival (PFS) and graft *versus* host disease (GVHD).
Methods: From May 2012 to September 2015 eighteen haplo-SCT were performed at our hospital on patients with HL who had relapsed after auto-SCT or had needed more than three lines of chemotherapy to reach any kind of response. The conditioning regimen consisted of cyclophosphamide (Cy) 14.5 mg/Kg on days -6 and -5, fludarabine 30 mg/m2 on days -6 to -2 and busulfan (Bu) on days -3 and -2. GVHD prophylaxis consisted of Cy 50 mg/Kg on days +3 and +4, followed by tacrolimus plus mycophenolate mofetil from day +5.
Results: The median age of the cohort was 31 years (19-58). The HCT-CI >2, EBMT score >3 and high DR index were 28%, 67% and 11%, respectively. Thirty-nine percent of all patients were not in complete remission and eleven percent were in progression at the time of transplantation. The median number of treatment lines before SCT was five (4-11) and brentuximab-vedotin was employed as bridge to the allo-SCT in 44% of cases. Thirty-nine of the patients had received a previous autograft and one patient both, auto-SCT and allo-SCT. Median time to haplo-SCT from diagnosis was 42 months. The stem cell sources were bone marrow (22%) and peripheral blood (78%). All the patients engrafted and full donor chimerism was reached in 100% of the entire cohort at day +30. The median times to neutrophil (>0.5 x 10e9/L) and platelet recovery (>20 x10e 9/L) were 18 (13-30) and 26 (10-44) days from SCT. Extramedullary toxicity grade 3-4 occurred in 2 cases. One of them developed severe hepatic veno-occlusive disease which was resolved with defibrotide. Seventeen patients were evaluable for acute and chronic GVHD. The 100-day cumulative incidence (CI) of grade II-IV acute GVHD was 35% (7% grade III-IV) with a median time of 33 days (23-80). The cumulative 3-year incidence of moderate chronic GVHD was 13%. With a median follow-up of 14 months (range 2-44), the CI of non-relapse mortality (NRM) and of relapse were 5% (1/18) and 42% (6/18), respectively. The actuarial OS and PFS at 45 months was 77% and 57%, respectively. Fifteen patients are alive, 12 disease free. The causes of death were relapse (n=2) and BK virus encephalitis (n=1). Relapses are being treated with brentuximab-vedotin followed by donor lymphocyte infusions (DLI), with no cases of GVHD.
Summary/Conclusions: Haplo-SCT with Bu based RIC and high-dose post-transplantation Cy offers high rates of remission in advanced HL, with low incidence of GVHD and NRM. In our experience, retreatment with brentuximab-vedotin combined with DLI is well tolerated and a good option to manage relapses after haplo-SCT.

E1505

UNRELATED TRANSPLANT FOR SEVERE APLASTIC ANEMIA (SAA): LONG TERM RESULTS AND RISK FACTOR ANALYSIS FOR OVERALL SURVIVAL

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Background: For patients with SAA, Transplantation from an unrelated donor (UD) is usually considered after failure of at least one course of immunessupression. This strategy is based on a relatively high risk of complications for UD transplant recipients, such as graft rejection, graft-*versus*-host disease (GvHD) and infections. However, the outcome of unrelated donor transplants has significantly improved in recent years, due to better donor selection, conditioning regimen otimization and better supportive care.
Aims: The authors aim to describe results from 51 patients with SAA who have received unrelated allogeneic transplants in a single reference institution from 1997 to 2014 and identify risk factors for survival.
Methods: Data of 51 patients were retrieved from the center databasis. Fisher exact test was used for categoric variables and Kaplan Meier for survival estimates. P level of significance was<0,05. Primary endpoint was overall survival. Secondary endpoints were engraftment, acute and chronic gvhd and identification of risk factors for survival.
Results: There were 30 females and 21 males. Median age was 15 years old (0-47). Median total number of cells infused was 3,4 x 108/kg.61% of the patients have received more than 50 transfusions previously. Conditioning regimen were: CY 120+TBI 1320 +/- ATG in 16 (31%) patients, Bu 12 mg/kg+ Cy 120+ ATG in 18 (35%), and Fludarabine+Cy+ATG in 8 (16%), Fludarabine, Cy+TBI 200 in 9 (18%) patients. Stem cell source was marrow in 84%, cord blood in 13% and peripheral blood in 3% of patients. Transplants were full matched in 32 (62%) patients, had one mismatch (out of 12) in 12 (24%) and 2 mismatches in 7 (14%) patients. Engraftment was complete as evaluated by donor chimerism at day 30 and 100 post transplant in 36 patients (71%), partial in 4 (8%) and graft failure was observed in 9 (18%) patients. Acute GVHD grade II-IV was seen in 9 patients (18%) and NIH moderate to severe chronic GVHD was seen in 8 (16%) patients. Median overall survival was 328 days (4-4287) and estimated 5 years overall survival was 55%. Risk factors for survival identified were: HLA mismatch and stem cell sources other than marrow.
Summary/Conclusions: Unrelated transplants are a feasible salvage therapy for patients with SAA refractory to immunessupression, being HLA compatibility and marrow stem cell source factors with a positive impact on survival.

E1506

DIFFERENT CLUSTERS OF IMMUNOLOGIC VARIABLES ARE ASSOCIATED WITH CHRONIC GVHD AND RELAPSE IN A DYNAMICAL MODEL

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Background: The long-term efficacy of allogeneic haematopoietic stem cell transplantation (SCT) relies primarily on the Graft-*versus*-tumor (GVT) effect, which partially overlaps with Graft *versus* Host disease (GvHD), the most common cause of morbidity and mortality in HSCT. Immune parameters that univocally associate with GVHD or GVT have not been identified yet.
Aims: In this study, lymphocyte subsets together with TCR-repertoire analysis, and index of thymic and bone marrow output were evaluated at different time points, in order to identify possible predictors of chronic GVHD and ineffective GVT.
Methods: Prospective evaluations of lymphocyte subsets, thymic and bone marrow output were performed in 40 patients before SCT, at 30, 90, 180 days and 1 year after SCT. CD4+/CD8+ naïve, central memory, effector memory, terminally differentiated effector memory (TEMRA) cells, subsets of regulatory T-lymphocytes, immature B cells, naïve, switched and unswitched memory B cells, memory double negative (IgD-CD27-) B cells were analysed by flow cytometry. Analysis of thymic and bone marrow output was performed by detection of T cell receptor excision circles (TRECs) and kappa-deleting recombination circles (KRECs). TRECs and KRECs were simultaneously quantified by a duplex quantitative Real-Time PCR. Heteroduplex assay was used to perform TCR-repertoire analysis. A 2-step multivariate analysis was performed using principal component analysis (PCA) and Cox regression analysis, to solve the problem of the high number of variables (immunological, patients- and trans-

plant related) in comparison with the relatively limited and heterogeneous pool of patients.
Results: Chronic GVHD was observed in 9 patients (median time: 7 months, range 4-10). In 2-step multivariate analysis, lower values of regulatory effector memory lymphocytes at day +30 and lower percentage of CD8+TEMRA cells at +90, together with lower values of immature B cells and KRECs at +180 were inversely correlated with chronic GVHD (HR 0,4; p=0,002). The relapse rate (30%; median time: 9 months, range 3-48) was used as clinical index of ineffective GVT. The following clusters (C) of immunological parameters were associated with relapse: C1(pre-transplant lower values of CD4+central memory, all regulatory, and regulatory central memory cells; HR 4,0 p=0,02); C2(pre-transplant higher percentage of mature B cells, lower values of switched memory B cells and KRECs at day+90; HR 0,1 p=0,008).
Summary/Conclusions: Different clusters of immunological parameters at different time points were evidenced as predictors of cGVHD and ineffective GVT, allowing a clear-cut distinction between these immunological reactions. Changes in pre- and post-transplant B-lymphopoietic microenvironment and imbalances in the subsets of B-cells may influence GVHD and GVT. The atypical association of regulatory T-cells with GVHD may be explained by the relative efficiency of different subsets of regulatory T-cells (naïve>memory), as shown in some experimental models. The correlation of CD8+TEMRA at +90 with chronic GVHD may early indicate a persistently activated and dysregulated immune system. The validation of these clusters of immunological parameters as specific early predictors of GVHD or GVT, even before SCT, could potentially allow the development of pre-emptive and targeted therapies.

E1507

THE JAK2 46/1 HAPLOTYPE IS ASSOCIATED WITH ACUTE GRAFT-VERSUS-HOST DISEASE IN ACUTE MYELOID LEUKEMIA PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has proven to be the most effective treatment option for certain hematological malignancies, however the favorable outcome of the procedure could be jeopardized by graft-*versus*-host disease (GvHD). Cytokines play a well established role in the mechanism of acute GvHD (aGvHD) and many of the involved cytokines relay their signal through the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway.
Aims: In the present work, we investigated whether recipient and donor JAK2 46/1 haplotype could affect allo-HSCT outcomes, such as aGvHD, relapse rate and mortality in AML patients.
Methods: We examined the association of recipient and donor JAK2 46/1 haplotypes and allo-HSCT outcome in a cohort of 124 adult patients diagnosed with AML, who received first allo-HSCT in complete remission (CR) between January 2007 and December 2013 at our single center. For identification of JAK2 rs12343867 from genomic DNA LightCycler melting curve analysis (LightCycler 480II, Roche Diagnostics) was performed.
Results: Presence of JAK2 46/1 haplotype was associated with higher frequency of aGvHD grades II-IV (carrier *vs* non-carrier recipients 42% vs.19% p=0.008 and carrier *vs* non-carrier donors 40% *vs* 21%, p=0.038). We confirmed significantly higher risk if both, recipient and donor were 46/1 haplotype carriers compared to those where recipient and donor were non-carriers (OR=5.242, 95% CI=1.784-15.404, p=0.003). Relapse free survival (RFS) did not differ between 46/1 haplotype carrier and non-carrier recipients (2-year RFS 69.1±6.1% *vs* 54.2±6.9%, respectively, p=0.25), but we found significant alterations in the cause of death between the subgroups. In the 46/1 haplotype group the major causes of death were transplantation related complications, including aGvHD (18/21, 86%), while 3 deaths (14%) were attributed to relapse. In the non-46/1 haplotype subgroup 48% died of relapsing leukemia, while non-relapse mortality accounted for 52% (p=0.024). If donors were 46/1 carriers, patients more often died of transplantation related complications (81% *vs* 50%, p=0.049). The relapse rate was comparable in patients transplanted from a carrier or a non-carrier donor. Survival analysis according to the donor haplotype showed a favorable RFS in the non-46/1 haplotype group (2-year RFS 71±6 *vs* 53±7%, p=0.033). The difference in 2-year RFS rates remained significant in the four recipient-donor groups [64±8% (non-carrier recipient, non-carrier donor), 82±8% (carrier recipient, non-carrier donor), 36±11% (non-carrier recipient, carrier donor) and 61±8% (carrier recipient, carrier donor); p=0.038).
Summary/Conclusions: Although the exact pathomechanism is yet to be explored, our findings suggest that recipient and donor JAK2 46/1 haplotypes might be involved in the regulation of aGvHD.

E1508

IN THE ERA OF TKIS, WHICH PH+ ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS ARE SUITABLE FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION?

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Background: Outcome of Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL) improved significantly with the introduction of tyrosine kinase inhibitors(TKIs).The role of autologous stem cell transplantation(auto-SCT) in this setting has gradually received increasing attention.
Aims: The aim of our study was to determine which Ph+ ALL patients are suitable for auto-SCT in the era of TKIs.
Methods: Fifty-nine Ph+ ALL patients received SCT and TKIs therapy were enrolled in this study,the auto-SCT group has 23 patients and the matched sibing donor(MSD-SCT) group has 36 patients.We analysized retrospectively the data according SCT type and the statue of molecular response.
Results: The 3-year overall survival(OS) rates of patients in the auto-HSCT group and MSD-SCT group were 67.1±10.3%, and 67.0±8.3% (*P*=0.701); the 3-year leukemia-free survival (LFS) rates were 53.9±10.9% and 61.3±10.5% (*P*=0.918); the incidence of replase(IR) were 46.3±11% and 25.5±7.9% (*P*=0.31); the transplantation-related mortality(TRM) were 0 and 14.7±6.1% (*P*=0.058). Twenty-seven patients reached molecular remission within 3 months after induction therapy and could stably maintain this response before transplantation(CMR3). The patients received the second generation TKIs(nilotinib or dasatinib) seemed to have a higher possibility to reach CCR3 than those received imatinib(70% *vs* 40.8% *P*=0.18). For these patients who reached CCR3, The 3-year OS of patients in the auto-HSCT group and MSD-SCT group were 90.9±8.7% and 78.7±11% (*P*=0.503); the 3-year LFS were 80.8±12.2% and 75.0±10.8% (*P*=0.661); the IR were 19.2±12.2% and 19.6±10.2% (*P*=0.909); the TRM were 0 and 6.2±6.1% (*P*=0.407).

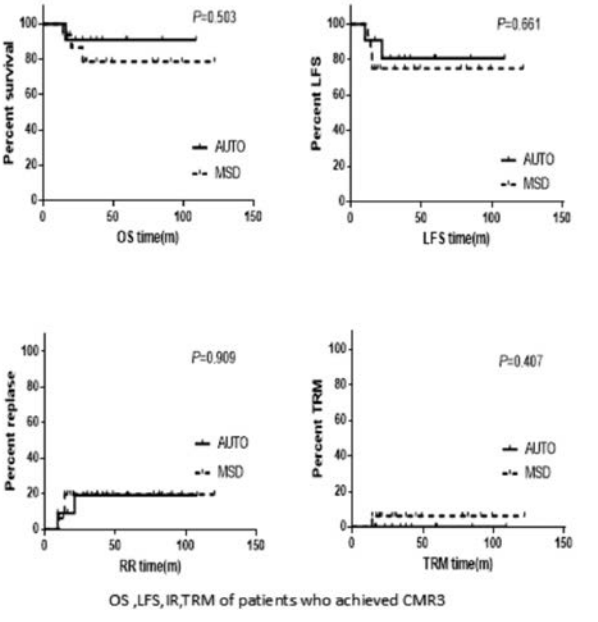

Figure 1.

Summary/Conclusions: Those patients who reached CMR3 were suitable for auto-SCT.The second generation TKIs could help more patiens benefit from auto-SCT.

E1509

COULD GRAFT *VERSUS* TUMOR EFFECT IMPROVE OUTCOMES IN MYELOID MALIGNANCIES WITH ACTIVE DISEASE UNDERGOING REDUCE INTENSITY ALLOGENEIC TRANSPLANT?

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Background: Allo-transplant (allo-HSCT) has become a significant treatment for refractory and relapsed haematological malignances; however, relapse remains a mayor cause of treatment failure. Reduce intensity conditioning (RIC) regimens have shown to be safe and effective in older patients or with comorbidities, but patients with chemorefractory disease still have high relapse rates and poor out-

comes. Although graft *versus* tumor effect (GVT) has been demonstrated in some malignancies, the benefit for relapsed acute myeloid leukaemia (AML) and myelodisplastic syndrome (MDS) is often limited because of its rapid growth.
Aims: Analysis of patients with myeloid malignancies (including AML, MDS and secondary myelofibrosis) receiving RIC allo-HSCT with or without complete response (CR) in terms of transplant related mortality (TRM), event free survival (EFS) and overall survival (OS) in order to investigate the GVT in both groups.
Methods: We analysed 169 patients who underwent to allo-HSCT in our centre between 1995-2015. Characteristics of both groups are described on table 1. Although cytogenetic abnormalities are not available at this moment, the data is been recording and this variable will be included in the final analysis.
Results: In the overall series and with a median follow-up of 36 months for patients alive, the estimated OS and EFS at one year, two years and five years was 71%, 64% and 58% and 61%, 51% and 45% respectively. The mainly cause of death was relapse in 32 patients (19%) and 25 (15%) due to TRM. In the Kaplan-Meier analysis the variables associated with OS (p<0,05) were: CR status, III-IV acute graft *versus* host disease (GVHD), development of cGVHD, fungal infection, thrombotic microangiopathy and veno-occlussive disease while just the development of cGVHD (p=0,001) with differences between grades: limited (p=0,046, HR 0,37, 0,14-0,98) and extensive (p=0,00, HR 0,24, 0,11- 0,52) and development of thrombotic microangiopathy (p=0,004, HR 4,6, 1,6-12,8), but not CR status at the moment of allo-HSCT (p=0,49), maintained significance in multivariate analysis. Regarding the outcomes on the basis of disease status at transplant, there were differences between CR and not CR patients in terms of OS (p=0,02, not achieved *vs* 32 months) and EFS (p=0,012; 71 *vs* 9 months) but there where no differences in PFS (p=0,6); attending the TRM (p=0,05, estimated 8% *vs* 22% at 1 year), there were differences manly due to the GVHD related mortality (p=0,05; 2% *vs* 17% at 1 year). Exploring the GVT in both groups separately: in the group of patients with CR, there were differences between patients without cGVHD compared with patients with limited or extensive cGVHD (p=0,00) with median OS not achieved in all groups; but in those patients without CR, just extensive cGVHD seems to be effective (p=0,00) with a median OS not achieved *vs* 10 and 28 months in those patients without and with limited cGVHD respectively.

Table 1.

Status at allo-HSCT (n = 169)	p	CR (n/%) (n = 93)	No CR (n/%) (n = 76)
Diagnosis			
• AML	p = 0,00	79 (84%)	31 (41%)
• MDS		14 (15%)	39 (51%)
• Secondary MF		1 (1%)	6 (8%)
Time from diagnosis	p = 0,014	5 months (2-123)	9 months (1-200)
Age	p = 0,18	56 years (18-69)	58 years (24-69)
Male	p = 0,8	51 (54%)	43 (57%)
Previous Auto-HSCT	p = 0,9	15 (16%)	10 (13%)
Donnor	p = 0,4		
• Sibling		63 (68%)	48 (63%)
• Unrelated [mismatch]		26 [11] (28%)	21 [5](28%)
• Haploidentical		4 (4%)	7 (9%)
Source peripheral blood	p = 0,04	83 (88%)	73 (97%)
FluBu Conditioning regimen (Vs others)	p = 0,3	80 (85%)	64 (85%)
GVHD prophylaxis	p = 0,06		
• TCR/Sirolimus		34 (36%)	38 (51%)
• Calcineurin inhibitor/MTX		48 (52%)	28 (37%)
• Others		11 (12%)	10 (12%)
In vivo T cell deplection	p = 0,68	4 (4%)	2 (3%)
Median CD34	p = 0,9	5,5 (2,2-11,2)	5,7 (2,3-12,2)
Acute GVHD			
• Grade II- IV	p = 0,34	42 (46%)	39 (54%)
• Grade III-IV	**p = 0,008**	**4 (4%)**	**13 (18%)**
Induced by early withdrawal IS treatment and/or DLI	**p = 0,03**	**7 (8%)**	**14 (21%)**
Median day aGVHD	p = 0,3	29 (5-196)	38 (15-252)
Chronic GVHD	**p = 0,03**		
• No cGVHD		**23 (27%)**	**27 (45%)**
• Limited		**22 (26%)**	**7 (12%)**
• Extensive		**40 (47%)**	**26 (43%)**
Induced by early withdrawal IS treatment and/or DLI	p = 0,18	21 (25%)	12 (17%)
Median day cGVHD	p = 0,06	182 (84-708)	207 (62-1258)
CMV reactivation	p = 0,24	24 (26%)	27 (36%)
Fungal infection	p = 0,08	21 (25%)	12 (17%)
Thrombotic microangiopathy	p = 0,5	5 (5%)	6 (8%)
Veno-occlusive disease	p = 0,6	3 (3%)	1 (1%)
Relapse		30 (32%)	23 (31%)
Death		28 (30%)	36 (48%)

Summary/Conclusions: In our series, the PFS of patients without CR at the moment of transplant was similar to those with CR, but the first group had worse OS, EFS and higher TRM due to a larger rate of severe acute GVHD with a high proportion of induced GVHD because the early withdrawal of the immunosuppressive treatment. On the other hand, patients without CR and development of extensive cGVHD had long term survival rates, similar to those patients with CR, reflecting the GVT. Efforts should be focus on avoid severe aGVHD maintaining the GVT in order to improve the TRM rates with new immunosuppressive strategies and improve the management of minimal residual disease.

E1510

PROTECTIVE EFFECT ON CHRONIC GRAFT-VERSUS-HOST DISEASE OCCURRENCE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION PATIENTS WITH EPSTEIN BARR VIRUS VIREMIA TREATED BY RITUXIMAB

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Background: Chronic graft-*versus*-host disease (cGVHD) is an important cause of late morbidity, mortality and impaired quality of life in patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT). It has been demonstrated that B cells are involved in cGVHD pathogenesis, which is evidenced by the presence of antibodies reactive to the host tissue in allo-HSCT patients with active cGVHD. It has also been proven that Rituximab could have a distinctly therapeutic effect on cGVHD in allo-HSCT patients. And now the prophylactic effect of Rituximab on cGVHD is being focused on. However, until now, no related studies have been conducted in Chinese patients.
Aims: To assess the prophylactic effect of Rituximab on cGVHD, a group of Chinese allo-HSCT patients receiving Rituximab to eliminate EBV viremia within 100 days following transplant is retrospectively evaluated.
Methods: Between January 2012 and August 2014, a total of 102 allo-HSCT patients from the First Affiliated Hospital of Soochow University, who underwent a myeloablative conditioning regimen and suffered EBV viremia within post-transplantation 100 days with a median time of 54 days (range 14-99), were included in this study. Among them, 50 (49%) patients (Rituximab group) received Rituximab treatment by 375mg/m2 weekly to deal with EBV viremia, while other 52 cases (control group) were treated by other anti-EBV agents. A competing risk model was adopted to compare the cumulative incidence of cGVHD, relapse rate and transplantation related mortality (TRM) between Rituximab group and control group. Death and relapse were treated as competing events in the analysis of cGVHD. Overall survival (OS) and progression-free survival (PFS) were estimated by the Kaplan-Meier method.
Results: All patients either in Rituximab group or in control group achieved viral load negativity except 3 patients who died before achieving viral negativity. In the Rituximab group, the median start time of Rituximab administration is post-transplantation 64 days (range 23-101) and the median count number of Rituximab administrations was 1 (range 1-4). The overall cumulative incidence of cGVHD in the cohort at 6-, 12-, 24-month were 23.5%, 42.2% and 46.1%, respectively. Retrospectively, the cumulative incidence of cGVHD in Rituximab group was lower at each time point (14.0% *vs* 32.7%, 34.0% *vs* 50%, 36.1% *vs* 54.1% at 6-, 12-, 24-month, respectively, P=0.059) when compared with controls. Moreover, there was no significant difference regarding cumulative relapse rate (P=0.48) and TRM (P=0.39) between two groups. Multivariable analyses corrected by other factors showed that Rituximab was one of the independent factors for the reduction of cumulative incidence of cGVHD (Hazard ratio (HR)=0.37, 95%CI=0.178-0.767, P=0.0075). Additionally, survival analyses showed that there was no significant difference between two groups regarding overall survival (OS) (P=0.667) or progression free survival (PFS) (P=0.571).
Summary/Conclusions: Rituximab administrated in post-transplantation early phase could reduce the cumulative incidence of cGVHD and play a protective role in cGVHD occurrence among allo-HSCT patients but without increasing relapse rate and TRM.

E1511

CHANGES OF PLASMA COMPLEMENT C3B, C5B AND VWF, ADAMTS13 IN PATIENTS WITH THROMBOTIC MICROANGIOPATHY AFTER HEMATOPOIETIC STEM-CELL TRANSPLANTATION

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Background: Transplant-associated microangiopathy (TMA) is an uncommon but devastating complication in patients undergoing hematopoietic stem-cell transplantation (HSCT). It may be confused with severe graft-*versus*-host disease (GVHD), infection, and other transplant related thrombotic diseases. Limited studies have shown the changes in plasma VWF/ADAMTS13 or complement activation markers in patients after HSCT. However, the role of VWF/ADAMTS13 and complement activation in patients with TMA and GVHD is not fully understood.
Aims: Current study is to investigate the alterations of plasma levels of C3b, C5b and VWF/ADAMTS13 in patients with TMA and to explore their roles in the pathogenesis and early diagnosis of transplant-associated TMA.
Methods: From 2011 to 2014, 14 patients with TMA were enrolled into the study in a single medical center. 71 other patients following HSCT were recruited as control subjects including 11 cases of hepatic vein occlusion disease (VOD), 20 cases of severe infections, 20 cases of severe II-IV° GVHD and 20 cases without complications. Blood sample was collected before transplantation and at the onset of transplantation related complication. Fluorescence resonance energy transfer substrate (FRETS)-VWF73 assay detected plasma ADAMTS13 activity. Collagen-binding assay and latex immunoassay determined VWF activity and VWF antigen, respectively. Plasma VWF multimer was determined by agarose gel electrophoresis and Western blot. Plasma levels of complement C3b and C5b were measured with ELISA.
Results: Compared with the levels before transplantation, plasma ADAMTS13

activity and VWF antigen or activity in the patients with TMA did not differ from those who developed TMA, with infection or GVHD or without any complication (p>0.05). However, plasma ADAMTS13 activity decreased and the ratio of VWF antigen/activity increased significantly in patients with VOD (p<0.05). Plasma VWF multimer distribution was similar in patients with infection, GVHD or without complication, but ultralarge multimers of VWF was present in patients with TMA and VOD. Plasma levels of complement C3b was increased in patients after HSCT (198.46 ng/ml ±14.78 ng/ml) compared with healthy subjects (85.02 ng/ml±8.50ng/ml) (p<0.05), but exhibited no difference in the other groups. Plasma C3b increased significantly in patients with TMA and GVHD (p<0.05). The plasma levels of C3b were higher in the TMA group (480.70 ng/ml±66.76ng/ml) than the GVHD group (298.50 ng/ml ±32.06 ng/) (p<0.05). Also, plasma levels of C5b in patients with TMA were significantly increased (1059.49 ng/ml ±85.57 ng/ml) as compared with those before transplantation (653.19ng/ml ±44.91ng/ml) and other groups (p<0.05).

Summary/Conclusions: We conclude that plasma ADAMTS 13 activity and the ratio of VWF antigen/activity remained stable in the patients with transplant-associated TMA, but the levels of complement C3b and C5b, particularly the C5b, increased significantly, suggesting the critical role of complement pathway in the pathogenesis of TMA. C5b may be a specific biomarker for early diagnosis of TMA but C3b is a marker for both TMA and GVHD.

E1512

DONOR KIR HAPLOTYPE B EXACERBATES ACUTE GVHD IN HLA-MISMATCHED HEMATOPOIETIC CELL TRANSPLANTATION: A SINGLE-CENTER RETROSPECTIVE ANALYSIS

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Background: Following allogeneic hematopoietic stem cell transplantation (allo-HSCT), natural killer (NK) cells play a role in immune responses such as graft *versus* tumor (GVT) and graft *versus* host disease (GVHD). Killer-cell immunoglobulin-like receptors (KIR) regulate the function of NK cells by interacting with MHC class I molecules. KIR haplotype A contains only one stimulatory KIR (2DS4), whereas haplotype B contains 2 to 5 stimulatory KIR genes. Previous studies have demonstrated the effect of donor KIR haplotypes on allo-HSCT outcomes. Some investigators showed that donor KIR haplotype B augmented GVT and suppressed GVHD, consequently improving survival. However, the association between donor KIR haplotypes and clinical outcomes after allo-HSCT remains controversial.

Aims: To investigate the impact of donor KIR haplotypes on clinical outcomes in a Japanese cohort from a single center.

Methods: We retrospectively analyzed the clinical outcomes of patients with hematological malignancies who underwent allo-HSCTs at Niigata University. From 1989 to 2011, 304 HSCTs were performed. DNA samples were available for 152 donor KIR haplotypes. Of these 152, HSCTs from HLA-haploidentical family donors, umbilical cord blood, and unrelated donors with administration of anti-thymocyte globulin (ATG), as well as HSCTs for non-malignant hematologic disorders, were excluded. The remaining 106 cases, including 44 AML, 28 ALL, 14 CML, 11 NHL, and 9 MDS, were enrolled. HLA-matched sibling donors and 10/10 (HLA-A, -B, -C, -DRB1, and -DQB1) HLA allele-matched unrelated donors were defined as the HLA full-match group. Donors with at least one HLA allele mismatch were defined as the HLA mismatch group. KIR genotyping was performed with a PCR-rSSO kit.

Results: There were 61 donors with KIR haplotype A (57.5%) and 45 with KIR haplotype B (42.5%). The distribution of KIR haplotypes in this cohort was similar to that previously reported in the Japanese population. A comparison of the two groups of donors with KIR haplotypes A and B revealed no significant differences in the overall survival, cumulative incidence of relapse, and non-relapse mortality, possibly due to the small sample size and the dominance of non-AML malignancies. The cumulative incidence of grade II-IV acute (a)GVHD was not significantly different (16.4% *vs* 33.3%; P=0.051). However, grade III-IV aGVHD was significantly more frequent in patients receiving grafts from KIR haplotype B donors (4.9% *vs* 20.0%; P=0.02). The cumulative incidence of severe aGVHD was evaluated according to the degree of HLA matching. In the HLA full-match group, there was no significant difference between KIR haplotype A and B in the cumulative incidence of grade III-IV aGVHD (5.7% *vs* 8.7%; P=0.68). In contrast, in the HLA mismatch group, the cumulative incidence of grade III-IV aGVHD was significantly higher in patients receiving grafts from KIR haplotype B donors (5.3% *vs* 37.5%; P=0.02). In multivariate analysis, donor KIR haplotype B was the only significant risk factor for grade III-IV aGVHD (hazard ratio 3.92; CI, 1.04-14.75; P=0.04).

Summary/Conclusions: These findings demonstrated that donor KIR haplotype B was an independent risk factor for severe aGVHD in HLA-mismatched HSCTs using conditioning regimens without ATG. This observation suggests that genetic determinants of the functional properties of donor NK cells can affect the severity of aGVHD, which is thought to be solely induced by donor T-cells. Furthermore, it indicates that under strong T-cell alloreactivity, NK cells may have a role in worsening aGVHD.

E1513

A TWO-CENTER RETROSPECTIVE COMPARISON OF BEAM *VS* BUCYVP16 CONDITIONING PRIOR TO AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH HODGKIN'S LYMPHOMA

S Singer[1,*], YA Efebera[2], Q Zhao[2], D Abounader[3], P Elder[2], CC Hofmeister[2], DM Benson[2], S Penza[2], L Andritsos[2], R Klisovic[2], S Vasu[2], W Blum[2], S Jaglowski[2], BM William[2], B Bolwell[3], B Pohlman[3], M Kalaycio[3], D Jagadeesh[3], B Hill[3], R Sobecks[3], N Majhail[3], SM Devine[2], R Dean[3]
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Background: High dose chemotherapy followed by autologous hematopoietic stem cell transplantation (AHSCT) is an optional treatment for patients with relapsed Hodgkin's Lymphoma (HL). A number of conditioning regimens are routinely used. However, the optimal conditioning regimen in terms of antitumor effect and toxicities remains unknown. We compare the outcomes of Carmustine, Etoposide, Cytarabine, Melphalan (BEAM) used at the Ohio State University to that of Busulfan, Cyclophosphamide, Etoposide (BUCYVP16) used at the Cleveland Clinic Foundation.

Aims: To compare the efficacy and toxicity of BEAM and BUCYVP16 conditioning regimens in patients with relapsed HL undergoing AHSCT.

Methods: We retrospectively analyzed patients treated with BEAM (n=128) or BUCYVP16 (n=105) followed by AHSCT between 2006 and 2014. Kaplan-Meier estimates were used to analyze progression free survival (PFS) and overall survival (OS). Cumulative incidence of relapse (CIR) was measured from transplant date until relapse, treating deaths as competing risks using Pepe and Mori test.

Results: Patient characteristics were similar for age (median 34 (19-73) *vs* 38 (19-69), p=0.51), disease stage (p=0.17), prior radiation exposure (p=0.21), comorbidity index (p=0.58), and response status at transplant (89.1% CR/PR *vs* 90.3%) for BEAM *vs* BUCYVP16 respectively. Differences were number of prior treatments (median 3 (1-10) *vs* 2 (1-5), p<0.01), and median CD34 dose infused (4.45 x10^6 *vs* 7.25, p<0.01) for BEAM *vs* BUCYVP16. Median follow-up from diagnosis and transplant was 7.0 years and 4.2 years respectively for BEAM and 6.5 years and 3.8 years for BUCYVP16. Median day to neutrophil engraftment was 10 (8-13) *vs* 10 (9-12) (p<0.01) and median time to platelet engraftment was 18 (13-70) *vs* 16 (7-49) (p<0.01) for BEAM *vs* BUCYVP16. Patients receiving BEAM had a lower CIR at 1, 3, and 5 years compared to BUCYVP16 (p<0.001). PFS at 1, 3 and 5 years from HSCT was 80%, 70% and 66% for BEAM *vs* 65%, 45% and 33% for BUCYVP16 respectively (P<0.001). OS from HSCT at 1, 3, and 5 years was also longer in the BEAM cohort: 94%, 88%, and 79% compared to 88%, 72%, and 54% in the BUCVY16 cohort (p<0.001, Figure 1). Grade 3/4 mucositis was higher for patients receiving BUCYVP16 (p=0.01). There were no differences in hemorrhagic cystitis, veno-occlusive disease or second primary malignancy.

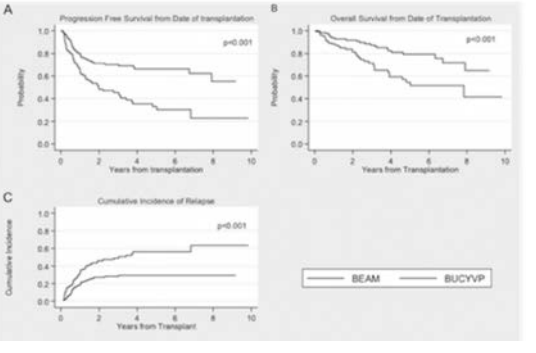

Figure 1.

Summary/Conclusions: This large retrospective study supports the use of BEAM as a conditioning regimen for AHSCT for relapsed HL. Both PFS and OS were longer for the BEAM cohort with a lower CIR.

E1514

A TWO-CENTER RETROSPECTIVE COMPARISON OF BEAM *VS* BUCYVP16 CONDITIONING PRIOR TO AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Background: High dose chemotherapy followed by autologous hematopoietic stem cell transplantation (AHSCT) has become the standard of care for patients with relapsed chemo-sensitive Non-Hodgkin's Lymphoma (NHL). Various conditioning regimens have been developed to improve outcome, but no regimen has proven superior. We compare the outcomes of Carmustine, Etoposide, Cytarabine, Melphalan (BEAM) used at the Ohio State University to that of Busulfan, Cyclophosphamide, Etoposide (BUCYVP16) used at the Cleveland Clinic Foundation.
Aims: To compare the efficacy and toxicity of BEAM and BUCYVP16 conditioning regimens in patients with relapsed NHL undergoing AHSCT.
Methods: We retrospectively analyzed patients treated with BEAM (n=269) or BUCYVP16 (n=409) followed by AHSCT between 2006 and 2014. Kaplan-Meier estimates were used to analyze progression free survival (PFS) and overall survival (OS). Cumulative incidence of relapse (CIR) was measured from transplant date until relapse, treating deaths as competing risks using Pepe and Mori test.
Results: Patient characteristics were similar for age (median 60 (20-77) *vs* 58 (27-78)), disease stage at diagnosis (p=0.29), prior radiation exposure (p=0.68), and response status at transplant (94.7% CR/PR *vs* 93.2%) for BEAM *vs* BUCYVP16 respectively. Differences were number of prior treatments (median 2 (1-8) *vs* 2 (1-13), p<0.01), comorbidity index (74.0% 0-3 *vs* 80.9%, p=0.03), and median CD34 dose infused (4.3 *vs* 6.02, p<0.01) for BEAM *vs* BUCYVP16. Median follow-up from diagnosis and transplant was 6.6 years and 4.0 years respectively for BEAM and 7.0 years and 4.3 years for BUCYVP16. Median day to neutrophil engraftment was 10 (8-19) *vs* 10 (9-15) (p<0.01) and median time to platelet engraftment was 18 (11-69) *vs* 16 (3-129) (p<0.01) for BEAM *vs* BUCYVP16. There were no statistical differences in CIR, PFS or OS. At 1, 3 and 5 years from AHSCT, PFS was 75%, 59%, and 46% *vs* 72%, 54%, and 44% (p=0.52); OS 88%, 75%, and 66% *vs* 83%, 69% and 59% (p=0.11) in the BEAM *vs* BUCYVP16 groups respectively (Figure 1). Veno-occlusive disease (VOD) was higher in the BEAM group (7 pts (2.6%) compared to none in BYCYVP16 (p<0.01)) but grade 3 or 4 mucositis was lower in BEAM (24.2%) compared to 56.1% in BUCYVP16 (p<0.01). There were no differences in hemorrhagic cystitis or second primary malignancy.

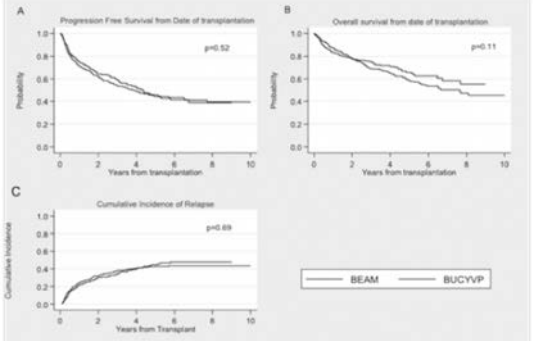

Figure 1.

Summary/Conclusions: This large retrospective study showed no statistical difference in PFS, OS, or CIR between BEAM and BUCVP16 conditioning regimens for AHSCT in relapsed NHL. Veno-occlusive disease occurred more frequently in the BEAM group, while grade 3 or 4 mucositis occurred more frequently in the BUCYVP16 group.

E1515

MINIMAL RESIDUAL DISEASE ANALYSIS USING DEEP SEQUENCING OF NPM1 AT THE TIME OF STEM CELL TRANSPLANTATION CAN PREDICT RELAPSE IN ACUTE MYELOID LEUKEMIA

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Background: Assessment of minimal residual disease (MRD) in acute myeloid leukemia (AML) has become increasingly more important for risk stratification, early detection of relapse and monitoring after stem cell transplantation (SCT). In AML with mutation in *NPM1*, RT-qPCR (reverse transcription-quantitative polymerase chain reaction) after first-line treatment has been shown to have high predictive value. However, there are numerous recurrent mutations in the *NPM1* gene and therefore specific MRD analyses of each individual mutation would require an overwhelming number of specific RT-qPCR assays. Using targeted deep sequencing, the same assay can be applied for all described *NPM1* mutations, and serve as a cost-effective alternative for MRD analysis also in cases with rare *NPM1* mutations.
Aims: The aim of this study was (i) to investigate whether deep sequencing may provide predictive information in the SCT setting, and (ii) to analyze potential relationship between deep sequencing and chimerism analyses post SCT.
Methods: A total of 37 bone marrow samples from 19 patients with AML with mutated *NPM1* were analyzed using targeted deep sequencing before (within one month) and three months after SCT. Sequencing was performed in multiplex on the Illumina MiSeq platform, using Truseq-library preparation, with coverages ranging between x6-18x10^5, a threshold of significance of 0.006% and a between run-CV of 2.1%. Calling of mutated and wild-type *NPM1* sequences was performed using an in house-script. Based on the linearity and sensitivity of the assay, *NPM1* MRD positivity was defined as variant allele frequency (VAF) ≥0.025% and *NPM1* MRD negativity as VAF<0.025%. Chimerism analysis with STR-PCR (short tandem repeat-polymerase chain reaction) was performed on bone marrow samples taken three months after SCT. Follow-up status of patients was collected from the local stem cell transplantation registry. The study was approved by the local ethics committee.
Results: Nine out of the 37 bone marrow samples displayed *NPM1* MRD positivity. Four of these were detected pre-SCT, and five post-SCT. In samples with *NPM1* MRD positivity, the *NPM1* mutation load ranged from 0.033-1.1% in VAF. Of the 5 patients with *NPM1* positivity post-SCT, only one had mixed chimerism, defined as >1% recipient donor T cells or CD34+ cells. There was no correlation between results from chimerism analysis and *NPM1* mutation load detected with deep sequencing. In patients with *NPM1* MRD positivity either pre- or post-SCT, the relapse-free and overall survival were significantly shorter compared with patients with *NPM1* MRD negativity at both time points (p=0.002 for both). In fact, all patients relapsing (n=5) showed *NPM1* MRD positivity in at least one of the samples.
Summary/Conclusions: In summary, MRD monitoring using targeted deep sequencing of *NPM1* is a highly sensitive technique, and in this study *NPM1* MRD positivity both pre- and post-SCT was highly predictive of relapse in AML after SCT. Chimerism analysis using STR-PCR appeared in this study to be less predictive of imminent relapse, at the time points analyzed. We conclude that detection of residual leukemic cells with deep sequencing is of value for clinical monitoring before and after SCT, allowing for early relapse-preventing intervention.

E1516

"REAL-LIFE" REPORT ON THE MANAGEMENT OF CHRONIC GRAFT-VERSUS-HOST DISEASE: A SURVEY CONDUCTED ON BEHALF OF GITMO (GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO)

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Background: Diagnosing, scoring and treating chronic graft-*versus*-host disease (cGVHD) is challenging because of polymorphic manifestations and lack of biomarkers for the diagnosis and assessment of disease activity. Several guidelines have been published, however the clinical practice remains demanding.
Aims: With this study we investigated the 'real life' management of cGVHD in 32 Italian Center members of GITMO (Gruppo Italiano Trapianto Midollo Osseo).
Methods: A detailed survey with 41 multiple-choice questions about the use of guidelines, and the first and further lines of treatment and management of cGVHD has been proposed to all adult and pediatric GITMO Centers (N=60)
Results: Thirty-two Centers replied, and according to the survey's results, 29 Centers referred to published guidelines for cGVHD management (mainly those proposed by National Institute of Health, NIH), however only 13 find them fully adequate. As shown in Figure 1, the main reasons for complain were related to second line treatment. According to NIH definition, most of the Centers (N=25) started treatment when severe cGVHD occurred, whereas 4 Centers also considered bad prognostic features regardless the grading. All Centers agreed on the use of prednisone as first line treatment, which was started at the dose of 1 mg/kg in 26/33 Centers. Prednisone alone was used in 4 Centers, while in the others it was associated to extracorporeal photo-apheresis (ECP, N=25), calcineurine inhibitors (CNI, N=17) and mycophenolate mofetil (MMF, N=11). A great inter-center variety has been reported regarding the duration of treatment, as well as the indication to and the choice of steroid-sparing agents. All but 6 Centers referred to NIH criteria to define response. Objective measurements (i.e. pulmonary function and lab tests), patient reports and ability to treatment discontinuation were scored as of great importance for response judgment, whereas physician opinion was scored as medium. In case of complete response, 30/32 Centers tapered steroid slowly, but there was no uniformity on the definition of slow taper (9/32 agreed on 10% reduction/week). Treatment failure, steroid refractoriness, intolerance or dependency were the main reasons for second line therapy. Sixteen Centers stated to have a policy for the choice of second line treatment, and the choice was customized according to organ involvement and patients condition in 24/32 Centers. Seven Centers declared a policy for third line of treatment. Overall, CNI, ECP and MMF were the most used treatments for refractory cGVHD: CNI regardless the involved organ, ECP and sirolimus for skin, lung, and gastrointestinal (GI) involvement, imatinib for skin and lung, infliximab and MMF for liver and GI, and rituximab for skin. ECP was available in 25/32 Centers. Finally, all responding Centers reported a strong need and willing to participate in prospective multicenter trials for both first and second line treatment of cGVHD; only 2 of them had a protocol open for refractory GVHD.

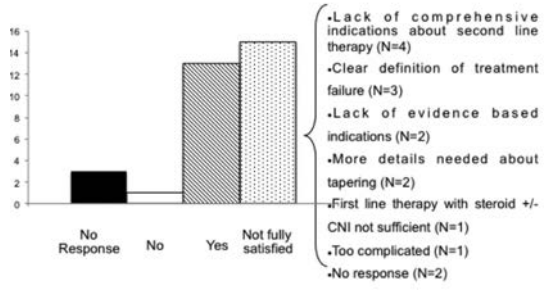

Figure 1. Are you satisfied from the current guidelines?

Summary/Conclusions: Despite the presence of guidelines, this survey showed a great disparity in the management of cGVHD, especially for refractory disease. The survey further emphasized the great interest and need for prospective trials investigating this setting.

E1517

A PHASE 2, MULTICENTER, SINGLE-ARM STUDY TO EVALUATE SAFETY AND EFFICACY OF DEFERASIROX AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH BETA-THALASSEMIA MAJOR

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Background: Hematopoietic stem cell transplantation (HSCT) is being increasingly used as curative therapy for severe disorders of the hematopoietic system such as thalassemia. The use of therapeutic phlebotomy post-HSCT is often difficult to perform in younger or anemic children. Deferiprone is shown to be related with bone marrow suppression and compliance to deferoxamine is low. Studies that evaluate the safety of deferasirox (DFX) in this setting, are limited.
Aims: The aim of this study is to evaluate safety and efficacy of DFX in Thalassemia Major patients in a Post Transplant setting.
Methods: This was a prospective, phase 2, multicenter, single-arm study to evaluate efficacy and safety of DFX in beta-thalassemia major (TM) patients who have undergone HSCT. The primary objective was to evaluate if DFX could provide safe chelation in patients with transfusional iron overload within a time period of 6 months to 2 years after HSCT. Transfusion independent patients aged ≥2 to<18 years old who had undergone HSCT with a washout period of at least 3 months after immunosuppressive therapies and had iron overload at screening defined by serum ferritin (SF) of >1000 µg/L or cardiac MRI T2*<20 ms or liver iron concentration (LIC, by MRI R2) of ≥5 mg/g were included in the study. Patients received DFX at an initial dose of 10 mg/kg/day with up titration every 3 months by 5 mg/kg/day per investigator judgment to the intended 20 mg/kg/day dose. Therapy continued for 52 weeks or until SF reached below 500 µg/L.
Results: Data from 27 patients enrolled (median (range) age 9.07 years (3-16), 70,4% males) were included in this analysis. One patient discontinued on week 14 due to withdrawal of consent. 20 patients were able to achieve the intended dose of 20 mg/kg/day. *Safety* From a total of 140 Adverse Events (AEs), 10 (7.1%) AEs in 4 patients were suspected to be related to study drug. The majority of AEs were of Grade I (n=81, 57.9%) or II (n=48, 34.3%). A total number of 6 (4.7%) SAE's were reported. 8 (5.7%) AEs resulted in study drug interruption or dose adjustment, 1 of which was suspected to be related to the study drug (ALT increase). On 51 (36.4%)AE's, no action was taken. Median ALT level decreased from 27 IU/L (range: 10-119) at baseline to 17 IU/L (range: 9-205) at week 52. Median AST level decreased from 35,5 IU/L (range: 17-66.5) to 26 IU/L (range:18-78) at week 52 (*Figure 1*). The median SCr was similar at baseline (0.4 mg/dL range: 0.2-0.7) and week 52 (0.4 mg/dL range: 0.2-1) Median cystatin C was similar at baseline (0.7 mg/mL range: 0.5-1) and week 52 (0.7 mg/mL range: 0.5-1) (*Figure 2*). No dose adjustments were required due to nephrotoxicity. Increased proteinuria was described in 9 (33.3%) patients, irrespective of dose at 52 weeks. No patient with proteinuria required dose adjustments. *Efficacy* SF significantly and consistently decreased through 52 weeks from a median of 1718 µg/L (range: 873.7-2919) to 845.3 µg/L (range: 146.2-2740), p<0.001. At week 52, 9 (33.3%) patients reached SF<500 µg/L, p<0.001. LIC also significantly decreased from a median of 8.60 mg/g (range: 2.8-43) to 4.1 mg/g (range 0.9-12.5), p<0.001. Cardiac T2* increased from a median of 25.95 ms (range: 4.5-51) to 28 ms (18.5-44), p=0.520.

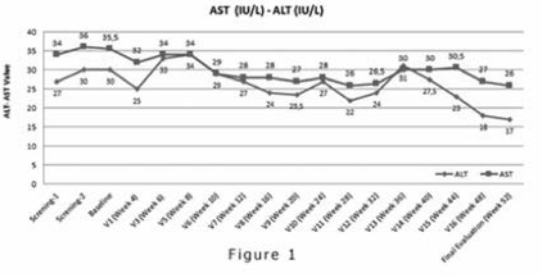

Figure 1

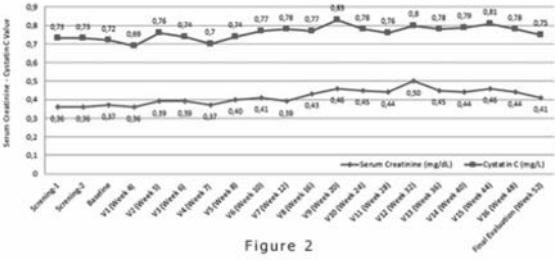

Figure 2

Figure 1.

Summary/Conclusions: Our findings suggest that DFX dose escalation up to 15-20 mg/kg/day is safe for TM patients following successful HSCT regarding nephrotoxicity and hepatotoxicity. 15-20 mg/kg/day deferasirox was also efficacious and this was evident through significant reductions of systemic and hepatic iron overload.

E1518

PARAMETERS OF PROTEIN METABOLISM AND THYROID FUNCTION AS PREDICTORS OF A SCORING SYSTEM FOR ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE

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Background: Some "classical" patient-, donor- and transplant characteristics, such as age, gender disparity, donor type, HLA-match, and source of stem cells, have been reported as predictors for acute and chronic GVHD. However, no studies analysed these "classical" variables together with parameters of metabolic and endocrine functions that may potentially influence the immune system.
Aims: Patient-and transplant variables together with index of liver and thyroid function, and some parameters of protein and lipid metabolism were retrospectively evaluated at different time points after transplantation, in order to identify possible predictors of acute and chronic GVHD and to calculate a risk score.
Methods: Clinical and transplant characteristics, number and type of infections before and after SCT were analysed in 200 patients. The following variables were also analysed pre-SCT, at day +7,+14,+21,+28, at+3 and +6 months: LDH, parameters of liver function; parameters of protein and lipid metabolism; thyroid function tests; autoimmune parameters; body mass index. A 2-step multivariate analysis was performed using principal component analysis and Cox regression analysis. Based on the regression coefficient of Cox analysis for each significant predictor, a scoring system for acute and chronic GVHD was calculated.
Results: In multivariate analysis, diagnosis of Myelodisplastic Syndrome or Chronic Myeloid Leukemia (p=0.0004), conditioning regimen including Total Body Irradiation (p=0.0003), and pre transplantation urea >34 mg/dl with +21 day urea >54 mg/dl (p=0.0008) were predictors for acute GVHD. Score values for each factor are 2, 1, 1, respectively and the system had a score range between 0 and 4. The probabilities of acute GVHD according to the sum scores ranged from 8% (score 0) to 98% (score 4). Female donor (p=0.0008), pre-SCT TSH values ≥2 mU/L with +28 day urea ≥39 mg/dl (p=0.02), +6 month total protein<5,5 g/dl with gamma-GT ≥347 U/L (p=0.0001) resulted predictors for moderate/severe chronic GVHD. Risk of chronic GVHD at +6,5 month ranged from 3% (score 0) to 97% (score 4).
Summary/Conclusions: Our study evidenced that factors other than those "classical" may be associated to GVHD. The scoring system included routine-parameters, which are easily available in clinical practice. Urea levels depend on the balance between protein intake, endogenous catabolism and urinary excretion. The inflammatory microenvironment of GVHD promotes muscle catabolism and hence, increased urea levels. Increased urea levels could be indirect index of increased uremic toxins as well, which may stimulate the production of pro-inflammatory cytokines and the activation of leukocytes. Increased urea levels and uremic toxins could also derive from a dysregulated metabolism of the gut microbiome that may influence immune system. Our findings suggest the usefulness to study in deep the complex network between metabolic/endocrine functions and immune system for a holistic approach of the transplant management.

E1519

DEFIBROTIDE FOR THE PREVENTION AND TREATMENT OF HEPATIC VENO-OCCLUSIVE DISEASE AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE

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Background: Hepatic veno-occlusive disease (VOD) is a common and serious complication of hemotopoietic stem cell transplantation (HSCT) in children.
Aims: We aimed to assess prospectively the use of prophylactic defibrotide in pediatric patients undergoing HSCT.
Methods: In this study, 76 patients who underwent HSCT were given defibrotide prophylaxis as 25 mg/kg per day in four divided intravenous infusions over 2h, starting on the same day as the pretransplantation conditioning regimen. The mean duration of use of defibrotide is 20 days as a prophylaxis.
Results: In this study, 76 patients were recruited, 53 male patients and 23 female patients, with the average of 9.3 years, range 1-20; 4% infants, 55% children and 41% adolescent. There were 33 patients with thalassemia major, 30 patients with leukemia, 7 patients with aplastic anemia, one patient with Diamond Blackfan anemia, two patients with congenitale dyserythropoetic anemia, one patient with osteopetrosis, one patient with famial hemophagocytic lymphohistiocytosis, and one patient with Kostman syndrome. All transplants were allogeneic. No serious side effects were seen. In eight patients developed clinical VOD (Seattle criteria). In these patients, defibrotide dose was increased to a treatment dose of 40-60 mg/kg per day. One infant patient with Kostman syndrome and one patient with aplastic anemia died due to hepatic and pulmonary veno-occlusive disease. After 24 months of follow up, 6 patients who developed VOD are being well and no patient have transplant related complications.
Summary/Conclusions: Hepatic veno-occlusive disease, which is caused by hepatocyte and sinusoidal vessel endothelium damage, can ocur early after HSCT, and in its severe form, may lead tol iver faillure, hepatorenal syndrom, portal hypertension, and eventually death from multiorgan faillure. In this prospective study, we demonstrated that the use of defibrotide is safe and effective in preventing and treating VOD in pediatric patients at high risk.

E1520

COMPARISON OF INTENSIFIED MYELOABLATIVE CONDITIONING REGIMEN WITHOUT ATG TO MYELOABLATIVE CONDITIONING REGIMEN FOR SINGLE-UNIT UMBILICAL CORD BLOOD TRANSPLANTATION IN HEMATOLOGICAL MALIGNANCIES

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Background: The superiority and safety of strengthening conditioning regimen for single-unit umbilical cord blood transplantation (sUCBT) in hematological malignancies remain controversial.
Aims: To define whether intensified myeloablative regimen is superior to conventional myeloablative regimen for single-unit umbilical cord blood transplantation (sUCBT) in hematological malignancies.
Methods: We retrospectively analyzed the clinical data of 251 hematological malignancies undergoing sUCBT from Apr 2000 to Dec 2014 at Anhui provincial hospital hematology department. In these patients, 216 received the intensified myeloablative conditioning regimen (IMCR), and 35 received the myeloablative conditioning regimen (MCR). We evaluated the effect of IMCR without ATG on patient outcomes. Among the IMCR group, 106 patient received TBI/Ara-C/CY regimen, 41 received Ara-C/Bu/CY regimen, 69 received Flu/Bu/CY regimen, and all received a combination of CsA and MMF for the prophylaxis of GVHD. For the MCR group, 35 patients received Bu/CY regimen using CsA/MMF±ATG or MTX for the prophylaxis of GVHD.
Results: The cumulative incidence of myeloid and platelet engraftment of the IMCR group was significantly higher than that in the MCR group (96.98% *vs* 82.81%, 85.89% *vs* 51.79%, P=0.000, 0.003, respectively). Corresponding incidences of transplantation-related mortality (TRM) by 180 days in the IMCR group and the MCR group were 19.50% *vs* 41.67% (P=0.0028), respectively. The incidence of CMV infection and pre-engraftment syndrome were significantly higher in the IMCR group compared with that in the MCR group (68.8% *vs* 40%, 82.9% vs.48.6%, P=0.001, 0.000, respectively). There were no differences in the incidence of grade II to IV aGVHD, grade III to IV aGVHD and 2-year cumulative incidence of relapse. Up to Oct. 2015,with a median follow-up of 30 months, the estimated 3-year overall survival and disease-free survival in the IMCR group were both significantly higher than that of the MCR group (62.4% *vs* 35.5%, 60.1% *vs* 35.5%, P=0.000,0.004, respectively). For 49 patients not in remission at transplantation in the IMCR group, the cumulative incidence of 2-year relapse was 21.92% (95%CI, 21.15-22.69%), which was comparable with patients in remission (P=0.115). The estimated 3-year overall survival and disease-free survival of advanced patients undergoing IMCR were 54.8% and 52.1%, respectively. For six patients older than 40 years, the incidences of myeloid, platelet engraftment and 180d TRM were not significantly different from patients between 20 and 40 year old and those younger than 20 year□100% vs.96.36% vs.96.73%, 66.67% vs.91.32% vs.84.75%, 16.67% vs.19.87% vs.18.48%, P=0.418, 0.405, 0.975, respectively).
Summary/Conclusions: This study is the first to show the superiority of intensified myeloablative regimen to conventional myeloablative regimen. A large-scale prospective study was needed to investigate its application.

E1521

TCRAB+/CD19+-DEPLETION IN HEMATOPOIETIC STEM CELLS TRANSPLANTATION FROM MATCHED UNRELATED AND HAPLOIDENTICAL DONORS IN PEDIATRIC ACUTE MYELOBLASTIC LEUKEMIA PATIENTS

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Background: Graft-*versus*-host disease (GvHD) and GvHD-associated morbidity and mortality are major obstacles to the success of transplantation from unrelated and haploidentical donors. Negative depletion of α/β(+) T cells and CD19+ B lymphocytes is a technology of graft manipulation with a potential to improve GvHD control and immune reconstitution.
Aims: The aim was to study retrospectively the outcomes of TCR-alpha/beta depleted transplants in a group of pediatric acute myeloid leukemia patients with the emphasis on two consequtive GVHD prophylaxis regimens.
Methods: A total of 59 pediatric patients with acute myeloblastic leukemia (17 female, 42 male, median age 9,1 years, range 0,6-22) underwent allogeneic HSCT between May 2012 and June 2015. Twenty two patients received haploidentical graft, 37 - a graft from matched unrelated donor. Disease status at transplant was CR1 in 40 pts., CR≥2 in 10 pts., active disease(AD) in 9 pts. Transplantation in CR1 was performed according to risk stratification scheme in the current institutional AML protocol. For all patients it was first HSCT.

TCRαβ+/CD19+ depletion of HSCT with CliniMACS technology was implemented in all cases. All patients received Treosulfan/Melphalan/Fludarabine as conditioning regimen. Two regimens of GvHD prophylaxis were used: regimen 1 (n=31): ATG (horse, ATGAM) 50 mg/kg and post-transplant Tacro/MTX (n=28), or no post-transplant prophylaxis (n=3); regimen 2 (n=28): ATG (rabbit, thymoglobuline) 5 mg/kg, rituximab 200mg/m^2 and post-transplant bortezomib (n= 27) or Tacro/MTX (n=1). The median dose of CD34+ cells in transplant was 7,9 x10^6/kg (range 1-21), TCRα/β - 15x10^3/kg (range 0,6-210).
Results: Primary engraftment of WBC and platelets was achieved in 58 (98,3%) patients at a median of 14 days, 1 pt. with AD had primary graft failure with no evidence of leukemia and was retransplanted from MUD. No case of graft rejection was registered. Early mortality was low with a +100-day pTRM - 3,4% (95% CI: 0,1-13.2), 3-year pTRM-10,8% (95%CI: 5-23). The 2 early deaths included viral infections (ADV), four late: bacterial sepsis in 1 pt. and viral infection(ADV and CMV) in 3 pts, all with extensive chronic GvHD. Cumulative incidence (CI) of acute GvHD grade ≥II was 20,3% (95% CI: 12-34), grade III–IV 8,5% (95%CI: 4-20) and chronic GvHD-28% (95% CI: 18-45). No correlation between graft composition and aGvHD was noted. CI of acute GvHD was significantly lower in a group with regimen 2: 7,4% (95% CI: 2-28) *vs* 31,3% (95% CI: 19-52) in group with regimen 1, p=0,028. Regimen 2 was also effective in prevention of chronic GvHD: CI at 1 year after HSCT was 14% *vs* 36%, p=0,085 Median time of follow-up for survivors is 1,9 years (range 7 months-3,5 years). CI of relapse at 3 year is 27,6% (95%CI: 18-43). Three year pEFS is 62% (95%CI: 49-75), 3-year pOS - 64% (95%CI: 50-78). There was no significant difference in survival and relapse rate according leukemia subtype, remission status and donor type.
Summary/Conclusions: We confirm that the depletion of TCR-alpha/beta and CD19 lymphocytes from the graft ensures high engraftment rate and acceptable transplant-related mortality in pediatric AML patients. All major outcomes were equivalent between transplantation from unrelated and haploidentical donor. GvHD prophylaxis including Thymoglobulin/Rituximab/Bortezomib improves GvHD control in recipients of TCRα/β- depleted grafts in comparison to ATGAM/Tacro/MTX apparently without loss of anti-leukemic activity.

E1522

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACQUIRED SEVERE APLASTIC ANEMIA

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Background: Acquired severe aplastic anemia (SAA) is a life-threatening disease and allo-geneic hematopoietic stem cell cell transplantation (HSCT) is a curative treatment. Recent researches indicate that haploidentical HSCT (haplo-HSCT) is an effective therapeutic option for SAA.
Aims: To evaluate the efficiacy and safety of haploidentical hematopoietic stem cell transplantation (haplo-HSCT) in patients with acquired severe aplastic anemia (SAA), who lacked suitable related or unrelated HLA-matched donors
Methods: 39 SAA patients underwent haplo-HSCT from Jul 2012 to Jun 2015 at our center. There were 23 males and 16 females at a median follow-up of 11 (range, from 0 to 36) months. The median time from diagnosis to transplantation was 1 (range, from 0.5 to 52) months. The median ages of SAA patients and related haploidentical donor were 23 years (range, 9 to 51years) and 45 (range, from 21 to 61) years, respectively. All patients were given BuCy plus ATG conditioning regimen. GVHD prophylaxis regimen consisted of cyclosporine A (CsA), mycophenolate motetil (MMF), and short-term methotrexate.
Results: Stem cells were collected from bone marrow in 23.08% (n=9) of patients, peripheral blood in 2.56% (n=1), bone marrow plus peripheral blood in 74.36% (n=29) patients. 36 patients received haplo-HSCT combined with the third part of cord blood transfusion 92.31%. The median stem cell dose transplanted was 9.76 (range, from 4.02 to 20.10)×10^8/kg for mononuclear cells, while 3.4 (range, from 1.05 to 8.60)×10^8/kg for CD34 cells. 36 patients achieved neutrophil engraftment at a median of 12 (range, from 9 to 28), and 29 patients achieved platelet engraftment at a median of 29 (range, from 10 to 26) days. Cumulative incidence of III°~IV° acute graft *versus* host disease (aGVHD) was 8.9±4.9%. 6 patients died of transplant-related mortality (TRM), including 4 from severe infection, 1 from TMA and 1 from encephalorrhagia. The 2-year overal survival rate of all patients was 83.2%±6.4%.
Summary/Conclusions: Haplo-HSCT is likely to be an option for SAA patients without suitable related or unrelated HLA-matched donors, in consideration of the acceptable TRM and severe GVHD incidences.

E1523

EBV AND CMV INFECTION IN RECIPIENTS OF HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANTATION RECEIVING TWO DIFFERENT DOSES OF ANTITHYMOCYTEGLOBULIN AS CONDITIONING REGIMEN

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Background: Antithymocyteglobulin (ATG) has been widely used to prevent acute graft-*versus*-host disease (aGVHD) in recipients receiving haploidentical hematopoietic stem cell transplantation (HSCT). Notwithstanding, immunosuppressive effect of ATG increases the risk for infections, especially viral infection. Therefore, the optimal dose of ATG which elicits adequate immune suppression and limits the development of viral infection/reactivation is needed to be studied.
Aims: To evaluate the effect of different doses of ATG on post-transplant viral infection, this multicenter prospective study was conducted to compare EBV and CMV infection in haploidentical HSCT recipients receiving 7.5 mg/kg or 10 mg/kg of ATG. Besides, the incidence of aGVHD was compared between the two arms.
Methods: Between January 2013 and August 2015, 217 consecutive patients with hematological malignancies undergoing haploidentical HSCT were enrolled in 4 hospitals. One hundred and ten patients were randomized to receive a total dosage of 7.5 mg/kg of ATG and 107 were randomized to receive 10 mg/kg of ATG.
Results: The 1-year cumulative incidence of EBV infection were 30.7±6.0% in 7.5 mg/kg of ATG arm and 43.1±6.0% in 10 mg/kg arm (*P*=0.107). CMV infection were comparable in the two arms (7.5 mg/kg arm: 79.6±3.9% *vs* 10 mg/kg arm: 84.1±3.6%, *P*=0.188). Acute GVHD grade II to IV within 100 days occurred in 35.9±5.2% of the patients with 7.5 mg/kg ATG and 19.2±4.8% of those with 10 mg/kg ATG (*P*=0.004). The incidence of aGVHD grade III to IV within day 100 were 14.1±3.9% in 7.5 mg/kg arm and 7.3±3.3% in 10 mg/kg arm (*P*=0.072). No difference in the 1-year cumulative overall survival was found (75.8±6.8% in 7.5 mg/kg arm *vs* 73.7±4.6% in 10 mg/kg arm, *P*=0.192).
Summary/Conclusions: Compared with 10 mg/kg of ATG, the application of 7.5 mg/kg might reduce the viral infection after haploidentical HSCT and not increase the risk for severe aGVHD.

E1524

AUTOLOGOUS STEM CELL TRANSPLANT WITH NON CRYOPRESERVED HEMATOPOIETIC STEM CELL IN ALGERIAN PATIENTS WITH MULTIPLE MYELOMA

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Background: For younger patients under 65 years of age, induction followed by high-dose chemotherapy with autologous stem cell transplantation (ASCT) is the standard treatment in multiple myeloma (MM). There is limited experience with non-cryopreserved autologous hematopoietic stem cell transplantation.
Aims: We evaluated the efficacy and safety of non-cryopreserved storage of ASCT in patients undergoing ASCT for MM.
Methods: From May 2009 to December 2013, 134 patients with MM were treated in our center in Oran. The median age at ASCT was 55 years (range; 27-67). There were 80 males and 54 females. The median harvested CD34$^+$ cell count was 3,5x10^6/kg (range; 1, 22 to 13, 24). Autologous stem cell was mobilized using G-CSF alone (10 µg/kg/day for 5 days). Leukapheresis to harvest stem cells were performed on day -2 and -1. The grafts were kept in a conventional blood bank refrigerator at +4°C until reinfusion on day 0. The conditioning regimen consisted of melphalan 200 mg/m^2.
Results: All patients had engraftment on the median of day 10 (range; 7 to 17) and platelet transfusion independence on the median of day 13 (range; 9 to 24). There was no graft failure. Mucositis grade 3/4 was seen in 68% patients. Transplant related mortality at 100 days was 2.9%. The overall response to transplant was 92%. In the 130 evaluable patients, the median post-transplant overall survival had not been reached. The estimated overall survival at 75 months was 63% with 95% confidence interval and the median post-transplant disease free Survival was 35 months (0.05%). 93 (72%) patients are alive and 75 (81%) without disease activity after a median follow-up of 35 months (range; 3 to 75).
Summary/Conclusions: We conclude that high dose chemotherapy and autologous transplant with non cryopreserved ASCT is a simple, effective and safe method for MM with equivalent results, and that cryopreservation is not necessary in the treatment of MM under our work conditions in developing countries.

E1525

SECONDARY ANTIFUNGAL PROPHYLAXIS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT: THE NIGUARDA HOSPITAL EXPERIENCE

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Background: As allogeneic hematopoietic SCT (HSCT) is the only curative treatment for the majority of acute leukemia patients (pts), clinicians encounter the therapeutic dilemma of balancing cytotoxic therapy and HSCT with the risk of invasive mold infections (IMIs) relapse-related morbidity and death. Use of effective, less toxic antifungals and routine early chest CT implementation, leading to prompt intensive antifungal therapy, improved responses and survival. These developments have increased the interest regarding the efficacy of secondary antifungal prophylaxis (SAP), but data are limited.

Aims: The aim of the study was to evaluate the IMI relapse rate and associated mortality rate after allogeneic HSCT in a cohort of pts with hematological malignancies. We assessed the HSCT outcome of pts with a history of IFI and the efficacy of SAP.
Methods: We retrospectively collected data of pts with IFI treated at Niguarda bone marrow transplant center between 2004 and 2016. Among the 322 HSCT recipients we identified 36 pts, median age 49 yrs (range 16-67), with a history of IFI. The underlying malignancies were AML (26), ALL (8), myelodysplastic syndrome (2). Hematologic disease status at transplant was CR (26), PR (6) and refractory disease (4). Conditioning regimens were myeloablative in 22 pts and reduced-intensity conditioning in 14.
Results: The IFI diagnosis according to the EORTC/MSG criteria was proven, probable and possible in 6 (2 A.fumigatus, 1 A.terreus, 1 Absidia Corymbifera and 2 Mucor), 5 and 25 pts, respectively. Sites of involvement included lung (31), sinuses (3), central nervous system (1) and breastbone (1). The median duration of antifungal treatment before SCT was 110 days (10-360) and the IFI status at transplantation was CR, PR (defined as presence of residual pulmonary localizations at CT scan but no symptoms) and active in 18, 16 and 2 pts, respectively. During the peritransplant period 31 pts received SAP with liposomal amphotericin (Amb), 1 pt received both Amb and Posaconazole, 2 pts Voriconazole, 2 pts Caspofungin. After engraftment 12 pts (1 active IFI, 5 PR and 6 CR) continued antifungal therapy: 6 received Voriconazole, 1 Posaconazole, 5 Amb for a median period of a month; 3 of Amb pts continued prophylaxis with Posaconazole (2) and Voriconazole (1) up to ciclosporine suspension. By day +100, IFI progression occurred in only 1 pt with active hematologic disease and proven aspergillosis who rapidly died. No fungal recurrence was present in the other pts, also in presence of CMV reactivation (15 pts). We observed 16 cases of aGVHD (12 with possible, 1 with probable and 3 with proven IFI) and 18 cases of cGVHD, treated with steroid therapy (median duration 17 months, 1-80). Among GVHD pts only one had a possible IFI reactivation (20 months from HSCT) in absence of SAP. With a median follow-up of 37 months (1–118) 20 pts died due to leukemia relapse (10), bacterial infections (6), GVHD/transplant toxicities (3), IFI (1).
Summary/Conclusions: In our experience (1 IFI-related death and 1 possible late IFI reactivation) with a correct SAP, prior IFI may no longer be considered a contraindication for allo-SCT. However, new data are needed to guide the use of SAP and to improve the cost-effectiveness of treatment. Determination of appropriate time to discontinue SAP is one of the many areas where more data are needed. Prolonged fungal pre-transplant therapy, aiming to achieve a clinically undetectable state of infection, followed by SAP during transplant may allow the SCT with reduced fungal reactivation.

E1526

EFFECT OF DONOR KIR GENOTYPE ON THE OUTCOME OF PEDIATRIC ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Natural Killer (NK) cells have recently attracted for the potential role in graft-*versus*-tumor effect and NK cell alloreactivity was investigated in several studies.
Aims: The impact of donor killer immunoglobulin-like receptor (KIR) phenotype and genotype on the outcome following allogeneic hematopoietic stem cell transplantation (HSCT) in children was evaluated.
Methods: We prospectively evaluated the gene content and expression of the KIR in children undergoing allogeneic HSCT for malignant diseases. The incompatibility between donor KIR and recipient KIR ligand (receptor-ligand (R-L) mismatch) was defined if the donor has an inhibitory receptor for which the cognate ligand is absent in the recipient. Donors were assigned to A or B KIR haplotype donor according to their gene content. A haplotype donor was defined as the individual having only genes of the group A KIR haplotypes. All other individuals having one or more B haplotype specific genes were defined as B haplotype donor.
Results: All 42 patients received allogeneic HSCT from sibling (n=11), unrelated donor (n=20), or haploidentical donor (n=11) between January 2011 and December 2013. Seventeen transplants were performed in patients with acute myeloid leukemia, 15 in patients with acute lymphoblastic leukemia, 8 in patients with neuroblastoma, and 2 in patients with other solid tumors. Five donor-recipient pairs had KIR R-L match and remaining 37 donor-recipient pairs had KIR R-L mismatch. The 2-year relapse-free survival (RFS) was 100% in recipients with R-L match and 69.3±8.9% in recipients with R-L mismatch, respectively (P=0.18). However, recipients who received HSCT from B haplotype donor had better RFS than those who received HSCT from A haplotype donor (93.3±6.4% *vs* 58.0±12.0%, P=0.03). Furthermore, there was no relapse in four recipients who received HSCT from donors having 2 or more B gene-content motifs. Survival benefit of B haplotype donors was found in both HLA-matched and mismatched transplants. Donor KIR genotype and R-L mismatch did not have any significant effect on rates of grade III-IV acute graft-*versus*-host disease (GVHD) or chronic GVHD. In multivariate analysis, A haplotype was the only independent factor predicting increased risk of relapse (RR 16.5, 95% CI 1.3-217.4, P=0.03).
Summary/Conclusions: This analysis has identified decreased relapse and improved RFS in patients who received allogeneic HSCT from donors having B haplotype. KIR genotyping should be considered for successful selection of a NK cell alloreactive donor.

E1527

DEVELOPMENT AND CLINICAL EVALUATION OF DIGITAL PCR ASSAYS FOR SENSITIVE CHIMERISM ANALYSIS BASED ON DELETION/INSERTION POLYMORPHISMS

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Background: Examination of chimerism has become a crucial means to monitor engraftment and, in the absence of specific disease markers, detect recurrence of leukemic cells after allogeneic stem cell transplantation. Various techniques have been established for chimerism analysis; the most often used STR amplification has high power of discrimination, but its detection limit is restrained to 1%>5%. Consequently, more sensitive approaches are required to ensure earlier detection of engraftment failure or relapses.
Aims: We have previously shown that digital PCR assays detecting deletion/insertion polymorphisms (DIPs) combine excellent sensitivity (routinely ≤0.1%) with accurate quantification over a large dynamic measurement range. Moreover, built on the dPCR principle, there is no need for standard curves and replicate measurements. Here we aimed at introducing a whole panel of digital-PCR based assays for routine chimerism measurement.
Methods: We selected a set of 53 DIPs and tested their suitability for duplex analysis in combination with a reference gene and a Y-chromosome specific locus. We identified 29 DIPs with high power of discrimination and good performance in duplex PCR.
Results: Assays were optimized and technically validated; applicability for diagnostics was confirmed on clinical samples in direct comparison with STR and qPCR. Finally, we established a screening plate for initial genotyping with DIP-specific digital duplex assays for convenient application of the system in every-day diagnostics.
Summary/Conclusions: In conclusion, we have developed and validated assays for regular chimerism determination including a screening plate for initial DIP assessment. The new tool ensures precise, robust and fast analysis of hematopoietic chimerism with a regular sensitivity of ≤0.1% (for ≥20 ng of genomic DNA). We propose that the new method will be highly useful for clinical routine diagnostics.

E1528

IRON-CHELATING THERAPY IMPROVES OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MDS

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Background: Iron overload (IO) is general among patients with myelodysplastic syndrome (MDS). IO increased the non-relapse mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Therefore, the application of iron chelating therapy in allo-HSCT attracts the extensive attention recently. r severe HC following HSCT.
Aims: To investigate the effect of iron chelating therapy on hematopoietic reconstitution and related complications of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with myelodysplastic syndrome (MDS).
Methods: Various clinical parameters were analyzed retrospectivly in 57 MDS patients with iron overload (IO)who received allogeneic transplantation. According to the level of serum ferritin (SF) before transplantation, we divided the patients into two groups: the effective treatment group (19 cases, SF<1000ng/ml) and iron overload group (38 cases, SF≥1000ng/ml).
Results: (1) 30/57 cases were received iron chelating treatment, 19/30 (63%) cases obtained obvious curative effect, the SF level before transplantation was<1000ng/ml, the median was 561(223-846) ng/ml. The effort of other 11/30 (37%) cases was not obvious, with the SF level before transplantation was ≥1000ng/ml,the median was 1262(1100-2352) ng/ml. 27/57 patients didn't received iron chelating therapy before transplantation, the SF level before transplantation was≥1000ng/ml,the median was 1540(1320-3112) ng/ml. (2) The rate of fully-engraftment in the effective treatment group and iron overload group was 19/19 (100%) and 34/38 (89%). And the latter group had 4/38 (11%) cases failed the hematopoietic reconstitution.The medium time of myeloid and platelet reconstitution of the 19 cases of effective treatment group was (12±2) and (16±6) days respectively, while those of 34 cases of iron overload group was (13±3)

and (18±6) days. The hematopoietic reconstruction was shorten in the former group, however the difference between the two groups had no statistical significance (*P*=0.441, *P*=0.579). (3) The infection rate of the effective treatment group and iron overload group was 7/19 (37%) patients and 28/34 (82%) patients respectively. The risk of infection of the effective treatment group was decreased significantly (*P*=0.002). (4) The incidence of aGVHD of the effective treatment group was 5/19 (26%) patients, all of which were I-II degree. The incidence of aGVHD of iron load group was 22/34 (65%) cases, with 16 cases of I-II degree, 6 cases of III-IV degree. The risk of aGVHD in the effective treatment group was significantly decreased (*P*=0.01). (5) The median disease-free survival (DFS) in effective treatment group was 28.9 (0.3-89.5) and 21.2 (0.1-81.0) months in iron load group during a median follow-up period of 22.0 (0.1-89.0) months.The DFS of effective treatment group was prolonged (*P*=0.053).
Summary/Conclusions: Effective iron chelating therapy before transplantation was helpful to hematopoietic reconstitution, and significantly reduced the incidence and degree of infection and aGVHD, decreased transplantation related mortality(TRM) and prolonged DFS, thereby improved the success rate of transplantation in MDS.

E1529

IMPACT OF CYCLOSPORINE-A CONCENTRATION IN T CELL-REPLETE HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Previous studies on HLA-identical allo-HSCT (allogeneic hematopoietic stem cell transplantation) have shown that level of blood CSA concentration during early stage after HSCT were significantly associated with risk of severe aGVHD (acute graft *versus* host disease). However, there remains a lack of data on T cell-replete haploidentical HSCT in which using ATG combined with CSA, MTX, MMF to prevent GVHD. Objective: This study was to investigate whether CSA (cyclosporine-A) levels impact on clinical outcomes of patients in the T cell-replete haploidentical allo-HSCT setting.
Aims: This study was to investigate whether CSA (cyclosporine-A) levels impact on clinical outcomes of patients in the T cell-replete haploidentical allo-sct (allogeneic stem cell transplantation) setting.
Methods: we retrospectively analyzed 140 consecutive patients who conducted T cell-replete haploidentical allo-sct in our institution to assess effect of CSA concentration in early stage on clinical outcomes including hematopoietic recovery, aGVHD (acute graft *versus* host disease), infection, DFS (disease free survival), and OS (overall survival).
Results: The median concentrations of CSA in the blood in the 1st, 2nd, 3rd and 4th week after allo-sct were 218ng/ml (rang:54-1377ng/ml), 235ng/ml (rang:27-1500ng/ml), 263ng/ml (rang:20-1500ng/ml), and 270ng/ml (rang:4-1500ng/ml); 46%, 40%, 27% and 18% of the patients had CsA blood levels below 200 ng/mL during these weeks. 39 patients developed grade 2–4 aGVHD for a cumulative incidence of 27.8% at a median of 32 days.CSA levels during 1st, 2nd, and 4th week didn't affect patients' hematopoietic recovery, aGVHD, infection, DFS, and OS significantly ($p>0.05$). However, patients having CSA concentration below 200ng/ml in the 3rd week had a higher cumulative incidence of grade 2-4 aGVHD ($p=0.02$). In a multivariate logistic regression analysis, low CSA concentration (below 200ng/ml) in the 3rd week remained the independent risk factor of grade 2-4 aGVHD ($p=0.02$). CSA level in the 3rd week was not associated with patients' engraftment, infection, DFS, and OS ($p>0.05$).
Summary/Conclusions: The analysis presented here emphasize that adequate management of CSA levels in the early stage, especially during the periengrafment period, can improve clinical outcomes in the T cell-replete haploidentical allo-sct setting.

E1530

HYPERBARIC OXYGEN THERAPY IS CLINICALLY EFFECTIVE FOR REFRACTORY VIRUS-ASSOCIATED HEMORRHAGIC CYSTITIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Late-onset hemorrhagic cystitis (HC) after allogeneic hematopoietic stem cell transplantation (HSCT) has been frequently associated with viral infection of adenovirus (ADV), BK virus (BKV), JC virus (JCV) or another viruses. Antiviral drugs are of limited efficacy, and the optimal treatment for HC has not yet been established. Hyperbaric oxygen (HBO) may benefit these patients, but efficacy and safety of HBO for HC has not been evaluated sufficiently.
Aims: To clarify the efficacy and safety of HBO therapy for viral HC, we retrospectively evaluated clinical data during the therapy.
Methods: In 199 cases of allogeneic HSCT from September 1994 to November 2015, we experienced 8 cases of refractory viral HC by ADV or JCV. We evaluated the effectiveness of HBO therapy in 8 patients with refractory HC after allogeneic HSCT at our institution.
Results: All 8 patients had macroscopic hematuria associated ADV or BKV infections. Patients received 100% oxygen in a hyperbaric chamber at 2.1 atmospheres for 90-120 min per day, 5 days per week, with 13 median treatments (range, 10-16). The 5 of 8 patients (62.5%) showed complete resolution of hematuria in 7 days. The pain during urination disappeared in all 8 patients (100%) in 5 days. Urinary DNA titers of ADV or BKV declined after HBO. Patients who started on HBO earlier after diagnosis of HC responded sooner and better than those who did on HBO later. Interestingly, 3 of 8 patients (37.5%) showed dramatic recovery from refractory acute graft *versus* host disease (GVHD) symptom accompanied by thrombotic microangiopathy (TMA), after HBO therapy for refractory virus-associated HC.
Summary/Conclusions: HBO therpy was generally well tolerated, and proved to be a reliable option for refractory viral HC cases to manage their condition.

E1531

THE INFLUENCE OF NK CELL ALLOREACTIVITY ON OUTCOME AFTER T-CELL-DEPLETED HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN PATIENTS WITH ACUTE LEUKEMIA

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Background: HSCT from human leukocyte antigen (HLA) haplotype-mismatched family members is the only curative option for patients with high-risk acute leukemia who do not have a matched donor. Natural killer (NK) cells are the first lymphoid cell population to reconstitute after allogeneic HSCT. It is believed that NK alloreactivity is regulated by quantitative differences in activating and inhibitory signals (mediated by activating and inhibitory killer cell immunoglobulin-like-receptors (KIRs), which recognize HLA class-I alleles (KIR ligands)). A number of studies demonstrated beneficial effects of donor activating KIRs and KIR-ligand mismatches on transplant outcome.
Aims: In the present study we tested if donor KIR haplotype, particular KIR genes and KIR-ligand mismatch have an effect on relapse incidence and event-free survival (EFS) in pediatric patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).
Methods: Fifty eight patients between 1/2012 and 6/2015 in complete remission with ALL(n=26) and AML(n=32) were included in this study. Patients received T-cell-depleted haploidentical transplants after myeloablative conditioning regimen and post-transplant tacrolimus (n=24) or bortezomib (n=34) as GVHD prophylaxis. In all cases GCSF-mobilized PBSC were depleted of TCR-alpha/beta and CD19+ cells with CliniMACS Plus. All samples were typed for HLA-A,-B,-C,-DRB1,-DQB1 by sequence-based typing. KIR genotype was performed by sequence-specific primers. "Receptor-ligand" model (at least one inhibitory KIR gene expressed in the donor in the abscence of the appropriate HLA-molecules in the recipient's ligand repertoire) was used to predict NK alloreactivity. The role of donor B *vs* A KIR haplotype and the number of total, inhibitory or activating KIR in the donor was evaluated. The effect of each individual KIR gene was analyzed. Cumulative incidence estimates were used for relapse and nonrelapse mortality, because they are competing risk. The Gray test compared univariate competing risk outcome. The Kaplan-Meier method evaluated EFS.
Results: All patients had primary engraftment (median 14 days for neutrophils and 15 days for platelets). The median number of total KIR genes in donor was 10(range, 7-15), activating 3(1-6), inhibitory 7(5-9). No significant effect of KIR genotype was found in patients treated with tacrolimus and in the whole cohort of patients. Incidence of relapse decreased for patients treated with bortezomib by: donor's B/x haplotype compared to A/A, 12±6.5 *vs* 22.2±13.6, p=0.05; higher total number of KIR genes (total KIR, 0 *vs* 29.4 ±10.9, p=0.005; activating KIR, 0 *vs* 29.4 ±10.9, p=0.004; inhibitory KIR, 8.7±5.9 *vs* 27.3 ±13.2, p=0.014; Influence of particular KIR genes on the incidence of relapse was detected: KIR2DS2(10±6.7 *vs* 21.4±10.7, p=0.05); KIR2DS3(0 *vs* 22.7±8.8, p=0.028); KIR2DL2(10±6.7 *vs* 21.4±10.7, p=0.05); KIR2DL5B(0 *vs* 21.7±8.5, p=0.035). No significant influence was observed for other KIR genes. Based on clinical studies "receptor-ligand" model was analyzed in patients with AML. There was no correlation between KIR-ligand mismatch and relapse risk and EFS (p=0.97 and p=0.50 respectively).
Summary/Conclusions: There is increasing evidence that impact of donor NK alloreactivity and KIR genotype is dependent on the transplant protocol. Our results suggest that the use of bortezomib instead of prolonged tacrolimus may contribute to the beneficial effects of donor NK cells.

E1532

ETANERCEPT FOR STEROID-REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE: A SINGLE CENTER EXPERIENCE

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Background: Acute graft-*versus*-host disease (aGVHD) is a major cause of morbidity and mortality after allogeneic stem cell transplantation (alloSCT). High dose systemic glucocorticosteroids (steroids) are currently recommended as first-line treatment for grade II-IV aGVHD resulting in overall complete responses (CR) in 40 to 50 percent of patients. Currently, there is no standard second-line treatment for steroid-refractory aGVHD (SR-aGVHD). Etanercept is a recombinant human tumor necrosis receptor fusion protein which inhibits the biological activity of tumor necrosis factor alpha (TNFα) involved in the pathophysiology of aGVHD. Studies that have investigated the use of anti-TNFα as primary as well as secondary treatment in aGVHD have shown promising results.[1,2]
Aims: Here we report the results of a retrospective analysis of patients with SR-aGVHD treated with etanercept in our center.
Methods: We studied the outcome of sixteen patients following alloSCT with SR-aGVHD, who gave consent for second-line treatment with the TNFα inhibitor etanercept (Enbrel, Pfizer) between January 2009 and April 2013. Etanercept was added to the initiated first-line treatment with high dose steroids combined with cyclosporine A (CsA) or mycophenolate mofetil (MMF) and administered subcutaneously twice weekly at a dose of 25 mg for a maximum of eight weeks.
Results: aGVHD developed at a median of 61 days post-transplantation. In all cases it concerned grade 3 aGVHD of the gut. Second-line treatment with etanercept was started at a median of 13 days after the initiation of first-line treatment. First-line treatment consisted of 2 mg/kg steroids combined either with CsA in fourteen patients or MMF in two patients and was continued during treatment with etanercept until a sustained response was achieved. The overall response rate was 50%, including a complete response (CR) in three patients (18.8%) and a partial response (PR) in five patients (31,3%). Responses were reached after a median duration of treatment of 9 days (range 5-40 days). Despite a promising initial response rate, five out of eight responding patients lost their response. One CR patient developed progressive GVHD after treatment with etanercept had to be discontinued because of the development of post-transplant EBV-related lymphoproliferative disorder. In addition, four PR patients lost their response during treatment with etanercept. Clinically significant infectious complications occurred in thirteen patients, including bacterial septicemia, viral infections and invasive pulmonary aspergillosis. Eventually, all sixteen patients died at a median of 57 days (range 1 –267 days) after initiation of etanercept. Death was caused by progressive GVHD in seven cases (43.8%), opportunistic infections in six cases (37.5%), cardiac death in two cases (12.5%) and relapse of the original malignancy in one case (6%).
Summary/Conclusions: Second-line treatment of SR-aGVHD of the gut with the TNFα inhibitor etanercept did not meet the promising results reported by Busca et al.[2] While etanercept showed a promising initial response rate of 50% in our patients, survival was very poor, which was mainly due to opportunistic infections in the context of ongoing or progressive GVHD, even in patients, who initially responded.

References

1. Levine JE, Paczesny S, Mineishi S, Braun T, Choi SW, Hutchinson RJ, et al. Etanercept plus methylprednisolone as initial therapy for acute graft-*versus*-host disease. Blood. 2008;111(4):2470-5.
2. Busca A, Locatelli F, Marmont F, Ceretto C, Falda M. Recombinant human soluble tumor necrosis factor receptor fusion protein as treatment for steroid refractory graft-*versus*-host disease following allogeneic hematopoietic stem cell transplantation. Am J Hematol. 2007;82(1):45-52.

E1533

HEMOCOAGULASE FOR THE TREATMENT OF SEVERE HEMORRHAGIC CYSTITIS FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Hemorrhagic cystitis (HC) is a common and potentially severe complication after allogeneic hematopoietic stem cell transplantation (HSCT). Despite many therapies have been tested in the treatment of severe HC, no definitive treatment has been established. Hemocoagulase is an established hemostatic agent, but its efficacy for HC has not been evaluated.
Aims: The aim of this study is to investigate the clinical efficacy of hemocoagulase for severe HC following HSCT.
Methods: We treated Twenty patients with severe HC (macroscopic hematuria with clots and macroscopic hematuria with renal or bladder dysfunction, with symptoms of cystitis) following HSCT with hemocoagulase. All the patients were initially treated with hyperhydration, forced diuresis and red cell and platelet transfusion support. For the patients with cytomegalovirus (CMV) replication in plasma determined by polymerase chain reaction, ganciclovir or foscarnet sodium was given. When patients developed macroscopic hematuria with clots, hemocoagulase was given, 1U iv twice per day for 5 days as a course. If the macroscopic hematuria disappeared at the sixth day after stopping the drug, hemocoagulase was no longer given. If not, the next course was given. During the treatment, the blood clotting factors was monitored for 2 to 3 times weekly. If the fibrinogen was decreased, the plasma or fibrinogen was transfused to correct the blood coagulation abnormality. The urine specimens reserved before and after hemocoagulase respectively were examined by naked eye and microscope to evaluate the efficacy.
Results: Patients included 12 males and 8 females with a median age of 27 years (range, 13-57years). The median time to onset of severe HC was 28 days after HSCT (range, 14 to 70 days). The HC was cured (disappearance of macroscopic hematuria without relapse) in 18 patients(2 cases were cured after 1 courses, 9 cases were cured after 2 courses, 2 cases were cured after 3 courses, 2 cases were cured after 4 courses, 2 cases were cured after 5 courses, 1 cases were cured after 9 courses), improved (amelioration of macroscopic hematuria) in 1 patients after 3 courses and uncontrolled (persistence of macroscopic hematuria with red cell transfusion requirements) in 1 patients who died of pulmonary infection and pneumorrhagia. For the patients with response, macroscopic hematuria disappeared at a median time of 28 days after the treatments (range, 4-127 days). Among 20 patients, 7 cases had no decrease of fibrinogen (2 cases were cured after 1 courses, 5 cases were cured after 2 courses), the other 13 cases had obvious decrease of fibrinogen, while after the infusion of plasma or fibrinogen, the fibrinogen recoveried. There was no patient required interruption of treatment.
Summary/Conclusions: Hemocoagulase seems to be a safe and effective drug for severe HC following HSCT.

E1534

HCT.CI PREDICTS MORBI-MORTALITY IN AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Hematopoietic Stem Cell Transplant Comorbidity Index (HCT.CI) score, described by Sorror, is a useful tool to assess the risk for Non Relapse Mortality (NRM) after Allogeneic HSCT. The impact of this score in Autologous HSCT is still to be confirmed.
Aims: To determine the impact of HCT.Ci score in the morbidity and mortality after autologous HSCT, assessing the 100 day morbidity defined as orothraqueal intubation (OTI), dialysis or vasopressors need, early mortality (before day 100) and long term NRM.
Methods: We retrospectively reviewed 473 medical records of patients that received an autologous HSCT in our centres between October 2002 and September 2015. Median age was 49 years (range 1-74 years), 58% were male, prevalent diseases were Multiple Myeloma (37%), Non Hodgkin Lymphoma (26%) and Hodgkin Lymphoma (20%), 29% were in complete remission, 42% received one chemotherapy scheme before transplant, 46% two schemes and 12% three or more (heavily pre-treated). Regarding comorbidities, 42% had low risk (LR) HCT.CI (score 0), 47% intermediate risk (IR) (1-2) and 12% high risk (HR) (≥3). For univariate analysis we use Chi2 for dichotomic variables, Kaplan-Meier for Overall Survival (OS) and cumulative incidence for NRM; for multivariate analysis we used logistic regression for dichotomic and Cox regression for time dependant variables.

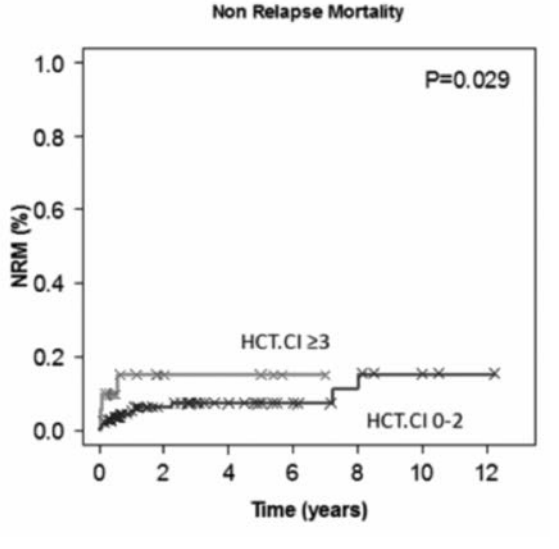

Figure 1.

Results: Median follow up was 1.3 years. Early mortality (day 100) was 4.4%, 9% required OTI, 8.5% vassopresors and 2.2% dialysis, 1-3 years NRM and OS were 7-9% and 85-79% respectively. High risk HCT.Ci patients had a significant

increase in 100 day mortality compared to low and intermediate risk (11% *vs* 4.1% *vs* 2.6% respectively, p=0.02), OTI (18% *vs* 8% *vs* 7%, p=0.02), dialysis (9% vs.2% *vs* 0.5%, p=0.001) and NRM (1-3 years HR *vs* LR/IR 15-15% *vs* 5-7%, p=0.02) (figure 1). After multivariate analysis these outcomes remain significant (comparing HR *vs* LR/IR): early mortality (OR 3.4, 95% CI 1.1-10.4), OTI (OR 2.9, 95% CI 1.3-6.7), dialysis (OR 8.8, 95% CI 2.4-32.6) and NRM (OR 2.6, 95% CI 1.05-6.6). Other than comorbidities, Multiple Myeloma patients had a significant reduction in NRM (OR 0.25, 95% CI 0.1-0.8) and OTI (OR 0.28, 95% CI 0.1-0.83); patients older than 60 years had a significant increase in NRM (OR 4.6, 95% CI 2.1-10.5) and heavily pre-treated patients had a significant increase in NRM (OR 3.3, 95% CI 1.4-7.9) and OTI (OR 2.4, 95% CI 1.1-5.6).
Summary/Conclusions: We observed that HCT.CI had a significant impact on Autologous HSCT treatment related mortality basically due to early toxicity express as 100 day mortality and the three main morbidity outcomes. This observation should be confirmed in larger series.

E1535

AUTOLOGOUS STEM CELL TRANSPLANTATION FOR INTRAVASCULAR LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY OF THE OF THE EUROPEAN SOCIETY FOR BLOOD AND MARROW TRANSPLANTATION LYMPHOMA WORKING PARTY

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Background: Intravascular large B-cell lymphoma (IVLBCL) represents a rare lymphoma subtype characterized by the selective growth of malignant cells within the lumina of small vessels. The combination of rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is widely used as first-line therapy but with about half of the patients being progressive within 2 years, outcome is inferior to non-intravascular diffuse large B-cell lymphoma.
Aims: Since the value and timing of autologous stem cell transplantation (autoSCT) in the treatment of IVLBCL is undefined, the objective of this study was to analyze for the first time the efficacy of autoSCT for IVLBCL in a larger cohort of Western patients.
Methods: This is a registry-based retrospective multicentre study including patients aged 18 years or above with histologically verified IVLBCL who underwent autoSCT between 1 January 2002 and 31 December 2013 and were registered with the European Society for Blood and Marrow Transplantation (EBMT) database. Of 97 patients identified in the EBMT registry, a full data set could be obtained for 19 patients. After exclusion of patients with a histopathology report not confirmative for the diagnosis of IVLBCL, the final study cohort consisted of 11 patients.
Results: The median age of the 11 patients was 55 (range 34-68) years. All had stage IV disease with CNS involvement in 5 patients. First-line treatment was mainly based on R-CHOP or a CHOP-like regimen. The median time from diagnosis to transplant was 6 months. Six patients were autografted in first remission while 5 patients received more than one line of treatment prior to autoSCT. Disease status at autoSCT was complete remission in 8 patients and partial remission in 3 patients. After a median follow-up of surviving patients of 51 months (range 12-95), 8 patients were alive and free of progression. One patient (transplanted in first remission) died treatment-related whereas 2 patients relapsed 15 and 27 months post autoSCT, both with more than one previous treatment line. No relapse occurred in patients autografted in first remission. 4-year progression-free survival of all 11 patients was 71%.
Summary/Conclusions: The outcome of this to date largest patient cohort compares favorable to the outcome after standard R-CHOP and is in line with the outcome of autoSCT in small Asian case series. Furthermore our data suggest that the outcome of autoSCT performed in first remission may be superior to that of autoSCT performed after multiple lines of therapy. Consolidative autoSCT performed in first remission after R-CHOP-like therapy results in favorable long-term outcome for patients with IVLBCL and thus appears to be a valuable treatment option for fit patients up to an age of 65-70 years.

E1536

RESULTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOGENOUS LEUKEMIA PATIENTS WHO FAILED TYROSINE KINASE INHIBITORS

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Background: Chronic Myelogenous Leukemia (CML) is a myeloproliferative neoplasm. The hallmark is the presence of a reciprocal t(9;22)(q34;q11.2)-Philadelphia chromosome (Ph), resulting in which produces a BCR-ABL fusion gene and therefore a BCR-ABL protein with constitutive tyrosine kinase activity. Tyrosine kinase inhibitors (TKI) are the first line of treatment. However, some patients are resistant. Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is indicated for patients with Chronic Myelogenous Leukemia (CML) who develop resistance to tyrosine kinase inhibitors (TKI).
Aims: The main aim is analyzing outcomes of HSCT in 49 patients with CML resistant to TKI and identifying risk factors for overall survival (OS) and disease free survival (DFS) in those patients.
Methods: We analyzed the outcomes of 49 patients resistant to TKIs transplanted at the BMT Center of Federal University of Paraná, from January 2001 to August 2015. The study is retrospective, observational and analytical, held from data record in either STMO database or medical chart. Informed consent was waived. Kaplan-Meyer method was used to build survival curves and Log-rank test to compare them. The established level of significance was $p<0.05$. The following risk factors were analyzed: donor and recipient sex, age at HSCT, pre-HSCT imatinib, second generation inhibitors or interferon, conditioning regimen, immunoprophylaxis, donor type, stem cell source, HLA compatibility, engraftment, acute graft *versus* host disease (aGVHD), chronic graft *versus* host disease (cGVHD), disease phase and previous response to TKI (hematologic and cytogenetic). Multivariate analysis was performed using Cox Regression model to identify risk factors for overall survival and disease-free survival.
Results: Chronic graft *versus* host disease (GVHD) (n=16) was associated to a higher OS (p=0,04) whereas acute GVHD (n=13) was associated to a lower OS. Regarding to DFS, a myeloablative (MA) conditioning regimen (n=45) lead to higher survival probability (p=0.00182). BCR-ABL mutation analysis was performed before HSCT in 55% of the patients (n=27) and found in 33% (n=9). The presence of mutations was a significant risk factor for OS (p=0.00366). The median OS was 373 days (33-3677) and the median DFS was 273 days (0-3677). Five years probability of survival was 41% for entire group.
Summary/Conclusions: HSCT is an important alternative for patients with TKI resistance. Chronic GVHD was associated with a 3,6 lower probability of death, while occurence of acute GVHD had a negative impact, increasing probability of death in 3 times. Worse outcome was showed for patients with pre-transplant mutations. Myeloablative conditioning was significantly associated with better DFS.

E1537

EQUIVALENT OUTCOME OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA PATIENTS WHO HAD T (8;21) WITH AND WITHOUT ADDITIONAL ABERATIONS

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Background: Although acute myeloid leukemia (AML) patients who had t(8;21) had favorable outcome after high-dose therapy with autologous stem cell transplantation(ASCT). It was still a heterogeneous disease. Limited data was reported about outcome in AML patients who had t(8;21) with additional cytogenetic abnormalities after ASCT.
Aims: In the current cohort study we investigated long-term survival of AML patients who had t(8;21) with and without additional aberations after ASCT.
Methods: Patients with de novel AML who had t(8;21) with or without additional cytogenetic abnormalities, younger than 60 years were eligible. G banding and FISH analysis were performed to test cytogenetic aberations. Peripheral blood stem cell (PBSC) harvesting was started if patients achieved CR1 and MRD level was less than 0.1% after 1 to 2 courses of induction therapy(3+7) and at least 2-coursrs of consolidation therapy including high-dose Ara-C. MRD was monitored using real-time PCR (RT-PCR) to quantify the RUNX1/RUNX1T, The conditioning regimen was busulphan/cytoxan.
Results: between March 2008 and March 2014, 27 eligible patients were enrolled. All enrolled patients were Chinese. Median age was 30 years (range, 13- 56 years). Fourteen patients were male and 13 were female. Eleven patients had additional aberrations, including –y (n=6), -x (n=2), del (8q) (n=1), del(9q) (n=1), del(9q) and –x (n=1). Patients' characteristics between the two groups were comparable. At the median follow-up 46 months (range,6.5 to 92 months) after consolidation therapy, the 5-year leukemia-free survival (LFS) and overall survival (OS) for the total patients were 88.9%±6.0% and 90.3% ±6.8% after consolidation therapy. For the patients with and without additional aberations, LFS were 87.5% and. 90.9%, p=0.832. OS were 90.8% and 90.9%, p=0.752, respectively. Two relapse patients after ASCT received haploidentical related transplantation and were still alive in CR after following-up 74 and 64 months.
Summary/Conclusions: AML patients who had t (8;21) with and without additional aberations had equivalent long-term survival after ASCT in Chinese, if patient achieved CR1 with MRD level less than 0.1% before transplant. Allogeneic HSCT including haploidentical related transplant is a salvage therapy for relapse patients after ASCT.

E1538

ANTIBACTERIAL PROPHYLAXIS REDUCES THE RISK OF BACTEREMIA IN PATIENTS WITH MULTIPLE MYELOMA AND LYMPHOMA UNDERGOING HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Bacterial infections are a major cause of morbidity and mortality in patients undergoing autologous stem cell transplantation (ASCT). However, whether these patients benefit from antibacterial prophylaxis is unclear. The few studies which addressed this issue are limited by small sample size or the use of multiple anti-microbial agents. Current guidelines recommend considering the use of antibacterial prophylaxis in patients with anticipated neutropenia of 7 days or more. At our center, patients undergoing ASCT were given ciprofloxacin for antibacterial prophylaxis until 2012. This change in policy provides a unique opportunity to study the potential benefit of antibacterial prophylaxis in ASCT.
Aims: To explore the efficacy and adverse effects of ciprofloxacin prophylaxis in patients undergoing ASCT.
Methods: This is a single center retrospective study. Data were collected from the medical records of all patients with a diagnosis of non-Hodgkin's lymphoma, Hodgkin's disease or multiple myeloma (MM) who underwent ASCT at Rabin Medical Center between 03/2007 and 12/2015. Until 10/2012 all patients were given oral ciprofloxacin 500 mg BID starting concomitantly with the conditioning regimen (BEAM for lymphoma and melphalan for multiple myeloma) until the first febrile episode or until engraftment. After 10/2012, our treatment policy has changed and we stopped using antibacterial prophylaxis in the ASCT setting.
Results: Between 03/2007 and 12/2015 303 patients received 327 high dose chemotherapy followed by ASCT. The median age at time of transplant was 55 years (range: 16 to 72) and 186 patients (57%) were males. The diagnosis was MM in 174 patients (46%), NHL in 110 patients (34%) and HD in 43 patients (13%). 154 consecutive patients were given ciprofloxacin prophylaxis and 171 patients were not given ciprofloxacin prophylaxis before and after 10/2012, respectively. Overall, 10% (n=32) of the patients had a bacteremia, 16% (n=27) in the group of patients who did not receive prophylaxis, compared to only 3% (n=5) in the group of patients who received prophylaxis (*P*<.0001). The rates of febrile neutropenia were 84% (N=129) in patients who received prophylaxis compared to 91% (155) in patients who did not receive antibacterial prophylaxis (P=0.06). The number needed to treat (NNT) in order to prevent one episode of bacteremia is 4.9. The frequency of all other infections, the duration of hospitalization, and the rates of all cause and infection-related mortality were not significantly different between groups. Of note, ciprofloxacin prophylaxis was not associated with an increased in bacterial resistance. Patients with lymphoma were younger (median 53 years, range: 19-72) than patients with MM (median 58, range: 33-68) but similar in all other demographic characteristics. The median duration of aplasia was 6 days (range: 4 to 13) in patients with MM after high-dose melphalan and 8 days (range 5 to 14) in patients with lymphoma after BEAM (p<0.0001). However, both groups seemed to equally benefit from antibacterial prophylaxis. In a multivariable analysis ciprofolxacin prophylaxis significantly decreased the odds of bacteremia (Odds Ratio: 0.19 (Confidence interval: 0.07 to 0.52)). Age, diagnosis, and the duration of aplasia were not associated with the risk of bacteremia
Summary/Conclusions: Ciprofloxacin prophylaxis markedly reduced the risk of bacteremia and neutropenic fever in lymphoma and MM patients undergoing ASCT without increasing the incidence of resistant bacterial strains in patients receiving ciprofloxacin prophylaxis. The NNT in order to prevent one episode of bacteremia is 4.9, suggesting that antibacterial prophylaxis should be considered in ASCT.

E1539

EFFECTIVE USE OF ORAL RIBAVIRIN FOR RESPIRATORY SYNCYTIAL VIRAL INFECTIONS IN ADULT ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

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Background: Respiratory syncytial virus (RSV) is a common cause of respiratory viral infections and is associated with increased morbidity and mortality in patients undergoing haematopoietic stem cell transplantation (HSCT). Little is known about the best management strategy in this high risk group and there is very little data on oral ribavirin treatment.
Aims: The outcome of adult allogeneic HSCT recipients with RSV infection treated with oral ribavirin was analysed at a single UK centre.
Methods: We performed a retrospective analysis of 23 consecutive RSV cases treated with oral Ribavirin between December 2010 and February 2015. Patient characteristics: male 12, median age 52 years (range 20 to 69). Underlying diagnoses: acute leukaemia 11, myelodysplasia 4, aplastic anaemia 3, multiple myeloma 3, chronic leukaemia 2. Allogeneic HSCT characteristics: 16 reduced intensity conditioning (RIC) and 7 myeloablative conditioning. RSV diagnosis was established by polymerase chain reaction (PCR) assay. At diagnosis, 7 patients presented with lower respiratory tract infection (LRTI) as defined by new infiltrate on chest X-ray (CXR), signs on auscultation or hypoxia, whereas 16 experienced upper RTI. All patients with a positive RSV PCR result received treatment. We initiated oral Ribavirin at a dose of 15mg/kg divided into 3 daily doses with no subsequent dose escalation. The median treatment duration was 10 days (range 5 to 47). Additional therapies were administered concomitantly: 3 patients received immunoglobulin replacement, 17 received antimicrobials and 3 patients also received antifungal treatment for suspected superimposed bacterial or fungal infection. Patients were retrospectively scored using the immunodeficiency scoring index for RSV (ISI-RSV) which takes into consideration neutropenia (<0.5x10^9/L), lymphopenia (<0.2x10^9/L), age (≥40 years), myeloablative conditioning regimen, graft *versus* host disease (GVHD acute/chronic), corticosteroid treatment and pre-engraftment period (or within 30 days of HSCT) to determine the risk of progression to LRTI. In our cohort, 14 patients were low risk with an ISI of 0-2 (2 patients ISI 0, 6 patients ISI 1, 6 patients ISI 2) and 9 patients were moderate risk with an ISI of 3-4 (8 patients ISI 3, 1 patient ISI 4) and therefore oral treatment was felt to have been appropriate, on retrospective analysis.
Results: The treatment was well tolerated with minor side effects in 2 patients. In total, 19 patients completed exclusively oral ribavirin treatment, whereas 4 were escalated to aerosolised ribavirin due to worsening symptoms. Only 7 patients required admission for intravenous antibiotics and/or aerosolised ribavirin. The median admission period was 7 days (range 1 to 16). None of the patients required intensive care admission. After a median follow-up of 17 months (range 4 to 48), 17 patients are alive and 6 died. One patient died of RSV pneumonitis complicated with bacterial and suspected fungal infection on the background of relapsed disease and 5 died of unrelated causes.
Summary/Conclusions: RSV in post allogeneic HSCT patients remains a challenge due to the high frequency of infection and increased morbidity and mortality associated with RSV LRTI. Prompt initiation of treatment is essential and may avoid unnecessary hospital admission. Our experience supports the use of oral ribavirin in selected adult HSCT recipients, after a careful risk assessment has been performed. Prospective studies and larger numbers of patients are needed to determine the optimal therapy for this patient group.

E1540

EFFICACY AND SAFETY OF BIOSIMILAR FILGRASTIM (ZARZIO®) AFTER AUTOLOGOUS STEM CELL TRANSPLANT: UPDATE OF A COMPARATIVE STUDY WITH LENOGRASTIM AND PEG-FILGRASTIM

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Background: Biosimilar Granulocyte Colony-Stimulating Factor (G-CSF) has been approved on the basis of comparable quality, safety and efficacy as the originator product. So far, biosimilar Filgrastim Zarzio® has been approved also for autologous peripheral stem cell mobilization and for prophylaxis of sever neutropenia duration following conditioning chemotherapy and stem cell infusion. However, there is still general skepticism about safety and efficacy of Zarzio® in these setting of patients, considering the lack of prospective studies with a long follow-up.
Aims: The aim of the study was to evaluate the efficacy and the safety of Zarzio® when used as hematologic recovery after autologous stem cell transplant (ASCT).
Methods: From March 2013 to November 2015, 114 consecutive adult patients with hematologic malignancies (Plasma cell disorders n=68; Non Hodgkin and Hodgkin's lymphomas n=42; others n=4) underwent ASCT in our Institution. Zarzio® was given at the dosage of 5 mcg/Kg beginning to day 3 from infusion of stem cells and continued until neutrophilis recovery. Hematologic recovery after ASCT was defined as an absolute neutrophilis count upper than 0.5x10^9/L and a platelets count upper than 20 x 10^9/L in three consecutive checks. This cohort of patients was compared with two historical cohorts of patients in our Institution: a) 99 consecutive adult patients treated with Lenograstim (Myelostim®) at dosage of 5 mcg/Kg daily given from day 3 after infusion from January 2009 to February 2013; b) 60 consecutive adult patients treated with peg-filgrastim (Neulasta®) at dosage of 6 mg single dose at day 3 after infusion from March 2006 to December 2008.
Results: The three patient cohorts were similar for all baseline features analyzed, without any significant differences in terms of sex, median age, diagnosis, median chemotherapy lines prior ASCT, disease status at ASCT, conditioning regimen and median infused CD34+ cells. We analyzed the time of hematologic recovery after stem cell infusion, the occurrence of fever of unknown origin (FUO) in neutropenia, documented infectious episodes and needing of intra-

venous antibiotics, number of red blood and platelet transfusions, the days of hospitalization and the transplant-related mortality (TRM). The results of the study show a significantly shorter time to neutrophilis and platelet recovery (P=0.001 and P=0.007, respectively) in the cohort of patients treated with Neulasta®, whereas no difference was observed among the other two groups. As for the other analyzed parameters, we didn't observe any significant difference among the three patient cohorts for all the other analyzed parameters. In particular, we did observe a similar incidence of FUO episodes (P=0.102), microbiologically documented infections (P=0.424) and needing of intravenous antibiotics (P=0.612). Moreover, we didn't find any significant differences as for transfusional needing (P=0.099), median hospitalization time (0.102) and TRM (P=0.709). No difference in terms of drug-related adverse events was observed in the three patient cohorts with no reported serious adverse events. Similar results were obtained performing two separate sub-analysis only for lymphoma or myeloma patients.

Summary/Conclusions: Despite the limitations due to the non-randomized nature of the study, from our data biosimilar Filgrastim (Zarzio®) seems to be substantially equivalent in terms of efficacy and safety to Lenograstim (Myelostim®) and Peg-Filgrastim (Neulasta®), when used for hematological recovery after ASCT in adult patients with hematologic malignancies.

E1541

EXTRACORPOREAL PHOTOPHERESIS FOR THE TREATEMENT OF ACUTE AND CHRONIC GRAFT *VERSUS* HOST DISEASE: A RETROSPECTIVE MULTICENTRIC ANALYSIS ON 94 PATIENTS

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Background: Extracorporeal photopheresis (ECP) is considered a valid second-line treatment of both acute and chronic GVHD, which represent the most frequent causes of morbidity and mortality after allo-SCT in hematological malignancies.

Aims: According to an observational multicentric study, we report here a retrospective analysis on the use and results of ECP in 94 patients with acute and chronic GVHD, recruited in 6 italian Centres.

Methods: 94 patients with aGVHD grado ≥ 2 (n=45) and cGVHD (n=49) were submitted to ECP after first-line steroid-based therapy. ECP treatment consisted on conventional bi-weekly procedure for 4 weeks. Then, most of the patients were treated with bi-weekly procedures every 2 weeks for 4 other weeks. Response to steroid was assessed after 7 days for aGVHD and 15 days for cGVHD.

Results: aGHVD: 45 patients with aGVHD were treated with ECP. In 22 (49%) ECP was used as salvage treatment, being these patients non responsive to steroid (NR). 19/22 (86%) achieved a CR after ECP, whose median duration was 77 days (range 20-1112). The median duration of steroid therapy was 101 days (range 24-772), with a median of 22 days (range 7-80) on full steroid dose. The median duration of steroid on tapering was 82 days (range 0-756). 8/22 (36%) patients developed a cGVHD (extensive in 6 cases and limited in 2 cases). After a median follow up of 419 days (range 61-2149), 11/22 (50%) patients are alive. In 23/45 cases (51%) ECP was used earlier, being these patients partially responsive to steroid. Among these patients, 22 (96%) achieved the CR after ECP. In this setting of patients, the median duration of steroid was 88 days (range 32-719), with a median of 18 days (range 4-69) on full dose steroid therapy. The median duration of steroid on tapering was 68 days (range 0-700). The median duration of ECP treatment was 194 days (range 22-933). 7/23 (30%) patients developed a cGHVD (extensive in 6 cases and limited in 1 case). With a median follow up of 741 days (range 81-1819), 17/22 (77%) patients are alive. Among the 45 patients with aGVHD, the projected 2 years OS was 50% for the patients with steroid-refractory aGVHD *versus* 80% for the patients with steroid partially responsive aGVHD (p=0,07), and the cumulative incidence of cGVHD at 1 year was 48% *vs* 23%, respectively (p=0,32). cGVHD: 49 patients with cGVHD were treated wiht ECP. 12/49 (24%) and 37/49 (76%) had limited and extensive cGVHD, respectively. The median time of cGVHD onset was 193 days (range 43-3756), the median time from steroid to ECP was 26 days (range 0-1347) and the median duration of ECP was 276 days (range 19-2861). The median duration of steroid therapy in this group was 276 days (range 19-2861). 22/49 patients (45%) and 17/49 patients (35%) achieved a CR and PR, respectively. After a median follow up of 811 days (range 152-4676), 44/49 patients (90%) are alive, 28 of whom (64%) on immunosuppressive therapy.

Summary/Conclusions: ECP confirmed to be an effective treatment in aGVHD steroid-resistant patients as it can induce a CR in more than 80% of the cases, with 50% of these patients becoming long-term survivors. In aGVHD steroid partially-responsive patients it can probably allow a faster steroid tapering, with more than 70% of patients becoming long term survivors. In cGVHD steroid-resistant patients, ECP are associated with an overall response rate of 80%, with 90% of patients who become long-term survivors.

E1542

IMPACT OF PLERIXAFOR ON HOSPITAL EFFICIENCY: A SINGLE CENTER EXPERIENCE

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Background: Plerixafor in combination with granulocyte colony-stimulating factor (G-CSF) has shown to increase mobilization of peripheral blood stem cells (PBSC) as compared to G-CSF alone in patients undergoing autologous stem cell transplantation (ASCT). However, up to 25% of patients treated with G-CSF alone still fail mobilization. Adding plerixafor to the regimen of poor mobilizers allows to rescue these patients from mobilization failure and to reduce the number of apheresis sessions. Furthermore, it was hypothesized that the use of plerixafor would lead to a better prediction of the number of apheresis time-slots needed and a reduction of time-slots lost. This in turn would free hospital resources which could be dedicated to other procedures.

Aims: The goal of this retrospective study was to capture the impact of plerixafor on mobilization outcome and on the efficiency of the apheresis department. Efficiency was described in terms of time-slots lost and the number of extracorporeal photopheresis (ECP) sessions carried out. ECP, whose benefit is generally recognized in patients with cutaneous T-cell lymphoma and graft *versus* host disease, is a procedure which carries some similarities with standard apheresis techniques and may therefore be carried out by the same staff provided some additional training. The demand for this procedure has steadily increased over the last years, but could not be matched by available health care resources.

Methods: Hospital records of patients treated before (2005-2008) and after (2009-2014) introduction of plerixafor were collected and analyzed retrospectively. Main outcomes were mobilization failure rate and number of time-slots lost due to insufficient mobilization, ie low CD34 count. Secondary endpoints included the number of apheresis sessions per patient, mean CD34+ cells collected, the number of ASCT and ECP sessions carried out, and costs per ASCT patient.

Results: Following plerixafor introduction, the mobilization failure rate dropped from 12% to 4% and the mean number of time-slots lost per patient dropped from 1.39 to 0.89. Additional drug costs due to plerixafor were partially balanced by a reduction in apheresis sessions, resulting in an additional cost of 700 € per ASCT candidate. More importantly, with the use of plerixafor, the availability of time-slots turned from erratic to predictable such that freed capacity could be dedicated to other apheresis procedures, and the number of ECP sessions increased from 0 in 2005 to 685 sessions in 2014 (see Figure).

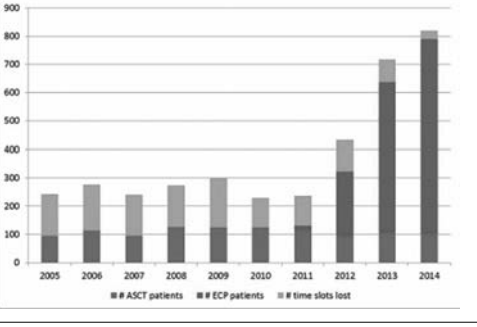

Figure 1.

Summary/Conclusions: Plerixafor not only improved mobilization outcomes for patients undergoing ASCT, but also reduced uncertainty around scheduling of apheresis sessions, leading to a decrease in time-slots lost. Freed resources were used to manage patients requiring ECP therapy, showing that the introduction of plerixafor had much wider implications beyond that of improvement in PBSC mobilization. Even though this result is not generalizable, in a climate of stretched resources it is important to consider the opportunity and redistribution investment possible with freed capacities.

E1543

A TREOSULFAN-BASED TOXICITY-REDUCED CONDITIONING REGIMEN IS SAFE AND FEASIBLE FOR CHILDREN WITH NON-MALIGNANT DISEASES BUT INCREASES REJECTION RATES IN STEM CELL TRANSPLANTATION OF HEMOGLOBINOPATHIES

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Background: Hematopoietic stem cell transplantation (HSCT) as a treatment of non-malignant diseases has improved over the last decades. Less toxic chemotherapy during conditioning aims at reducing transplant related mortality with the risk of an increased rejection rate. Non-malignant disorders are chemo-naïve and carry an increased risk of transplant rejection compared to malignant diseases.
Aims: Aim of the present study was to evaluate safety and feasibility of a treosulfan-based toxicity-reduced conditioning regimen in pediatric patients undergoing HSCT.
Methods: This retrospective study analyzed the safety and feasibility of a treosulfan-based conditioning between 2009 and 2013 in 31 pediatric HSCT recipients (17 males, 14 females) with a median age of 7.8 years (range 0.1-18.2 years), undergoing allogeneic HSCT for non-malignant diseases, thalassemia (n=11), leukodystrophies (n=6), severe combined immunodeficiency (n=4), sickle cell disease (n=3), familial hemophagocytic lymphohystiocytosis (n=3) and others (n=4). The chemotherapy during the conditioning regimen consisted of fludarabine 150 mg/m^2 in 5 single doses (5 x 30 mg/m^2) between day -7 and day -3. Treosulfan was given in a dosage of 42 g/m^2 in 3 single doses (3 x 14 g/m^2) between day -6 and day -4. On day -2, thiotepa was applied in a dosage of 10 mg/kg bodyweight per day, twice a day (2 x 5 mg/kg BW). Between day -6 and day -3, patients received treatment with 4.5 mg /kg BW anti-thymocyte globulin (ATG) in 4 dosages (1 x 0.5 mg/kg BW as test dosage; 1 x 1.0 mg/kg BW; 2 x 1.5 mg/kg BW). Patients received transplants from a matched sibling donor (MSD, n=15), a matched unrelated donor (MUD, n=12), a mismatched unrelated donor (MMUD, n=1), a matched family donor (MFD, n=2), or a mismatched family donor (MMFD, n=1). The median observation period was 2.0 years (range 34 days - 5.6 years) and included the time of measurement directly before the start of conditioning until December 2014.
Results: Primary engraftment occurred in 97% of the patients, with a median time of 15 days after transplantation to reach >1000 leukocytes per µl peripheral blood and a median time of 21 days to reach platelet counts, which did not require transfusions anymore. After re-transplantation, final engraftment occurred in 67% of the patients. Graft-*versus*-Host Disease (GvHD) grade II and III-IV occurred in 6% and 9% of the patients, and chronic GvHD occurred in 0% of the patients. Rate of other grade IV side effects was low, except for grade IV stomatitis that occurred in 42% of the cases. Transplant related mortality (TRM) was observed in 7% of the patients after one year. HSCT recipients with hemoglobinopathies were accompanied by a rejection rate of 31% compared to 0% rejection rate among all other patients. Mixed chimerism occurred in 71% of the cases. Mixed chimerism >50% occurred in 42% of the cases and mixed chimerism >80% occurred in 23% of the cases.
Summary/Conclusions: In conclusion, a treosulfan-based conditioning regimen resulted in excellent engraftment rates with low toxicity in children with non-malignant diseases. However, in children with hemoglobinopathies, the rejection rate was not acceptable occurring in around one third of the cases. Future prospective controlled studies will have to clarify the long-term outcome.

E1544

POST-TRANSPLANT CYCLOPHOSPHAMIDE AS GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN PEDIATRIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES TRANSPLANTED FROM HAPLOIDENTICAL AND MATCHED UNRELATED DONORS

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Background: Standard GvHD prophylaxis regimens impair the graft-*versus*-tumor (GVT) effect, delay immune reconstitution and are associated with high rate of infections. High-dose post-transplantation cyclophosphamide (PTCy) targets alloreactive donor T cells proliferating early after BMT, promotes regulatory T cell and permits rapid immune reconstitution.
Aims: In this pilot trial we evaluate the safety and effects of PTCy in unmanipulated haploidentical and matched unrelated transplantation (MUD) in pediatric patients with hematologic malignancies.
Methods: Twenty eight pediatric patients with high risk hematologic malignancies underwent unmanipulated allogeneic bone marrow (BM) (n=26) or peripheral blood stem cell (PBSC)(n=2) transplantation followed by PTCy between April 2014 and September 2015 with a median follow-up of 9 months (1,5-19). Nineteen patients were transplanted from haploidentical donors and 9 from MUD. The median age was 10,4 years (range 1,9-18); 14 patients with ALL, 4 AML, 8 JMML and 2 with HD; 16 patients were in remission (CR) (14-ALL, 1-AML, 1-HD), 12 had active disease (AD) (8-JMML, 3-AML, 1-HD). In 11 pts this was a second allograft. Twenty-six pts received myeloablative Treosulfan/Melphalan/Fludarabine and 2 pts - reduced-intensity Fludarabine/TAO 2Gy/Thiotepa as conditioning regimen. GvHD prophylaxis consisted of PTCy on day +3, +4 and tacrolimus from day +5 in all pts., 12 (43%) pts. also received ATG (rabbit, thymoglobuline) at 5 mg/kg/course.
Results: Primary engraftment was achieved in 27(96,4%) of 28 pts., the median time to neutrophil and platelet recovery was 20 days (14-45) and 21 days (10-97). Twenty seven (96,4%) patients were in full donor chimerism on day +30. Early mortality was 14,9% (95% CI: 6-36), causes of death included viral infections (n=3); GvHD and viral infection (n=1). Cumulative incidence (CI) of acute GvHD grade ≥II was 50% (95%CI:34-74), grade III–IV - 34% (95%CI:19-62) and chronic GvHD-19% (95%CI:8-45). No correlation between the use of ATG and acute/chronic GvHD was noted. CI of relapse was 15,4% (95% CI: 6-38) and was significantly lower in CR *vs* AD group: 0% *vs* 36% (95% CI: 17-77), p=0,015. One year event-free survival (EFS) was 69,7% (95%CI: 52-87) and overall survival (OS) - 76% (95%CI: 59-93). One year pOS was significantly lower in the ATG group 58% (95%CI: 30-86) *vs* non-ATG group 93% (95%CI: 79-100), p=0,039. One year pEFS was 87% for patients in remission and 48% for patients with active disease (p=0,028). There was no significant difference in survival and relapse rate according to donor type.
Summary/Conclusions: Our study demonstrates the high rate of engraftment with acceptable transplant-related mortality in pediatric patients with hematological malignancies after unmanipulated HSCT with posttransplant cyclophosphamide. The data need to be confirmed in a larger trial with long-term follow-up.

E1545

ALLOGENEIC HEMATOPOIETIC TRANSPLANTATION FOR NON-HODGKIN'S LYMPHOMAS A RETROSPECTIVE ANALYSIS OF 77 CASES

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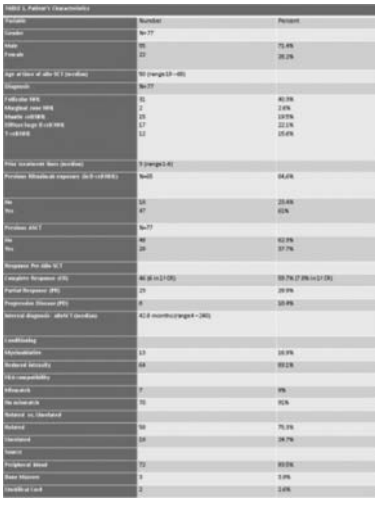
Background: Non-Hodgkin's lymphomas (NHL) are a heterogeneous group of malignancies characterized by uncontrolled proliferation of clonal lymphocytes, which have varied aggressiveness. Allogeneic stem-cell transplantation (allo-SCT) is a therapeutic option for advanced and otherwise incurable NHL. The major drawbacks associated with this therapy are graft-*versus*-host disease (GVHD) and infections. On the positive side, it is becoming clear that in the allo-SCT setting there is a graft-*versus*-lymphoma effect. Selection of the appropriate patients and donors, as well as the transplantation timing still is a matter of debate.
Aims: To investigate the factors that influenced the outcome after allo-SCT for NHL over a 20 years period in a Spanish University hospital
Methods: Retrospective analysis of patients with NHL who received an allo-SCT between January 1995 and December 2014. Statistical analyses were performed using the IBM SPSS Statistics version 21. Survival curves were estimated by the Kaplan-Meier method and compared using the log-rank test. Variables were selected based on previous studies: age (≤60 *vs* >60 years), gender (male *vs* female), previous ASCT, disease status pre allo-SCT, number of chemotherapy lines before allo-SCT, intensity of conditioning regimen (ablative *vs* reduced intensity), HLA compatibility (no mismatch *vs* mismatch), related donor *vs* unrelated donor. Cox regression method was used to develop a prognostic model of OS.

Table 1.



Results: The characteristics of the series are summarized in Table 1. Indolent NHL (iNHL) accounted for 43% of patients (n=33) and aggressive NHL 57% (n= 44). Within aggressive NHL, 16% were of T-cell origin.The median time follow-up after alloSCT was 23 months (range 0-232). The OS at median time was 53.5% and the PFS was 53.8% (only 9 progressed). The main cause of death (n= 48) were GVHD 37.5% (n=18), infections 22.9% (n=11), secondary malignancies 8.3% (n=4) and veno-oclussive disease 4.2% (n=2). Age and response pre allo-SCT had statistically significant impact on OS in multivariate analysis (p<0.01) The OS for patients ≤60 years old vs.>60 years old were 58.% and 24%, respectively.The OS for patients in CR pre allo-SCT was 60% and for patients in PR was 55%. All patients with progressive disease were death at median time of follow-up.
Summary/Conclusions: Allo-SCT is a feasible option for high risk of relapse lymphoma patients. Old patients and those with non-responsive disease before allo-SCT should be considered for alternative therapies. The results are remarkable in younger patients with sensitive NHL.

E1546

BEING OVERWEIGHT/OBESE PRIOR TO ALLOJENEIK HEMATOPOIETIC STEM CELL TRANSPLANTATION (ALLO-HSCT) IS RELATED TO BETTER OVERALL SURVIVAL (OS) AND DECREASED NON-RELASE MORTALITY

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Background: Obesity is an increasingly important health problem in developed countries and a cause of variability in treatment outcomes. The effect of being owerweight or obese had been studied in various populations in patients undergoing Allo-HSCT. Sorror et. al reported obesity as a factor in hemetopoietic cell transplant-specific comorbidity index and increased risk of non-relapse mortality (NRM).
Aims: In this study, we evaluated the impact of obesity on acute graft *vs* host disease (aGVHD) risk, overall survival (OS), relapse rate (RR), relapse free survival (RFS) and NRM in patients undergoing Allo-HSCT.
Methods: Retrospectively, 245 patients who underwent allo-HSCT between 2000 and 2015 at Ankara University School of Medicine was included in our study. Patients were classified into four groups based on pretransplant BMI values according to World Health Organization as follows: underweight (BMI<18.5 kg/m^2, n=17), normal (18.5 ≤BMI<25 mg/kg/m^2, n=116), overweight (25 ≤BMI<30 mg/kg/m^2, n=78) and obese (BMI ≥30 mg/kg/m^2, n=34). Patients received weight adjusted chemotherapy doses. Characteristics of patients were compared using the Chi-squared test for categorical variables. The probilibity of OS was calculated by the Kaplan-Meier method.

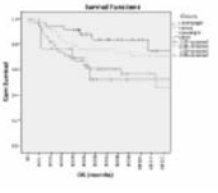

Figure 1.

Table 1.

Table 1. Patient Characteristics

	Underweight (n=17)	Normal (n=116)	Overweight (n=78)	Obese (n=34)	P
Mean Age, years	36.0	37.9	33.5	38.9	0.362
Sex (F/M)	8/7	47/69	37/41	18/16	0.428
Stem Cell Source					
BM	5	7	14	9	0.033
PBSC	10	104	61	25	
BM+PBSC		5	3		
Donor					
Related	5	56	28	5	0.093
Unrelated	10	60	50	29	
HLA Status					
Fullmatch	11	81	47	26	0.396
Mismatch	3	22	22	4	
missing	4	13	9	4	
Diagnosis					
Acute Leukemia	8	72	50	18	
Bone Marrow Failure	4	16	13	3	0.0081
CML					
Lymphoma		7	5	7	
Others	1	10	8	4	
	4	11	4	3	
Conditioning					
Myeloablative	9	89	66	29	0.803
Reduced-intensity	8	27	12	5	

Results: The patient characteristics are given in the table. Mean age of the group was 36.8 (range, 16-71). Overweight and obese patients received more bone marrow as a stem cell source (P=0.033). Bone marrow failure syndromes were more common diagnosis in normal and underweight recipients (p=0.0001). Myeloablative conditioning were prefered in overweight and obese group (p=0.002). Five year OS in underweight, normal, overweight and obese groups were as follows: 51%, 60%, 74% and 83% (P=0.005). Relaps rate was higher in underweight and normal compared to overweight and obese but not statistically different (23% *vs* 19%, P=0.53). 2-year RFS in underweight, normal, overweight and obese groups were as follows: 33%, 29%, 41%, 50%, P=0.069). The incidence of aGVHD detected higher in obese and overweight group however not statistically significant (44% *vs* 40%, p=0.6). NRM was lower in overweight and obese group (12% *vs* 31%, P=0.001).
Summary/Conclusions: Interestingly, we have demostrated that being overweight/obese improved OS and decreased NRM in our cohort. Relapse rate was higher in underweight and normal weight group however not statistically significant.

E1547

EXPRESSION OF CD143 ON CD34-POSITIVE CELLS IN PERIPHERAL BLOOD AND APHERESIS PRODUCTS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AT THE MOBILIZATION OF PERIPHERAL BLOOD PROGENITOR CELLS (PBPCS)

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Background: Angiotensin converting enzyme (CD143) is a part of the renin-angiotensin system catalyzing the cleavage of angiotensin I to angiotensin II. CD143 is expressed in blood-forming tissues of the human embryo: para-aortic splanchnopleura, yolk sac, aorta-gonad-mesonephros, liver and bone marrow. Transplantation into NOD/SCID mice and cultivation have shown that CD34+/CD143+ cell progenitors, but not CD34+/CD143-, sustain multilineage hematopoietic cell engraftment and have long-term culture-initiating cell potential.
Aims: To estimate of CD143 expression on CD34+ cells in peripheral blood and apheresis products in patients with hematological malignancies before and after the mobilization of PBPCs.
Methods: The study included 31 patients: 16 women and 15 men aged 22-68 years old (median 52). 29 patients were diagnosed with multiple myeloma and 2 - Hodgkin's lymphoma. Analysis of CD34+ cells was performed by flow cytometry (FACSCantoII, Becton Dickinson) using monoclonal antibodies: CD34 PE, CD45 FITC, and CD143 APC (Becton Dickinson). The percentage of CD34+ among CD45+ cells and the percentage of CD34+/CD143+ in CD34+ population were calculated in peripheral blood of patients before mobilization and in peripheral blood and apheresis products on the day of leukapheresis. The mobilization was performed with cytotoxic chemotherapy (cyclophosphamide - 4 g/m^2 - 24 patients, patient - 2 DHAP)+5 mg/kg G-CSF (CC+G-CSF) in 26 cases and with G-CSF (10 mg/kg) only - in 5 cases. The control group included peripheral blood samples from 10 healthy donors without mobilization.
Results: The percentage of CD34+ cells in peripheral blood before mobilization from all patients was 0.03±0.01% compared to healthy control group with 0.024±0.004% (p=0.62). The percentage of CD34+/CD143+ was 12.33±1.55% (3.42±1.43% in healthy donors, p=0.004). After the mobilization percentage of CD34+ cells in peripheral blood was 0.08±0.05% when mobilization was performed with G-CSF, and in case of using CC+G-CSF the percentage of CD34+ was 0.83±0.18% (p=0.09). The percentage of CD34+/CD143+ cells with G-CSF mobilization was 28.32±4.06%, compared to 46.07±2.96% (p=0.01) in patients with CC+G-CSF mobilization. The apheresis products contained 0.36±0.11% of CD34+ cells after mobilization with G-CSF, and 1.55±0.25% of CD34+ cells after mobilization with CC+G-CSF (p=0.048). The percentage of CD34+/CD143+ cells in apheresis products was 29.16±5.99% when mobilization was carried out with G-CSF and 47.96±2.77% (p=0.01) in patients with CC+G-CSF mobilization.
Summary/Conclusions: Thus, increase of CD34+/CD143+ cell number in peripheral blood and apheresis products after mobilization has been shown. Increase of CD34+/CD143+ cells in patients after mobilization of PBPCs with chemotherapy and G-CSF was significantly greater in contrast to the mobilization with G-CSF alone. Patients with hematological malignancies before mobilization had higher percentage of CD34+/CD143+ cells in peripheral blood then healthy donors that may be due to previous chemotherapy.

E1548

A COMPARISON OF ORIGINATOR G-CSFS *VERSUS* BIOSIMILAR G-CSFS AFTER AUTOGRAFTING IN HEMATOLOGICAL MALIGNANCIES

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Background: Autografting is widely used for the treatment of hematological malignancies, especially for lymphoproliferative diseases. In recent years, biosimilar G-CSFs (BioG-CSFs) have gradually been introduced into clinical practice to mobilize peripheral blood hematopoietic stem cells (CD34+ cells) and reduce the duration of neutropenia.
Aims: This study was designed to assess, in the setting of "real life" clinical practice, the role of BioG-CSFs and to compare them with a historical patient cohort treated with "originator" G-CSFs after autografting. The study was prompted by the growing propensity for the use of biosimilar growth factors to reduce overall costs and by the imminent implementation of other novel biosimilar molecules.
Methods: The study period was 2006-2015 during which standard policies for autografting did not change. Primary endpoints were post transplant engraftment kinetics, transfusion requirements and duration of hospitalization. Secondary objective was one-year overall survival. Day of neutrophil engraftment was defined as the first of 3 consecutive days of absolute neutrophil count ≥500/ul whereas day of platelet engraftment was defined as the first of 7 consecutive days of platelets ≥20.000/ul unsupported by transfusion.
Results: 276 patients were studied for a total of 354 transplants. Underlying diseases included multiple myeloma (n=204), non-Hodgkin lymphoma (n=56), Hodgkin lymphoma (n=9), chronic lymphocytic leukemia (n=2), acute myeloid leukemia (n=4) and histiocytic sarcoma (n=1). Seventy-eight multiple myeloma patients received double autologous transplant. Conditionings were as follows: melphalan 200 mg/m^2 for myeloma; busulfan/cyclophosphamide for acute myeloid leukemia; BEAM or FEAM-like for lymphoproliferative disorders. Overall,148 and 206 patients received originator G-CSFs and BioG-CSFs respectively. Infused CD34+ cells were 4.99 (IQR 3.77-5.05) x10^6/kg/body weight and 4.17 (IQR 3.66-5.00) x10^6/kg/body weight in the originator G-CSFs and BioG-CSFs cohorts. Cumulative incidences of neutrophil recovery at day +15 and +25 were 95.3% (95% CI 91.7% - 98.8%) and 99.3% (95% CI 97.6% - 100%) and 96.1% (95% CI 93.4% - 98.8%) and 98.5% (95% CI 96.8% - 100%) in the originator G-CSFs and BioG-CSFs groups respectively (p=0.980). These data were confirmed by Propensity Score adjustment, HR 1.03 (95% CI 0.87-1.2), p= 0.763. Cumulative incidence of platelet recovery at day +30 was 98.6% (95% CI 96.5% - 100%) and 96.1% (95% CI 93.4% - 98.8%) in the originator G-CSFs and BioG-CSFs cohorts (p<0.001). Of note, no differences between cohorts were found in a) median duration of neutropenia (median: 7 and 6 days, p=0.180) b) platelet (median 1 pool/patient in both, p=0.441) and red blood cell (median: 0/patient in both, p=0.703) transfusion requirements, hospital stay (median: 20 days in both, p=0.270). Interestingly, engraftment kinetics after the second transplant were faster for both neutrophil and platelet recoveries (HR 1.34 (95% CI 1.1-1.62), p=0.003 and HR 1.30 (95% CI 1.01-1.67), p=0.038, respectively, by multivariate models). No severe adverse reactions attributable to growth factors were documented. Finally, one-year overall survival was comparable between cohorts (p=0.901).

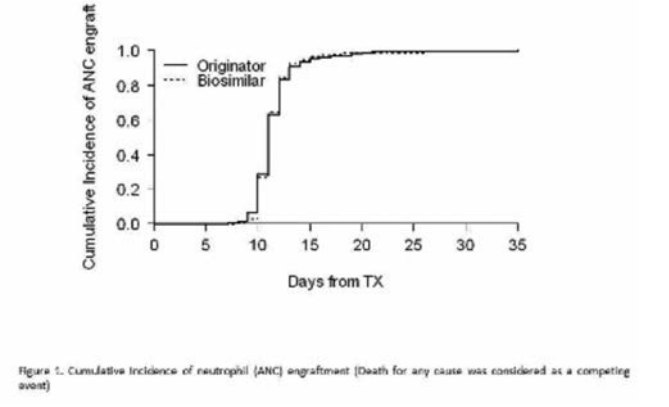

Figure 1. Cumulative incidence of neutrophil (ANC) engraftment (Death for any cause was considered as a competing event)

Figure 1.

Summary/Conclusions: In this sizable study, BioG-CSFs were as effective as originator G-CSFs. Moreover, their extensive use led to a significant cost containment.

E1549

FRONTLINE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR AGGRESSIVE B AND T CELL LYMPHOMA

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Background: Either autologous or allogeneic haematopoietic stem cell transplantation is the salvage and even curative treatment for relapsed lymphoma. For high risk aggressive B cell or T cell lymphoma, frontline auto-transplantation can also improve survival.
Aims: We investigated the outcome between frontline *versus* salvage autotransplantation for aggressive lymphoma including B cell, T cell, and Hodgkin lymphomas.
Methods: For high risk aggressive B cell lymphoma (stage III and IV or IPI score above 3) and aggressive T cell lymphoma except ALK+ ALCL, we retrospectively compared the outcome of frontline autologous transplantation and non-transplant or salvage transplant patients.
Results: Between 2001 and 2015, we have 95 patients undergoing autologous (N=67) and allogeneic (N=28) haematopoietic stem cell transplantation. The 5-year overall survival was 70.4% and 59.1%, respectively. For high risk aggressive B cell lymphoma undergoing frontline autotransplant, the overall survival was 81.8%. During the same period, 5-year overall survival of stage III and IV diffuse large B cell lymphoma patients treated at our institute were 58% and 45%, respectively. For aggressive T cell lymphoma, 66.7% *versus* 0% of 5-year overall survival for frontline *versus* salvage autotransplant.
Summary/Conclusions: Frontline autotransplant should be considered in selected high risk aggressive B cell lymphoma patients and is very important for aggressive T cell lymphoma patients.

LB2269

PHASE 2A STUDY OF ALXN1007, A NOVEL C5A INHIBITOR, IN SUBJECTS WITH NEWLY DIAGNOSED ACUTE GRAFT-VERSUS-HOST DISEASE (GVHD) INVOLVING THE LOWER GASTROINTESTINAL TRACT

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Background: Murine models implicate complement in multiple steps of the acute GVHD cascade. Transplantation of donor T cells deficient in C3a and C5a as well as pharmacologic exposure to C5a receptor blockade have all been shown to reduce GVHD lethality in mice. In humans, complement activation has been correlated with gastrointestinal (GI) GVHD.
Aims: For this reason, we performed a multicenter Phase 2a trial (NCT02245412) of ALXN1007, a recombinant humanized monoclonal C5a antibody, in patients with newly diagnosed acute GVHD of the lower GI tract.
Methods: Between 14 Nov 2014 and 13 Nov 2015, a total of 15 patients with biopsy-confirmed acute GVHD of the lower GI tract consented, enrolled, and received weekly intravenous administration of 10 mg/kg ALXN1007, in combination with methylprednisolone at an initial dose of 2 mg/kg, through day 56. The primary objective was to assess the day 28 overall acute GVHD response rate along with safety and pharmacokinetics/pharmacodynamics (PK/PD).
Results: Median age was 60 years (range, 25-69); 60% were male, and acute myeloid leukemia was the most common diagnosis (33%). At enrollment, lower GI stages 1, 2, and 3 were present in 7, 2, and 6 patients, respectively. Day 28 overall acute GVHD response rate was 77% (2 patients were nonevaluable due to leukemia relapse at day 18 or early withdrawal). Complete GI GVHD response rates at days 28 and 56 were 69% and 77%, respectively. Day 56 overall acute GVHD response rate, day 180 nonrelapse mortality, and overall survival were 77%, 12.5%, and 69.2%, respectively. PK/PD analyses suggest that the dose may have been suboptimal with 10/13 patients achieving <90% inhibition of C5a. Day 28 response rate was higher in those achieving 90% inhibition (3/3) *versus* in those with less C5a inhibition (6/10). The drug was well tolerated with just 1 grade 2 infusion-related reaction and no grade 3 or higher adverse events attributable to the study drug.
Summary/Conclusions: The efficacy and safety data suggest that ALXN1007 is a promising therapy for patients with acute GVHD involving the lower GI tract. The level of C5a inhibition might be an important biologic parameter to predict clinical response. PK/PD analyses suggested higher doses and frequency may be needed to optimize C5a inhibition and maximize clinical response. For this reason, the trial has been amended to test 20 mg/kg weekly and twice-weekly dosing.

LB2270

RABBIT ANTITHYMOCYTE GLOBULIN *VERSUS* ALEMTUZUMAB IN ALLOGENEIC STEM CELL TRANSPLANTATIONA META-ANALYSIS

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Background: Rabbit antithymocyte globulin (ATG) and alemtuzumab have been used for graft-versus-host disease(GVHD) prophylaxis in allogeneic haematopoietic stem cell transplantation, but which is more efficient remain unclear.
Aims: This study is to analysize the role of rabbit antithymocyte globulin *versus* alemtuzumab in allogeneic stem cell transplantation.
Methods: we perform a meta-analysis of all studies comparing rabbit antithymocyte globulin (ATG) and alemtuzumab for graft-versus-host disease(GVHD) prophylaxis in allogeneic haematopoietic stem cell transplantation to evaluate their benefits and drawbacks. There are 7 studies (one prospective and six retrospective)for comparing ATG *vs* alemtuzumab in GVHD prophylaxis with 622 patients.
Results: Our results showed that the incidence of grade II-IV acute GVHD(RR 1.51, 95% CI 0.97-2.34, P=0.07), incidence of grade III-IV acute GVHD (RR 1.48, 95% CI 0.63-3.47, P=0.37) had a statistically non-significant reduction in alemtuzumab group, however , alemtuzumab significantly impaired OS (HR 0.61 (95% CI 0.41-0.90, P=0.01) compared with ATG. The incidence of overall chronic GVHD(RR 0.97, 95% CI 0.67-1.40, P=0.87) and the incidence of relapse(RR 1.03, 95% CI 0.72-1.47, P=0.88) were similar in the two groups.
Summary/Conclusions: We propose that using alemtuzumab for GVHD prophylaxis is beneficial for allogeneic stem cell transplantation due to the efficacy in grade III/IV acute GVHD, but OS is impaired compared with ATG group.

LB2271

A PRELIMINARY DISCUSSION ON THE RELATIONSHIP BETWEEN SERUM BIOMARKERS AND AGVHD IN PATIENTS WITH ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective means for the treatment of a variety of hematologic malignancies. Acute graft *versus* host disease (aGVHD) is a common complication after allo-HSCT. And it seriously affects the quality of life and survival rate of patients and is also the main cause of non relapse mortality in allo-HSCT patients. The diagnosis of aGVHD mainly depends on the clinical manifestations and pathological examination of the damaged target organs. But its clinical symptoms are changeable and lack of specificity, the pathological examination often in clinical diagnosis and treatment because of various reasons can not be carried out.
Aims: To explore the relationship between serum biomarkers and aGVHD in allo-HSCT patients at different time points, and to provide theoretical basis for early intervention and prognosis evaluation of aGVHD.
Methods: Collection the serum samples of patients with allo-HSCT in November 2014 to November 2015 in the Department of Hematology, Xinqiao Hospital, Third Military Medical University . 10 patients with aGVHD were selected as the case group, and 10 patients without aGVHD were randomly selected as the control group. Protein chip technology was used to detect the content of IL-1β, IL-7, IL-8, ST2, vWF in plasma of the two groups patients in 7 days before transplantion and 7 days, 14 days, 28 days, 56 days, 100 days after transplantation.
Results: Compared aGVHD group and non-aGVHD group, 7 days and 56 days after transplantion, the level of ST2 in serum were significantly increased (P<0.05); 28 days, 56 days and 100 days after transplantion, the level of IL-7 in plasma were significantly increased (P<0.05); 56 days after transplantion, the level of IL-1β in plasma were significantly increased (P<0.05); Serum IL-8 and vWF were not significantly different between two groups in every time points.
Summary/Conclusions: The content of IL-1β, IL-7, ST2 in serum of patients with aGVHD after transplantation is higher than that of patients who were not aGVHD, and they can be used as the auxiliary diagnosis index of aGVHD and is helpful to early diagnosis and intervention of aGVHD. Monitoring of serum biomarkers for aGVHD immune targeted therapy provides a new direction.

Thrombosis and vascular biology

E1550

TLR2-PI3K/AKT SIGNALING PATHWAY INVOLVED IN PLATELET ACTIVATION INDUCED BY GROUP B STREPTOCOCCI

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Background: Platelets not only play an inportmant role in the initiation of hemostasis and thrombosis,but also participate in immune and inflammatory response. Most studies focus on the platelet-bacteria interaction and demonstrate that bacteria are capable of bind to,aggreating and activating platelets. Human platelets are reported to express several groups of TLRs, which particiated in the inflammation process. and monitoring host infection.Recent data from our laboratory demostrated that Group B streptococci(GBS) could induce platelet aggregation and up-regulate the expression of CD62P and further study shows that platelet TLR2 might involve in the activation.GBS,or streptococcus agalactiae,is one of the most common cause of life-threatening sepsis,pneumonia and meningitis in neonates,pregnant women,the elderly and immunocompromised adults.Therefore,illuminating the mechanisms of GBS-induced platelet activation is important for providing the basis for platelets in defense against infection and immunity.Since increasing reports have shown that the PI3-K/Akt signaling pathway regulates platelet activation and hemostasis,it is possible to research the TLR2 related signaling pathway.
Aims: Exploring whether TLR2-PI3K/Akt signaling pathway involved in the platelet activation induced by GBS.
Methods: 1. Platelets were from healthy volunteers (all genders, 25-52 years old) who had not taken any anti-platelet drugs (like aspirins, clopidogrel and abciximab) during the previous 30 days. GBS 639 were isolated from sepsis patients. 2. Platelet aggregation, the expression of platelet CD62P and PAC-1 were used as the indicator of platelet activation. GBS-induced platelet aggregation was assayed by light transmission; platelet TLR2, CD62P and PAC-1 expression were determined by flow cytometry; AKT and AKT phosphorylation expression were determined by RT-PCR or western blot assay. In some experiments, platelets were pre-incubated with PI3-K specific inhibitors LY294002 or anti-TLR2 monoclonal antibody.. 3. Statistical analysis: Data are reported as the mean±SD. Treatment groups were compared with the appropriate control (s), and statistical significance was examined using the two-tailed t-test. Differences were considered significant when P=0.05. All values were analyzed using SPSS version 17.0 software.
Results: 1. Platelet activation and TLR2 protein expression: Platelet aggregation, surface expression of TLR2, CD62P and PAC-1 induced by GBS were increased significantly on the platelets upon activation with GBS 639. However incubated with anti-TLR2 monoclonal antibody, they all decreased. 2. PI3-K/Akt signaling pathway: Real-time PCR showed that the PI3-K and Akt mRNA expression levels were increased significantly in the platelets stimulated with GBS 639. Western blot results showed that of Akt phosphorylation triggered by GBS was occurred as early as 15 min and increased gradually to reach a peak at 2 h post-infection and no significant changes were observed in total Akt protein expression during the infection. However, the expression of p-Akt, platelet aggregation and surface expression of CD62P and PAC-1 induced by GBS were significantly inhibited in the presence of a PI3-K inhibitor LY294002. 3. The relationship between TLR2 and PI3-K/Akt signaling pathway in platelet activation: Platelet p-Akt expression levels induced by GBS were significantly decreased after the activity of platelet TLR2 was blocked by anti-TLR2 monoclonal antibody, and no significant changes in total Akt protein expression.
Summary/Conclusions: GBS induced platelet activation through the TLR2-PI3-K/Akt signaling pathway in human platelets.

E1551

FUNCTIONAL ANALYSIS OF VON WILLEBRAND FACTOR IN MOUSE MODEL OF ACUTE ISCHEMIA-REPERFUSION KIDNEY INJURY

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Background: Acute kidney injury (AKI), an abrupt loss of renal function, is often seen in clinical settings and may become fatal. The adhesive protein von Willebrand factor (VWF) plays a pivotal role in platelet thrombus formation under high shear stress conditions and is recently understood as a key protein in a cross-talk between inflammation and thrombosis.
Aims: We therefore assumed that VWF may be involved in the pathophysiology of AKI, the major cause of which could be an insufficient renal circulation and/or inflammatory cell infiltration in the kidney. To test this hypothesis, we studied the relevant role of VWF in AKI in mouse model of acute ischemia-reperfusion (I/R) kidney injury.
Methods: Mice (male, 8-12 weeks of age), whose right kidney were surgically removed 1 week prior to the kidney I/R experiment, were anesthetized with inhaled isoflurane and then placed in an abdominal position on a heating pad. Surgical incision was given on the left side of back and the left kidney was brought out and kept outside during the operation. Both renal artery and vein

were clamped at the renal hilus by a clamping clip for 30 min ischemia. Then a clip was taken off to provoke the reperfusion of renal blood flow, which was monitored by Laser Doppler flowmetry. The kidney was then put back in a body and skin incision was closed. The renal blood flow was measured again 30 h after reperfusion and mice were then sacrificed for blood collection.
Results: We compared 15 wild-type (WT) and 16 VWF-gene deleted (knock-out; KO) mice (from The Jackson Laboratory, Bar Harbor, ME). Excess blood loss was not observed in all mice (WT or KO) during whole surgical process. Although no difference was seen immediately after reperfusion, significantly ($p<0.05$) higher renal blood flow at 30 h after reperfusion was confirmed in VWF-KO mice, as compared to WT (KO; 24.0±2.3 *vs* WT; 15.1±1.46 ml/min/100g of kidney weight, and the reperfusion/base flow ratio: KO; 1.0±0.07 *vs* WT; 0.6 ±0.07). Consistent with the renal blood flow data, the serum creatinine value at 30 h after reperfusion were significantly ($p<0.05$) lower in VWF-KO mice than WT (KO; 2.77±0.11 *vs* WT; 3.15±0.11 mg/dl).
Summary/Conclusions: Our results suggest that VWF does play a role in the pathogenesis of AKI, in which VWF-dependent thrombotic or inflammatory responses may trigger thrombotic ischemia or endothelial damages of vascular bed in the kidney. Thus, proper functional regulation of VWF would be beneficial for better microcirculation and vessel functions in the kidney, suggesting a novel therapeutic potential against AKI.

E1552

INCREASED PLATELET LEUKOCYTE INTERACTIONS IN TYPE 2 DIABETIC INDIVIDUALS COMPARED TO HEALTHY CONTROLS

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Background: Type 2 diabetes mellitus (T2DM) is considered as a form of low grade inflammatory response. Activated platelets are involved in inflammatory processes and bind to inflammatory cells monocytes and neutrophils to form platelet leukocyte aggregates (PLAs). Platelet bound neutrophils and monocytes become activated (expressing CD69) and undergo a phenotypic change into pro-coagulant phenotypes expressing tissue factor (CD142).
Aims: 1) To investigate the baseline levels of platelet activation in pre-diabetes and diabetes and compare this to normal controls. 2) To investigate the percentage of monocytes and neutrophils forming aggregates with platelets (PMA's and PNA's) in pre-diabetes and diabetes and comparing this to normal individuals. 3) To investigate the up regulation of pro-thrombotic and activation antigens on the surface of monocytes and neutrophils (Tissue Factor and CD69) in pre-diabetic and diabetic individuals.
Methods: Peripheral blood was collected into 4.5ml sodium citrate tubes containing 3.2% sodium citrate, samples were analysed within 1 hour of collection on the Navios 8 colour flow cytometer (Beckman Coulter, Miami, Florida). To ensure reporting on standardized results, flow check pro fluorescent labelled beads (Beckman Coulter, Miami, Florida) were used to standardise the optical path and laminar flow of the instrument. Blood was also collected into serum separator tubes (SST), heparin and EDTA for both biochemical and haematology testing. 50µl of citrated blood was incubated with 5µl of an antibody cocktail containing the antibodies CD42b-FITC, CD142-PE, CD69-PC5, CD16-PC7 and CD14-APC.After 10 minute incubation in the dark at room temperature each sample was lysed with 500µl Versalyse lysing solution for 15 minutes. 500µl of PBS was added to the sample and it was immediately analysed on the flow cytometer.
Results: Our results showed increased levels of platelet leukocyte aggregates%PLA in pre-diabetes and diabetes therefore indicating increased interactions between platelets and leukocytes in the peripheral blood of pre-DM and DM individuals. The diabetic group showed increased baseline levels of circulating platelets monocyte aggregates (%PMAs) median 49.04[36.78-62] compared to the control group 7.2[5.4-9], $p<0.0001$. Baseline platelet neutrophil aggregates (%PNAs) were significantly increased in the DM group [median 13.56(10.17-16.95)] compared to the control group [median 6.01(4.51-7.51)] $p<0.0001$. We also showed increased levels of tissue factor expression, a main part of the coagulation cascade, on the monocytes and neutrophils possibly is causing the hyper-coaguable state seen in diabetic individuals causing CVDs. Tissue factor (TF) expression on both monocytes for the diabetic group were significantly higher than the control group and correlated with increased levels of%PMAs ($r=0.6744$, $p<0.0001$).
Summary/Conclusions: In this study we showed increased PLAs in diabetic individuals than normal controls which may be responsible for the cardiovascular complications in diabetic patients. The increased tissue factor expression on monocytes and neutrophils in diabetic individuals may also contribute to the pro-coagulant state.

E1553

ANTICOAGULATION THERAPY AND A CAREFUL INTERDISCIPLINARY MANAGEMENT OF SPLANCHNIC VEIN THROMBOSIS: A SAFE AND EFFECTIVE APPROACH

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Background: Splanchnic vein thrombosis (SVT) are unusual thrombosis that may be associated with underlying disorders either local or systemic. The optimal initial treatment of SVT is uncertain and limited data regarding safety and efficacy of anticoagulation are available at present.
Aims: In this report, we present our recent experience with SVT and discuss our treatment algorithm.
Methods: We retrospectively reviewed the records of 38 patients diagnosed as SVT that attended our anticoagulation clinic until June 2015. All clinical, laboratory, therapeutic and outcome data were collected for each patient. In patients who were not actively bleeding, anticoagulation treatment was started as soon as possible according to the platelet count. For a platelet count >50.000/mcl, we administered initial full dose low-molecular-weight heparin (LMWH) and antivitamin-K (AVK; target INR range 2-3 or 1.8-2.5 in the presence of additional risk factors for bleeding), half or prophylactic dose of LMWH for a platelet count between 30.000/mcl and 50.000/mcl and no treatment below 30.000/mcl. Moreover an appropriate prophylaxis with beta-blockers or with endoscopic therapies was extensively employed in in our cohort of cirrhotic SVT. The quality of oral anticoagulation was assessed by the time in therapeutic range (TTR). The response to initial anticoagulant therapy was evaluated as vessel recanalization and computed according to the Kaplan-Meier method. Major bleeding (ISTH definition) and vascular events (arterial and venous thrombosis) were also assessed.
Results: Thirty eight patients were included (median age 51 years; 71.1% males); portal vein thrombosis was the most common site of thrombosis (52.6%), followed by multiple venous segments involvement (34.2%). Hepatic cirrhosis was the common cause (44.7%) while myeloproliferative neoplasms were identified in four patients (10.5%). Thirteen patients (34.2%) were diagnosed incidentally. Thirty five patients (92.1%) were treated with anticoagulation (47.4% with AVK; median TTR 57%). Twenty three patients remained on anticoagulant treatment during the whole study period. During a median treatment duration of 27 months, the incidence rate of vascular events was 2.1 per 100 patient-years (1.6 and 0.8 per 100 patient-years among patients treated with LMWH alone or AVK respectively). Major bleeding occurred in one patient treated with AVK, corresponding to an incidence rate of 0.8 per 100 patient-years. Twenty six patients (68.4%) obtained vessel recanalization or thrombosis resolution. The probability of recanalization of the occluded vessels was 50% at 30 months. The median follow-up was 30.9 months (2-213); four patients (26.7%) died for causes not related to anticoagulation (cirrhosis, cancer).
Summary/Conclusions: The use of our treatment algorithm and a careful interdisciplinary management of SVT, appears to be safe and effective even for patients with major risk factors for bleeding (e.g. liver cirrhosis).

E1554

FIBRINOGEN GENETIC VARIABILITY AND COAGULATION PROCESS MEDIATE ATHEROSCLEROSIS MANIFESTATIONS AND AFFECT THE EXTENT OF THE DISEASE

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Background:
Aims: Evidence suggests that altered coagulation and fibrinogen genetic variability may contribute to atherogenesis. Therefore, we examined the combined effects of the rs1800790, rs2070011 fibrinogen polymorphisms on coagulation and endothelial function as well as the effect of coagulation on atherosclerotic manifestations.
Methods: In the present study we enrolled 422 patients with stable angina (SA) and 277 controls. The rs1800790 (G455A) and the rs2070011 (G58A) polymorphisms were detected by appropriate genotyping. Fibrinogen and D-Dimer levels, as well as factors (f) V, X activity were measured by standard coagulometry techniques. Flow-mediated dilation (FMD) was assessed by brachial ultrasound.
Results: We have shown that the 455AA homozygosity was associated with increased fibrinogen levels in both groups ($p=0.035$ controls and $p<0.001$ SA). In addition, homozygotes for the 58A allele had lower fibrinogen levels in controls ($p=0.038$). Both the 58AA ($p=0.027$) and 455AA homozygotes ($p=0.022$) had higher levels of D-Dimer in the SA group. The 455AA homozygotes had also increased fV activity in the SA group ($p=0.048$). No effects were observed on fX activity and FMD. Further analysis revealed that fibrinogen levels were strongly associated with SA (1.005 [1.003-1.007], $p<0.001$) as well as the presence of myocardial infarction (MI) (1.003 [1.001-1.005], $p=0.001$). Similarly, D-Dimer levels were also strong predictors of CAD (1.001 [1.001-1.002], $p<0.001$), but not of MI (1.000 [1.001-1.001], $p=0.083$). Surprisingly, when fV and fX activ-

ities were examined for possible associations with clinical presentations, we found that fV activity was associated with increased number of diseased vessels (1.016 [1.001-1.030], p=0.037), while fX activity was strongly related to MI (0.985 [0.973-0.997], p=0.013).
Summary/Conclusions: Our results demonstrate that the rs1800790 variant increases significantly fibrinogen, D-Dimers levels and fV activity, contributing to atherogenesis. Moreover, fibrinogen and D-Dimers levels are strong predictors of SA and MI. Interestingly, in our study we have shown for the first time that fV and fX are associated with the number of diseased vessels and the risk of MI respectively.

E1555

INTERVENTION TO OPTIMIZE VENOUS THROMBOEMBOLISM PREVENTION IN A COMMUNITY TEACHING HOSPITAL
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Background: According to the U.S. Surgeon General, venous thromboembolism (VTE) affects 350,000 to 600,000 Americans each year and is implicated in over 100,000 deaths (The Surgeon General's Call to Action to Prevent Deep Vein Thrombosis and Pulmonary Embolism. Rockville, MD: Office of the Surgeon General, US; 2008.). Noting that only half of eligible patients in our inpatient medical service were receiving VTE prophylaxis (VTEP), our hospital instituted an intervention to improve performance on this critical measure.
Aims: To evaluate the efficacy of an electronic medical record (EMR)-based intervention to optimize VTEP among patients admitted to our institution.
Methods: In February of 2011, our hospital's information technology department instituted an automated prompt in the EMR system designed to increase the number of VTEP-eligible patients receiving VTEP. The prompt guides resident physicians in the process of admitting patients to a "hard stop" screen entreating them to identify VTE risk factors in the patient and to choose from a list of evidence-based VTEP therapies (including but not limited to heparin, sequential compression devices, and an option to not order VTEP if contraindicated). Concurrently, an educational presentation was offered to residents explaining the intervention and re-educating them on evidence-based VTEP guidelines. The computerized prompt remains in use today.
Results: Data covering 964 patients admitted in the month prior to the implementation of the VTEP prompt and 673 patients from after implementation were collected and analyzed. Prior to the implementation of the VTEP prompt, 49.0% of 964 patients were found to have received VTEP therapy. Following prompt implementation, that number rose 16.9% to 65.9% of patients. Data is continually collected and assessed semiannually.
Summary/Conclusions: As advances in medical technology continue to improve patient care, optimization of medication utilization becomes increasingly important. With underuse of critical medications raising the risk of iatrogenic illness, it remains the responsibility of the patient care team to engage in best practices to minimize these risks. Our intervention has demonstrated that VTEP utilization may be optimized by the use of a low-cost, easily implementable, and easily replicable intervention that effectively leverages the EMR system.

E1556

ERYTROCYTE-RELATED PHENOTYPES AND GENETIC SUSCEPTIBILITY TO THROMBOSIS
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Background: Venous thromboembolism (VTE) is a common disease that involves the interaction of genetic and environmental risk factors. Previous studies have estimated a heritability of approximately 60% for the risk of VTE. Using variance analysis, intermediate phenotypes related to thrombosis have identified genetic risk factors. Erythrocyte-related phenotypes are considered good risk factors.
Aims: To investigate the relationship of erythrocyte-related phenotypes with the risk of VTE.
Methods: Our study used the Genetic Analysis of Idiopathic Thrombophilia 2 (GAIT 2 Project) sample, which consisted of extended pedigrees (935 individuals). The sample had 120 subjects with thromboembolism (86 with VTE, 47 with arterial thrombosis and 13 with both). The following possible risk factors were evaluated: Red blood cell and indices, reticulocytes, serum iron, total iron binding capacity, serum ferritin, serum transferrin receptor (STFR), haptoglobin, serum vitamin B12 (B12), serum folate and red blood cell folate. Using the variance component method, heritability (h^2), the household effect and all of the phenotypic, genetic and environmental correlations with VTE were estimated.
Results: The h^2 of VTE was 0.67. Most erythrocyte parameters showed significantly high h^2. In addition, VTE was correlated with: hematocrit (0.52, p=0.01), red blood cell distribution width (RDW) (0.28, p=0.05), immature reticulocyte fraction (IRF) (0.45, p=0.008), transferrin saturation index (-0.7, p=0.05), STFR (0.4, p=0.006), and B12 (0.34, p=0.03).
Summary/Conclusions: We demonstrated a genetic relationship between erythrocyte phenotypes and VTE, and we identified 6 intermediate phenotypes as risk factors. We suggest that these risk factors will be useful in the search for thrombosis-related genes.
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E1557

INTENSIVE HEMATOLOGY SCREENING FOR MYELOPROLIFERATIVE NEOPLASM AND EARLY RADIOLOGICAL INTERVENTION IS ASSOCIATED WITH EXCELLENT OUTCOMES IN BUDD CHIARI SYNDROME
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Background: Budd Chiari Syndrome (BCS) is a rare and life-threatening disorder, resulting from thrombosis of the hepatic veins. Aetiologies include prothrombotic states such as Myeloproliferative Neoplasm (MPN). Current treatment strategies involve a step-wise approach of anticoagulation followed successively by radiological and surgical interventions, and ultimately liver transplantation.
Aims: We aimed to retrospectively describe our institution's experience with the management of patients with BCS, including the diagnosis (by bone marrow biopsy and molecular testing for JAK2 and calreticulin mutations) and management of concomitant MPN.
Methods: In this retrospective study, approved by the hospital's Research Ethics committee, all cases of primary BCS, including new and recurrent presentations, presenting between January 2000 and August 2012 were identified from the hospital's computerised database.
Results: 27 patients with primary BCS presented during the study period. Twenty-four patients (89%) had identifiable risk factors, with the commonest being MPN, detected in 17/24 (71%) of the tested patients. This included 4 patients who initially presented with normal peripheral blood counts. The MPN diagnosis was made on the basis of JAK2V617F positivity (n=13), either alone (n= 8) or with a confirmatory bone marrow biopsy (n=5); or on marrow biopsy alone (n=4). The calreticulin mutation was detected in 2/9 tested patients (one patient with JAK2 negative MPN and one with ET with unknown JAK2 status). Subtypes of MPN included: Polycythaemia Vera (n=8), Essential Thrombocytosis(n=6), MPN unclassified (n=2) and Chronic Myeloid Leukaemia (n=1). An additional thrombophilic state was present in six of the MPN patients: the oral contraceptive pill (n=4), factor V Leiden heterozygosity (n=1), anticardiolipin antibody and recent in-vitro fertilisation (IVF) treatment (n=1). Of the six patients with a known diagnosis of MPN prior to BCS, three were on treatment with hydroxyurea, aspirin or venesection but none were anticoagulated prior to BCS diagnosis. Of the 11 patients with newly diagnosed MPN, additional treatment included hydroxyurea alone (n=3), venesection alone (n= 2), hydroxyurea and venesection (n=2), hydroxyurea and splenectomy (n= 1) and interferon (n= 1). Three patients with MPN were not given additional cytoreductive treatment: two in the context of normal peripheral blood counts, and one diagnosed just prior to death. All patients in the study were anticoagulated with warfarin or Low Molecular Weight Heparin, and 2 of the MPN patients also had aspirin. 25 of the 27 (92.6%) patients also had upfront radiological interventions, consisting of TIPS (transjugular intrahepatic portosystemic shunt) in 18 (67%) patients and/or angioplasty/stenting in 11 (40%). No patient developed TIPS failure. At a median follow up of 59 months (range 2 to 248 months), the overall survival was 96% at one year and 81% at five years, much greater than predicted by the patients' median Rotterdam score of 1.16 (range 0.07-2.11). No patients required liver transplantation.
Summary/Conclusions: There is a high incidence of MPN in patients with primary BCS, predominantly JAK2V617F positive, including patients with normal peripheral blood counts at the time of diagnosis. All BCS patients should therefore have JAK2 testing and, if negative, calreticulin mutation testing. A bone marrow biopsy should be considered if these are negative, in the context of unexplained polycythaemia or thrombocytosis. Furthermore, we advocate early radiological hepatic decompression as an alternative to the step-wise approach, as we have demonstrated excellent medium term outcomes with no TIPS failure and no patients requiring transplantation.

E1558

FIBRIN SHEATHS ON CENTRAL VEIN CATHETERS AS A RISK FACTOR FOR DEEP VEIN THROMBOSIS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Indwelling of central vein catheter (CVC) is well known as a risk factor for deep vein thrombosis (DVT) especially in patients with malignancies. The term «CVC-related thrombosis» is attributed to mural thrombosis that

involves CVC, adheres to vessel wall and partially or totally occludes the vessel lumen. However, presence of CVC in a central vein may result in emerging not only «true» mural thrombosis but so-called fibrin sheaths (FS) that envelope CVC but do not involve venous wall. Despite high FS incidence data on its clinical importance is scarce.
Aims: To investigate the role of FS in DVT development; to reveal the efficacy of anticoagulant therapy in patients with DVT with previous FS.
Methods: We analyzed data on 182 CVC inserted in 113 patients with acute lymphoblastic leukemia (ALL) aged from 1 to 19 years. Data were collected retrospectively from electronic hospital charts. The diagnosis of DVT and FS was made by means of echocardiography (ECHO) and Doppler ultrasound scanning (DUS) of extracranial brachiocephalic veins. DUS has been performed at different time after CVC implantation depending on clinical indications and physician's opinion. A «p» value<0,05 was considered statistically significant. Data were analyzed using the IBM SPSS for Windows Software. Univariant logistic regression analysis was performed for estimation of FS significance for DVT development. Between group comparisons were performed by chi-square or Fisher's exact test depending on sample value.
Results: FS were detected on 63 (35%) CVC. Incidence of FS was 2,71 events per 1000 catheter days. Catheter-related DVT was revealed in 51 (28%) cases. In 24 of them FS preceded DVT emerging. Presence of FS was significantly associated with subsequent DVT (odds ratio (OR) 2,75, 95% confidential interval (CI) 1,32-5,74, p=0,003). After detecting of FS, anticoagulant prophylaxis was started in 31 cases. In this group DVT subsequently developed in association with 15 (48%) CVC. In group that did not receive anticoagulants DVT developed in 9 (32%) cases. In both groups there was one occlusive DVT. Difference in DVT incidence between groups with and without anticoagulant prophylaxis after FS detecting was statistically insignificant (p=0,908). FS resolution occurred independently of anticoagulants usage (p=0,598).

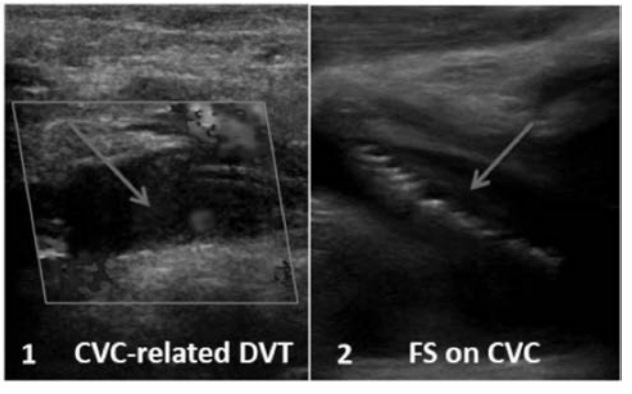

Figure 1.

Summary/Conclusions: According to our data, FS was detected on 35% of CVC. Presence of FS was associated with subsequent CVC-related DVT emerging. Efficiency of anticoagulants for FS resolving is doubtful. The question whether anticoagulant prophylaxis of DVT should be started after FS detecting remains unclear. Large prospective studies are required to resolve this issue.

E1559

FIBRINOGEN GENETIC VARIABILITY ACCELERATES ATHEROGENESIS BY ALTERING THE INFLAMMATORY PROCESS AND COAGULATION IN PATIENTS WITH DIABETES MELLITUS TYPE 2 AND HYPERTENSION
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Background:
Aims: Hypertension (HTN) and diabetes mellitus (DM) are significant risk factors for atherosclerosis and characterised by altered coagulation and inflammatory process. In the present study we aimed to investigate the role of fibrinogen genetic variants (rs180070 and rs2070011) on coagulation and inflammation in patients with combined DM and HTN
Methods: 744 subjects (HTN and DM) were enrolled in our study. Fibrinogen polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Fibrinogen, interleukin-6 (IL-6), high sensitivity C-reactive protein (hsCRP) CD40L, D-dimers and factors V, X activity were measured with appropriate laboratory methods.
Results: In HTN patients, hsCRP levels were higher in the group of patients with higher fibrinogen levels compared to patients with lower and intermediate fibrinogen levels [0.28 (0.12–0.42) *vs* 0.17 (0.04–0.38) *vs* 0.18 (0.09–0.39), p=0.032)]. In addition, we found a significant effect of the rs180070 polymophism on IL-6 levels among HTN and DM. In HTN, IL-6 levels were higher in AA homozygotes of the rs180070 compared to all other genotypes (AG or GG) (4.96±0.69 *vs* 3.7±0.2, p=0.046). Similarly, DM patients homozygotes for the A allele (rs180070) had higher IL-6 levels (6.72±0.99 *vs* 4.01±0.35, p=0.016). In multivariable analysis, the only independent predictor of IL-6 levels in DM was the AA genotype [β(SE): 0.216 (1.76), P=0.047]. In hypertensives, AA (rs180070) homozygosity was the only adjusted independent predictor of IL-6 levels [β(SE): 0.151 (0.642), P=0.032]. Subgroup analysis of HTN and DM patients did not reveal any differences of CD40L levels in the presence of AA genotype (rs180070) (p=0.919 for DM and p=0.144 for HTN). We did not observe any significant change in IL-6 or CD40L levels with the presence of the AA genotype of the rs2070011 among the studied subgroups. Finally, we found that the AA homozygosity (rs180070) was associated with increases D-dimer levels in both DM and HTN (DM: 741.6±109,4 *vs* 487.3±35.6, p=0.028, HTN 623.3±79.6 *vs* 388.6±23.5,p=0.048).
Summary/Conclusions: The AA homozygosity (rs180070) was associated with higher IL-6 levels and CD40L concentrations. We also showe for the first time, to our knowledge, that the AA homozygosity (rs180070) is associated with increased D-dimer levels. Our results of this study showed that higher levels of fibrinogen were associated with elevated D-dimer levels. Homozygotic status for the rs180070 polymorphism was also related to higher D-dimer levels both in HTN and DM patients.

E1560

VENOUS THROMBOSIS IN PUERPERIUM IS ALSO DOMINANTLY LOCALIZED IN LEFT LEG, BUT TENDS TO BE MORE MASSIVE THAN THROMBOSIS DURING GESTATION
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Background: During pregnancy and puerperium different factors play role in pathogenesis of venous thrombosis, and they may influence on localization and extent of thrombosis. Compression of iliac veins by gravid uterus is responsible for dominance of thrombosis in left leg during gestation. On the other hand, after delivery and during puerperium blood hypercoagulability plays a key role in pathogenesis of thrombosis, but little is known about anatomic distribution and extent of venous thrombosis in legs during puerperium.
Aims: To investigate the anatomic distribution and extent of deep vein thrombosis of the lower extremity during pregnancy and puerperium.
Methods: Retrospective analysis of 174 consecutive women with pregnancy related venous thrombosis of lower extremities that were referred to our institution from January 2004 to December 2015 has been performed. The localisation of thrombosis was classified as a thrombosis of left or right leg, proximal, distal or massive (proximal+distal). Thrombosis of superficial veins was excluded. All thrombotic episodes were confirmed with duplex ultrasonography. Descriptive statistics and chi square test were used for statistical analysis.
Results: Out of 174 women with pregnancy related thrombosis, 26 (15,5%) developed thrombosis during the first trimester, 23 (13,3%) during second, 37 (21,2%) during third and 87 (50%) during puerperium. Thrombosis of the left leg developed 127 (73%) women, and of the right leg 40 (23%). Bilateral thrombosis occurred in 7 (4%) of women. Prevalence of left leg thrombosis was 66%, 78%, 83%, 69% during first, second, third trimester and puerperium, respectively. Prevalence of right leg thrombosis was 33%, 13%, 13%, 26%. Left leg thrombosis was more frequent throughout gestation but also during puerperium than thrombosis in right leg (p<0,05). In our group of patients, 68 (39%) developed massive thrombosis, 81 (47%) proximal and 25 (14%) distal thrombosis. Prevalence of massive thrombosis during first, second, third trimester and puerperium was 41%, 30%, 13%, 52% respectively, of isolated proximal veins was 48%, 57%, 84%, 27%, and of distal veins 11%, 13%, 3%, 21%. The difference between different periods of pregnancy and puerperium regarding distribution and extent of thrombosis was statistical significant (p<0,05). Before the delivery most common localization of thrombosis was in proximal veins, while massive thrombosis occurred most frequently in puerperium.
Summary/Conclusions: We observed that deep vein thrombosis of the left leg occurred more frequently than thrombosis of the right leg not only during gestation but also during puerperium. This indicates that significant proportion of thromboses in pueperium might have initiated in last period of gestation and become clinically overt only after delivery. More massive thrombosis observed in puerperium points to importance of blood hypercoagulability on extent of thrombosis.

E1561

A NOVEL ADAMTS13 MUTATION IN A PEDIATRIC PATIENT WITH CONGENITAL AND ACQUIRED ADAMTS13 DEFICIENCY
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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening disorder caused by the deficiency of von Willebrand factor (VWF) cleaving protease, ADAMTS13. Severe ADAMTS13 deficiency (activity levels<10% of normal) can be due either to anti-ADAMTS13 autoantibodies (acquired TTP) or to ADAMTS13 gene mutations (congenital TTP).
Aims: In this report, we describe the novel mutation and clinical history of a pediatric patient with both congenital and acquired ADAMTS13 deficiency.
Methods: A 8 years old girl presented with easy bruising and thrombocytopenia. She was diagnosed with immune thrombocytopenic purpura (ITP) when she was 8 months old and followed with mild thrombocytopenia. Family history was unremarkable. At admission, blood work-up exhibited anemia, thrombocytopenia, fragmented erythrocytes, and schistocytes. Elevated serum LDH was detected. Renal function tests were impaired. TTP was diagnosed and started double volume plasma exchange (PEX) immediately. She was unresponsive to plasma exchange therapy. We used high dose methylprednisolone, acetylcysteine, cyclosporin, rituximab, vincristine and cyclophosphamide therapies unfortunately, there was no response. In spite of splenectomy, clinical status was deteriorated. We continued PEX beside all these therapies. During the treatment she had serious complications related to TTP as partial retina detachment, convulsion, renal failure and myocardial hypertrophy. During the treatment she had to be transfused with 118 units erythrocyte and despite of all efforts she died at the nineth month of TTP attack.
Results: The patient had undetectable ADAMTS13 activity (measured by FRETS-VWF73 assay) and antigen (measured by Technozym ADAMTS13 Antigen assay) and a high titer of anti-ADAMTS13 IgG. ADAMTS13 activity of the patient's mother was 60%. According to these results congenital and acquired ADAMTS13 deficiency seemed to be together in one patient. The genetic analysis identified the presence of a nonsense mutation in homozygous state on the *ADAMTS13* gene, a C to T substitution at nucleotide589 in the exon 6 (c.589C>T) leading to the substitution of the Glutamine 197 with a premature stop codon (p.Gln197X) in the metalloprotease domain in homozygous state. The mutation was also confirmed in heterozygous state in the patient's mother (her father died in an accident years ago).
Summary/Conclusions: This patient is unique with complex phenotype (congenital and acquired ADAMTS13 deficiency together) and novel mutation in ADAMTS13 gene. Similar complex phenotype is reported before and in that case ticlodipine is the trigger factor for acquired TTP. We could not detect any trigger factor for acquired TTP as drugs or infections. We also measure a high titer of anti-ADAMTS13 IgG at admission before the first plasma exchange.
The novel mutation has not been previously reported in the literature and there aren't any data from in vitro expression studies. However, due to its location within the metalloprotease domain, it is most probably responsible for the deficiency of protein product and its activity. This phenotipic complexity and mutation could be a novel type of TTP with severe clinical status and unresponsive to treatment.

E1562

PEDIATRIC VENOUS THROMBOEMBOLISM: A SINGLE CENTRE EXPERIENCE
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Background: Pediatric venous thromboembolism (VTE) and associated complications are rare but increasing. We reviewed our experience of pediatric VTE in 20 years.
Aims: To identify predictors of pediatric VTE, efficacy of treatment and prevalence of complications.
Methods: A retrospective chart review of patients aged 1 to 18 years with VTE was done.Data were collected on demographics, risk factors, thrombophilia work-up, treatment, and relapse. Neonatal and catheter related thrombosis cases were excluded.
Results: Sixty-five pediatric patients (M/F: 1.5) were recruited. Median age at diagnosis was 8.7 years (range: 1 month -17 years). Thrombotic locations were cerebral veins (n=34), lower extremities (n=12), upper extremities (n=7), DVT & pulmonary embolism (n=1), splenic and/or portal veins (n=7), renal vein (n=2), mesenteric veins (n=1), and purpura fulminans (n=1). In 35 patients (53%), a probable acquired risk factor was identified; the most common risks were leukemia, mastoiditis, vasculitis, congenital heart defect and infections. Thrombophilia work-up showed FV Leiden mutation (n=12), low protein C (n=12), high FVIII levels (n=10), low anti-thrombin-3 (n=4), high homocytein level (n=3), prothrombin 20210a mutation (n=3) in 33 patients (50%). Fifty-four patients received anti-coagulant therapy; the majority (n=49) received low molecular weight heparin (LMWH) and acetylsalicylic acid (n=4). Three received warfarin, one was on dabigatran study and one received rivoraxabin. Low molecular weight was given as single dose at a dose of 100 u/kg to all patients. Five had recurrent thrombosis under treatment, one was on dabigatran and the rest were on LMWH. One patient with vasculitis had post-thrombotic syndrome.
Summary/Conclusions: The etiology of pediatric venous thromboembolic disease (VTE) is multifactorial, and in most children, 1 or more clinical and inherited risk factors are present. In our experience low dose LMWH may be used with success in pediatric VTE.

E1563

ANTITHROMBOTIC EFFECTS OF ARGININ-CONTAINING GLYPROLINE
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Background: It has been shown that glyproline peptides have the anticoagulant, fibrinolytic and antiplatelet action in the organism. The decrease of platelet aggregation can be enhanced by adding to the molecule of glyprolines arginine, which causes the release of NO in the bloodstream.
Aims: The aim of this study was to determine the antithrombotic effects of arginine-containing glyproline Pro-Gly-Pro-Arg (PGPR) in rats with experimental venous thrombosis.
Methods: The peptide PGPR was administered to experimental rats intranasally for 4 days in a daily dose of 1 mg/kg. Control animals similarly received saline. Then venous thrombosis (Wessler model) was simulated in rats. The degree of clot formation was assessed by weight of thrombus (mg). The state of the hemostasis system was estimated used standard methods: determination of anticoagulant activity (APTT), fibrinolytic activity on fibrin plates, ADP-induced aggregation of platelets.
Results: It is established that preliminary injection of PGPR lead to decrease of thrombus formation in experimental rats on 60%. Thrombus formation in control rats-100%. Moreover in the blood plasma of experimental animals anticoagulant and fibrinolytic activity were increased by 21% and 30% respectively. Platelet aggregation was decreased at 25% compared with the control.
Summary/Conclusions: Thus arginine-containing glyproline Pro-Gly-Pro-Arg showed anticoagulant, fibrinolytic and antiplatelet properties. Therefore this drug had significant antithrombotic effects, and prevented the thrombus formation in the organism.

E1564

IMPACT ON MOTHER AND NEWBORN OF ANTITHROMBIN III DEFICIENCY DURING PREGNANCY
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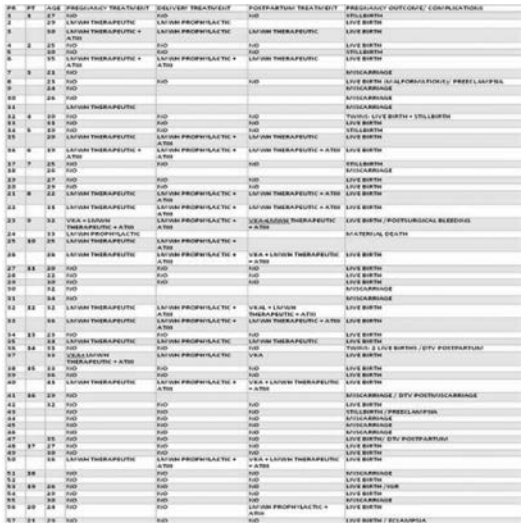
Background: During pregnancy, the risk of suffering thromboembolic disease increases fivefold. The most significant risk factors are a prior history of thrombosis followed by the presence of thrombophilia, with ATIII deficiency associated to a greater risk of thrombosis and complications during pregnancy (including miscarriage, intrauterine growth restriction, fetal death, placental abruption, pre-eclampsia and HELLP syndrome). The ideal treatment for such women remains undefined, given that limited clinical data is available and most cases are reported on an individual basis or on a small scale. It is widely accepted that most of these patients should receive thromboembolic prophylaxis, although the dosage, duration and type of anticoagulant remains a contentious issue.
Aims: Evaluate the impact of antithrombin deficiency and the different treatments in pregnant women.
Methods: Retrospective 30-year study of pregnant women with ATIII deficiency. We analysed a total of 57 pregnancies in 21 women diagnosed with ATIII deficiency (10 due to family history, 2 due to a child having shown the deficiency and the remainder due to personal history). The average age was 29 (19-41). 4 pregnant women displayed cardiovascular risk factors (1 high blood pressure, 1 smoker, 1 dyslipidemia and the other a smoker plus sufferer of dyslipidemia) and 8 had additional thrombophilia (6 with heterozygous Factor V Leiden, one protein S deficiency and one combined homozygous factor XII deficiency and heterozygous Factor V Leiden).
Results: Of the 57 pregnant women, 2 of whom were carrying twins, 38 received no treatment as the antithrombin III deficiency was diagnosed at a later date. From this group, 22 resulted in live births, 12 miscarried, 6 suffered an intrauterine stillbirth and 2 experienced intrauterine growth retardation. In terms of maternal complications that went untreated, 2 women developed pre-eclampsia and there were 5 thrombotic events (2 pulmonary thromboembolisms, one of which with sinus-related thrombosis and 4 deep vein thromboses). As part of 6 pregnancies, patients received Acenocumarol due to a history of thromboses. 19 pregnant women received treatment: 15 low molecular weight heparin (LMWH) in therapeutic doses subject to Anti Xa control (6 received Acenocumarol previously due to a history of thromboses), 1 LMWH in intermediate doses and 3 in prophylactic doses. At the same time, ATIII 50UI/kg/72 hours was administered to 4 patients and 2 took Acenocumarol from week 12. During the peripartum period, two received LMWH, another ATIII and 13 ATIII linked to LMWH in prophylactic doses. Post-partum, 9 patients received Acenocumarol (6 having received bridging treatment via ATIII and LMWH and 3 just via LMWH) and 10 LMWH (5 of which with ATIII). From amongst the 19 pregnancies as part of which treatment was administered, 17 resulted in live births. Complications recorded included one pre-eclampsia, one miscarriage and one maternal death due to severe sinus thrombosis, all three of which occurred in patients receiving a prophylactic dose of Heparin. The only adverse effect of the treatment involved bleeding from a caesarean wound in one of the patients receiving ATIII and LMWH. 10 received an epidural with no adverse effects.

Table 1.



Summary/Conclusions: In our experience, the rate of maternal-fetal complications in patients with an ATIII deficiency is clearly higher than amongst the general population; furthermore, there is a higher number of adverse effects in the group receiving no treatment. Although in the series of cases in hand, therapy using LMWH and/or ATIII has been demonstrated as an effective and safe strategy, these patients must be subject to strict monitoring, as complications can occur throughout the pregnancy and post-partum period, particularly in the case of inadequate thromboprophylaxis. Further research is needed to establish and standardise action protocols.

E1565

REVIEW OF PATIENTS WITH CEREBRAL SINUS THROMBOSIS OVER A 7 YEAR PERIOD: SINGLE CENTRE EXPERIENCE FROM A UNIVERSITY HOSPITAL IN THE UNITED KINGDOM

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Background: Cerebral sinus venous thrombosis (CVST) is a rare form of venous thromboembolism, usually affecting younger individuals. It has a wide range of clinical presentation and sometimes difficult to diagnose.
Aims: We present a retrospective review of 83 patients who were diagnosed and treated for CVST over a 7 year period at a University hospital in the United Kingdom
Methods: Electronic clinical records of all patients who were diagnosed and treated for CVST between 1st January 2008 and 31st December 2014 were reviewed.
Results: Eighty three patients (45 females, 38 males) with median age 35 years (range between 2 months - 80 years) were diagnosed and treated for CVST during this period. Headache was the most common presentation reported by 70% patients, followed by seizures (19%), motor deficit & aphasia (18%), altered sensorium (13%), confusion (7%), photophobia(6%), and cranial nerve palsy(5%). Twenty five (30%) of patients had intracranial bleed at the time of presentation. Diagnosis of CVST was confirmed by contrast head CT (14%), CT venogram (54%), contrast brain MR (16%) and MR venogram (20%). Twenty patients (24%) developed CVST during pregnancy, puerpurium or due to oral contraceptive use. Cancer (glioblastoma 1, meningioma 4, acute leukaemia 3, other cancers 8), neurosurgery (n=11), head injury (n=6) and ENT infections (n=9) were provoking factors in (50%) of the patients. No provocation could be identified in 16 patients (19%). The commonest affected site involved was the transverse sinus (62%) followed by sigmoid sinus (51%) and superior saggital sinus (44%). In over half of the patients (n=47) more than one sinus was involved with thrombosis. Patients were treated with anticoagulation (83%), hemi-craniotomy (4%), endovascular surgery (3%). No anticoagulation was given in 14 (16%) patients. After a follow-up of 24-84months, 54% of patients had a complete recovery. Residual neurologic impairment was seen in 25 patients (intracranial HT, persistent headaches, secondary epilepsy). Thirteen patients died (median age =57, range14-71 years). Cause of death was unrelated to CVST in 10 patients (progressive cancer 9; cerebral abscess 1). Adequate follow up clinical information was not available for 5 patients
Summary/Conclusions: CVST is a rare site thrombosis usually affects younger individuals and has a varied clinical presentation. Clinician should have a low index of suspicion to ensure prompt diagnosis and effective treatment. With modern management majority of patients can expect a complete recovery with no residual sequelae.

Transfusion medicine

E1566

WHAT DO FINAL YEAR MEDICAL STUDENTS KNOW ABOUT TRANSFUSION MEDICINE?

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Background: Blood component transfusion is a common procedure performed among different specialities. Every year transfusion errors are reported as cause of mortality and major morbidities. To improve the safety of the procedure the staff involved have to be adequately trained. Including transfusion training in medical school can improve patient safety. In UK most medical schools undergraduate curriculum includes transfusion related topics, but there are no national survey to assess the quality of teaching or the knowledge of the students.
Aims: To investigate the knowledge of final year medical students on transfusion medicine topics and evaluate if interprofessional teaching delivered with the collaboration of a transfusion practitioner and the pathology laboratory can improve their knowledge.
Methods: Topics relevant to transfusion safety were extrapolated analysing SHOT reports. Nineteen final year medical students were offered a two hours teaching involving case based discussion, visit to the transfusion laboratory and application of the knowledge acquired through simulation. The teaching session was delivered by the local haematology teaching fellow with the help of a transfusion practitioner and the collaboration of the transfusion laboratory. Students' knowledge was tested before and after the teaching session with an open answer test.
Results: There was a significant improvement between the average score in the pre-teaching and post-teaching test (36% *vs* 73%). Topics where the difference in the percentage of students who were able to answer correctly was more evident where: patient monitoring (0% *vs* 94%), indication for irradiated products (0% *vs* 78%), blood component prescriptions (5% *vs* 100%), acute transfusion reactions (16% *vs* 94%). Improvement of knowledge was observed also for: delayed transfusion reactions (0% *vs* 44%), understanding of FFP compatibility (0% *vs* 44%) and understanding of group and save test (0% *vs* 44%), interestingly in the group that had practical teaching in the transfusion laboratory on this topic the rate of correct answer was 100%.
Summary/Conclusions: Transfusion medicine topics are covered during medical school, however when tested, this group of final year medical students were unable to answer correctly to question about essential practical aspects of the transfusion process. Improving education in transfusion medicine for medical students will help them in their future role as doctors and can improve patient safety. A short teaching session in collaboration with transfusion practitioner and transfusion laboratory was an effective way to improve students short term knowledge. Further studies are required to find the best way to improve short term and long term knowledge on transfusion medicine.

E1567

BLOOD TRANSFUSION IN CARDIAC SURGERY IS ASSOCIATED WITH WORSE PROGNOSIS

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Background: Transfusion has been associated with a worse prognosis in patients undergoing cardiac surgery so restrictive attitude to transfuse had been proposed.
Aims: In order to assess the impact of transfusion in patients undergoing cardiac surgery we reviewed patients admitted to cardiac ICU.
Methods: Data concerning age, sex, reason for ICU admission, type of surgery, transfusion, preoperative haemoglobin, length of stay in the ICU, ICU survival, EUROSCORE prior to surgery and extracorporeal time from patients admitted in cardiac ICU of our hospital between January 2004 and June 2014 were collected. Univariate analysis with different variables and then a logistic regression model was
Results: We analysed data from 4,315 patients (63% male / 37% female) admitted to the unit during this period. Median age of 67.88 years (0-91 years). 93% were postoperative cardiac surgery. The most common reasons for surgery were valvular surgery (34.5%) coronary surgery (28%), coronary and valve surgery (18%). 3282 of patients (76.1%) were transfused. Patients undergoing valvular surgery were most frequently transfused than those who underwent coronary surgery (77% *versus* 66%). Transfused patients had lower haemoglobin at the time of surgery than those who were transfused (12.7±1.7 *versus* 14, 51±1.33, p<0.001). Of the total patients analysed, 23.7% had Hb<12 g / dl at the time of surgery. Of these, 97% were transfused compared to 69%

who had Hb>12 g / dl (p<0.001). Patients with Hb level>12 g / dl had a survival of 95% *versus* 88% in the group with Hb<12 g / dl (p<0.001). 97% of non-transfused patients survive *versus* 88% of transfused patients (p<0.001) with a relative risk of survival of 3.87 (95% CI 2.67-5,99) within the group without transfusion. For most common types of surgery:

Table 1.

			survival		OR (IC95%) survival
coronary surgery	Transfusion	yes	94%	P < 0.001	
		no	99%		8,26 (2.12-32.15
Valvular surgery	Transfusion	yes	92%	P < 0.001	
		no	99%		20.8 (2.9-144)
Valvular + coronary surgery	Transfusion	yes	92%	P < 0.001	
		no	98%		3.83 (0.97-15)

Patients who were transfused had a shorter average length of stay in the ICU than those who were not (8.46±3.95 days *versus* 15±3.4, p<0.001)
In the logistic regression analysis, the only variable that showed significant for survival was transfusion.
Summary/Conclusions: Transfusion is associated with a negative effect on the survival of patients undergoing cardiac surgery, 24% of patients undergoing cardiac surgery have anaemia at surgery. Patients with a better hemoglobin prior to surgery have a lower risk of being transfused so it must be necessary to establish measures to improve the haemoglobin prior to cardiac surgery in order to improve outcome of this patients.

E1568

TRANSFUSION PRACTICES USED IN TERMINAL STAGE -CANCER-PATIENTS
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Background: Despite the increased attention to quality palliative care, dying of oncology patients often take place in the ward or intensive care unit rather than hospice and with an intensive level of medical care.
Aims: To investigate the transfusion practises for the terminally ill cancer patients as part of their medical care during the last seven days of life.
Methods: We conducted a retrospective review of the causes of consecutive deaths oncology patients who succumbed in two Oncology Hospitals between April 2015 and June 2015. Patients were categorized as: solid tumor-patients, haematological malignancy-patients and no cancer-patients. We recorded the pretransfusion tests performed for these patients (ABO grouping, detection and identification tests for unexpected alloantibodies, crossmatching), as well as the transfusion episodes per patient and the number of transfused units per transfusion episode, during the last seven days of life. X^2-test and one-way ANOVA tests were used for the statistical analysis. The number of transfused platelets corresponds to equivalent number of platelets random-donor.
Results: 211 patients [116 males (55%) and 93 females (45%)] of mean age 67 years old (range 18-92) had died during the study period. Of them, 163 (77.25%) were solid tumor-patients, 11 (5.22%) were haematological malignancy-patients and 37 (17.53%) were no cancer-patients. The number of pretransfusion tests performed and blood product units transfused, are shown in the Table.

Table 1.

	TOTAL	NO CANCER	SOLID TUMORS	HAEMATOLOGY MALIGNANCIES	p-value
PATIENTS	211	37	163	11	
PRETRANSFUSION TESTS	143 (67,7%)	17 (45,9%)	115 (70,6%)	11 (100%)	0,01
RED BLOOD CELL TRANSFUSIONS					
Patients (%)	54 (25,6%)	9 (24,3%)	37 (22,7%)	8 (72,7%)	0,02
RBC units (total)	125	18 (14,4%)	86 (68,8%)	21 (16,8%)	NS
Episodes/patient		2	1,94	2,1	NS
RBC units/patient		2	2,32	2,62	NS
RBC units/episode		1,03	1,09	1,25	NS
PLATELET TRANSFUSIONS					
Patients (%)	20 (9,5%)	0 (0%)	12 (7,36%)	8 (72,7%)	0,001
PLT units (total)	295	0	157(46,4%)	158 (53,6%)	NS
Episodes/patient		0	2,25	4,66	0,013
PLT units/patient		0	13,41	19,75	NS
PLT units/episode		0	4,66	4,26	NS
FRESH FROZEN PLASMA TRANSFUSIONS					
Patients (%)	23 (9,95%)	2 (5,4%)	17 (10,43%)	2 (18,1%)	—
FFP units (total)	122	20 (16,4%)	93 (76,23%)	9 (7,37%)	NS
Episodes/patient		3,5	2,88	2,5	NS
FFP units/patient		6	5,47	10	NS
FFP units/episode		2,9	3,02	2,25	NS

Summary/Conclusions: 1. Blood transfusion practices remained intensive in terminal stage-cancer-patients and does not seem to be different compared to non-cancer patients. 2. Patients with haematological malignancies had even more intensive transfusion practises especially regarding the platelet- transfusion- episodes. 3. Cooperation with local hospital Transfusion Medicine Committee and the Ethics Committee, and proper allocation of medical resources could result in providing the optimal end-of-life care for cancer patients. The blood is a valuable but limited source. The health services have the duty to achieve both the maximum "beneficity" by transfusing blood products to the terminally ill patients, and "justice" by not depriving other patients of necessary transfusions.

E1569

THE SHP-2/ERK2-MEDIATED SIGNALING REGULATES BRANCHED I FORMATION BY CONTROLLING THE C/EBPA BINDING TO IGNTC PROMOTER REGION IN THE ERYTHROID-DIFFERENTIATION
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Background: The straight and branched repeats of poly-LacNAc chains characterize the histo-blood group i and I antigens, respectively. In the human hematopoietic cell model, the transcription factor CCAAT/enhancer binding protein a (C/EBPa) regulates branched I antigen formation through *IGnTC* expression in both erythroid- and granulocytic-differentiation. However, the detail mechanisms for the regulation on the I synthesis along these different lineages remain unclear.
Aims: To investigate the regulatory mechanisms for branched I antigen formation during the erythroid- and granulocytic-differentiation.
Methods: The sodium butyrate (SB)- and retinoic acid (RA)-induced differentiated K-562 cells were served as the erythroid- and granulocytic-differentiation model, respectively. The wild type and mutant form of ERK2 and SHP-2-overexpressing K-562 cells were used to study the involvement of MAP kinase-mediated pathways and upstream signaling molecule in the I antigen formation. CHIP analysis was used to compare the binding affinity of C/EBPa to *IGnTC* promoter.
Results: The ERK2-mediated signaling specifically regulated the synthesis of I antigens during the erythroid-differentiation, but not for granulocytic-differentiation. SHP-2 acted as upstream regulator of ERK2 to control the phosphorylation status of Ser21 on C/EBPa, which affect the binding affinity to *IGnTC* regulatory region, and to govern the I antigen formation.
Summary/Conclusions: In this K-562 cell model when induced by SB, the SHP-2-ERK2 signaling is specific for the branched poly-LacNAc formation during erythroid-differentiation.

E1570

PEGYLATED CARBOXYHEMOGLOBIN BOVINE EXHIBITS SUPERIOR RESUSCITATIVE CAPACITY IN RODENT MODEL OF SEVERE HEMORRHAGE
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Background: PEGylated Carboxyhemoglobin bovine (PEG-HbCO) is a novel biological therapy designed to release carbon monoxide and then transfer oxygen to hypoxic tissue. Prior *in vitro* cell culture and *in vivo* animal models including stroke and myocardial infarction have demonstrated the PEG-HbCO exhibits both anti-inflammatory and anti-hypoxia activities. Severe hemorrhage results in rapid hypotension and tissue hypoxia. Fluid restoration and pressors are standard of care that positively impact blood pressure and circulatory volume, but unfortunately they have minimal impact towards resolution of tissue hypoxia and systemic inflammation resulting from hemorrhagic shock.
Aims: A rat model of severe hemorrhage was developed to evaluate the effects of PEG-HbCO on cardiovascular and respiratory macro function in comparison to the colloid volume expander, Hetastarch. Additionally, microscopic measurements of microcirculation and tissue oxygenation were compared between the two resuscitative agents as well as circulating markers of inflammation and hypoxia.
Methods: Male Sprague-Dawley rats underwent a controlled 45% blood volume hemorrhage at 3.5 ml x min^{-1} x kg^{-1} through a cannulated carotid artery and were maintained in a hypovolemic, hemorrhagic shock phase for 30 minutes. Resuscitation fluids were infused at a rate of 3.5 ml x min^{-1} x kg^{-1} to a volume equal to 20% of the total estimated blood volume through a cannulated jugular vein. Systemic measurements were recorded via a cannulated femoral artery that was connected to a pressure transducer while microcirculatory parameters were collected through phosphorescence quenching and intravital microscopic examination of the exteriorized spinotrapezius muscle.
Results: Compared to baseline, the 45% hemorrhage produced a significant reduction in heart rate, blood pressure, arterial diameter and interstitial fluid oxygen partial pressure (ISF PO_2). Resuscitation with either PEG-HbCO or Hetastarch improved animal heart rate and mean arterial pressure systemic parameters towards pre-conditional baseline levels, but only PEG-HbCO -treated animals showed an improvement in ISF PO_2. The impact of improved systemic variables was evident in mortality with untreated animals (sham) expiring an hour after hemorrhage, while Hetastarch resuscitated animals expired after 4 hours. PEG-HbCO animals survived for the entire 8 hour observation period. In addition to extended survival times, PEG-HbCO animals showed steady systemic and microcirculatory parameters. Hetastarch resuscitated animals, how-

ever, developed rigor mortis in their limbs at approximately 3 hours post-hemorrhage, which was concurrent with a rapid decline in systemic variables. Additionally, only PEG-HbCO yielded reduction in hypoxia and inflammatory gene expression levels as compared to Hetastarch and sham control animals.
Summary/Conclusions: Systemic hypoxia is a major factor in trauma-related mortality and early intervention can improve survival rates. This study showed that PEG-HbCO has a unique capacity to restore macro and microcirculatory functions in addition to reducing inflammation and tissue hypoxia. Moreover, reestablishing blood pressure is acutely important for post-hemorrhage survival and restoring oxygen delivery to peripheral tissues is critical for improving long-term outcomes.

E1571

Abstract withdrawn.

E1572

FIVE YEARS OF HEMOVIGILANCE REPORTS OF COMPLICATIONS OF THE BLOOD DONATION REPORTED AT A TERTIARY CARE CENTRE IN KARACHI

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Background: There is a minor chance of risk among blood donors. Even though blood donors are usually screened for the presence of risk factors, sometimes blood donations can put a person at panic.
Aims: The safety of the blood supply depends on the actions to protect both; blood transfusion recipient and the blood donor. Hemovigilance practice of learning of complications of blood donation and protecting them from such complications is the best way to minimize the risk to blood donor.
Methods: Comprehensive blood donor hemovigilance program was studied at Dr. Ishrat ul Ebad Khan Institute of blood diseases, Karachi from 2010 to 2015. Outlines of reported and communicated complications were collected after whole blood donation. Analysis was done by general logistic regression.
Results: Complications after 30,000 Whole blood donation procedures calculated 1620 total. (54 per 1,000 donations). The majority of the complications were faint and pre-faint reaction with light headedness (58.6%), Sore arm (24%), Bruises and hematoma (14.4%). Minor complications were Agitation/sweating (2%) and arterial puncture (1%). Markers of the complications were age, sex, race, weight, blood pressure and donation status. All associated independently after whole blood donation. Age and first-time status were associated with a significantly higher risk of complications with 18-22 years old at higher risk compared to 23 to 50 years old. First-time donor were at higher risk compared to repeat donor.
Summary/Conclusions: The results of this study are helpful in identifying and understanding the promoter to complication of blood donation. Donor age and status were strong predictors of complications. The remedies and specific areas of care should be provided.

E1573

A NEW METHOD FOR ACTIVATING PLATELETS IN PLATELET-RICH PLASMA TO USE IN REGENERATIVE MEDICINE: A CYCLE OF FREEZING AND THAWING

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Background: Platelet-rich plasma (PRP) is a platelet-derived product, which exhibits regenerative properties and have been widely used in regenerative medicine. Platelet-rich plasma can be used with or without previous platelet activation, for what purpose is to be used. There are different ways of activating platelets. Activating platelets by freezing cycles is a new, not widely known and applied method.
Aims: The objective of the study was to assess whether and to which amount a cycle of freezing/thawing may affect the amount of growth factors in autologous platelet-rich plasma.
Methods: Fifteen ml of whole blood was obtained by venipuncture and collected in two citrated tubes from 20 healthy, male volunteer donors to obtain PRP. After preparation of PRP with standard methods, they were divided into two groups. First group was activated by adding 10% calcium gluconate to finalize PRP with volume ratio of 1:10. Second group was cryopreserved at -80°C for 24 hours. Of the obtained PRP, insulin-like growth factor-1 (IGF-1) platelet-derived growth factor (PDGF-BB), basic fibroblast growth factor (bFGF) concentrations were measures using the ELISA kit following the manufacturer's instructions.
Results: The mean platelet count of the donors and the autologous PRPs were 238,5±44,7 x 10^3 / µl and 544,7±161,5 x 10^3 / µl, respectively. Overall, there was an increase in the concentration of the insulin-like growth factor-1 (IGF-1) platelet-derived growth factor (PDGF-BB) and basic fibroblast growth factor (bFGF) after a cycle of freezing and thawing. But only the increase in PDGF levels were significant (218,90±30 and 78,95±49,42 respectively; p: 0,0001). There was not a statistically significant difference between the GF levels of freeze thawed and calcium activated PRPs. A cycle of freezing/thawing was the only independent factor associated with growth factor yield in multivariate model. In summary overall, there was an increase in the concentration of the three GFs, but only PDGF was significant. There was not a significant difference between the GF levels of post-freezed and calcium activated PRP. A cycle of freezing/thawing was the only independent factor associated with growth factor yield in the multivariate model.
Summary/Conclusions: With its features of being simple, inexpensive and easy for standardization; a cycle of freezing/thawing may be the method of choice in the future for PRP activation procedure, especially for obtaining PRPs rich in PDGF, without inducing fibrin matrix.
This study is done with the support of the Turkish Society of Hematology, with the approval number of 2014-8.

E1574

PERFORMING ELUTION TEST WITH MONOSPECIFIC ANTIHUMAN GLOBULIN UNMASKS MULTIPLE IMMUNOGLOBULIN COATING; IMPLICATIONS ON IMPROVING MULTIPLE IMMUNOGLOBULIN DETECTION SENSITIVITY IN AIHA SCREENING

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Background: Autoimmune Haemolytic Anemia (AIHA) basic screening includes: (i) Direct Antiglobulin Test (DAT) using monospecific antihuman globulin (MAHG), namely anti-IgG, anti-IgA, anti-IgM, anti-C3c and anti-C3d, to detect the autoantibody immunoglobulin (Ig) class(es) or complement fragments on erythrocyte membrane; (ii) Indirect Antiglobulin Test (IAT) using polyspecific antihuman globulin (PAHG), comprised of anti-IgG and anti-C3d, to identify autoantibody(ies) in serum or red blood cells (RBCs) elution. Current elution techniques are valuable for allowing the identification of antibody specificity and the elimination of false positive DAT cases. The use of only anti-IgG in IAT implies that: (a) Non-IgG classes causing AIHA will be missed in elution test, rendering false negative results, (b) Elution test in AIHA caused by multiple Ig classes would allow non-IgG classes to be missed, generating misleading results.
Aims: To highlight that in DAT (+) RBCs coated with multiple Igs, DAT is not sufficient to unmask all the putative Ig classes and to suggest a method that potently unmasks multiple Ig coating.
Methods: Acid elution (DiaCidel/BIO-RAD) was performed on 59 patients' DAT (+) RBCs. The eluates were screened by IAT against a commercially available panel of RBCs (ID-DiaPanel) using PAHG Liss/Coombs gel cards (Biorad, Switzerland). An additional method using MAHG gel cards (DC-Screening I/BIO-RAD) in elution IAT was also applied. Seven patients were excluded from the study since their DAT positivity was due to alloantibodies following recent transfusion or non-specific reactions. Of the remaining 52 patients, only 21 (40.38%) fulfilled the diagnostic criteria for autoimmune haemolysis; 17 presented with AIHA and 4 with Evans syndrome. DAT positivity without haemolysis was observed in 31 (59.61%) patients, representing a random finding during pretransfusion testing.
Results: Initial screening of blood samples by DAT identified 49 warm and 2 cold autoantibodies, as well as one mixed type autoantibody. When AIHA was caused by multiple Ig classes, the use of MAHG in elution IAT allowed the identification of Ig classes that were not detected by the standard DAT. In detail, six DAT (+) and IgM (-) cases by DAT were identified as IgM (+) by elution IAT; three IgG (-) DAT (+) cases were found to be IgG (+) by elution IAT; and five DAT (+) IgA (-) cases by DAT were identified as IgA (+) by elution IAT. Overall, in 14 out of 52 cases (26.92%), the DAT missed to identify multiple Igs coating. Even though elution IAT with PAHG would identify the three IgG (-) DAT (+) cases, still 11 cases with multiple Igs (21.15%) would have been missed, a statistically significant difference (p=0.012). It is notable that 11 of the 14 missed cases (78.57%) presented with severe autoimmune haemolysis.
Summary/Conclusions: In cases of AIHA caused by multiple Ig classes, we assume that monospecific antiserums in DAT bind mainly with the dominant Ig class due to the high concentration of bound antibodies on the RBC membrane (like the prozone phenomenon). This constraint is bypassed when Igs are discarded from the RBC membrane in elution, since they are available to react with PAHG by IAT. However, the latter applies only for IgGs, since non-IgGs are not detectable by PAHG. In conclusion, we report an efficient method for unmasking non-IgGs participating in multiple Ig coating, i.e., the use of MAHG

in IAT elution. This method may have clinical implications, because AIHA is frequently severe when multiple Ig coating occurs.

E1575

BIOTINYLATION OF ERYTHROCYTES FOR CLINICAL RESEARCH

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Background: Within Sanquin there are currently several clinical transfusion protocols in development or already realized, where the objective is to assess the efficacy and safety of erythrocyte transfusions in certain categories of patients (MDS patients, IC patients). This question arises from the fact that up to 25% of the donor erythrocytes are cleared within 24 hours after a transfusion, a phenomenon that is becoming more and more linked to side effects of transfusion in the receiver. The mechanisms that ensure that erythrocytes are cleared are to a large extent still unclear. Identification of the routes that lead to the clearance of erythrocytes are especially important for the transfusion practice.
Aims: The goal is therefore to develop a standardized labelled blood product (GMP) that can be used for clinical research and evaluation of new blood products.
Methods: The yield after transfusion is difficult to measure. Although autologous red blood cells (RBCs) have a different genetic make-up than the transfused products, it is difficult to distinguish donor from recipient RBCs. Previously, donor erythrocytes were labelled with Chromium-51, a radioactive label a procedure that is not allowed in the Netherlands. Another option is to discriminate on the basis of differences in minor blood group antigens. This, however, is laborious and not always possible. A third possibility is to label erythrocytes with biotin, also known as vitamin B8. The advantage of labelling with biotin is that different concentrations of biotin labelling can be used, allowing the detection of several RBC transfusions in 1 patient.
Results: Former studies have shown that labelling with biotin is safe, also upon multiple exposures. We found that labelling with biotin does not affect the life span or function of the RBCs, and remains stable over time, also when the erythrocyte concentrate (EC) needs to be irradiated to prevent Graft *vs* Host disease. Furthermore, labeling with different densities of biotin gives reliable identification of different populations of donor RBCs. However, about 12,5% of healthy volunteers may develop antibodies against biotin, without clinical consequences, and resolve spontaneously within a year time. Lastly, biotin can be stored in different concentrations, in bags that are compatible with blood bags, without loss of quality, and with preservation of properties.
Summary/Conclusions: Having arrived at the end of the validation study, it shows that EC that are labeled with biotin, can be produced in a standardized manner under GMP conditions, and can be used for clinical research and evaluation of new blood products.

E1576

KNOWLEDGE, ATTITUDE AND PRACTICE ABOUT THE VOLUNTARY BLOOD DONATION AMONG THE YOUNG STUDENT POPULATION OF KARACHI

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Background: Safe blood is a crucial and irreplaceable component in the medical management of many diseases. The Voluntary non-remunerated blood donation is the ideal sources of quality blood, which forms less than 15% of the demand of the blood in Pakistan. Motivation among the youth, particularly students, is essential to make voluntary blood movement more successful.
Aims: To assess the knowledge, attitude and practice regarding the voluntary blood donation among the young student population of Karachi so that an effective approach can be made regarding motivation enrollment of voluntary non remunerated blood donors in future in Pakistan
Methods: A cross sectional prospective study was conducted among 600 students from different universities and colleges of Karachi. A well-structured and pre-tested questionnaire, in English, was used to access the knowledge, attitudes and practices about voluntary blood donation. A scoring mechanism was used to understand overall knowledge level. The participants were given a briefing about the objectives of the study and confidentiality about the personal data. Obtained data was analyzed by using statistical package of social sciences (SPSS) version 17.0 in computer. Statistical significance level was set at p=0.05.
Results: The sample population consisted of 54% male and 46% female students in the age group of 18-28 years. Only 65% of the students have heard about voluntary blood donation and 28% of the students have given blood once in their lifetime and among them 19% are blood donors at the moment. 42% of the participants believed that there is a specific reason why they don't donate blood and 59% believed that there is a risk involved for the donors, when donating blood. 80% students wanted to promote voluntary blood donation. Fear and lack of awareness on blood donation are the reasons for not donating blood. Students gather information about voluntary blood donation from several sources mostly schools, colleges, family and friends.
Summary/Conclusions: This study showed that myths and misconceptions are leading the youngsters not to donate blood. Study also showed how increasing awareness and marketing through different ways can boost the culture of voluntary blood donation in society. Student population can be motivated to participate in different ways. There is a dire need to mobilize the electronic media for educating our youth about voluntary blood donation due to its access to masses.

E1577

CURRENT STATUS OF INCIDENTS CAUSED BY HEMOSIDEROSIS IN PATIENTS WITH HEMATOLOGIC DISEASES: RESULTS FROM SPANISH HAEMOVIGILANCE SYSTEM

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Background: The Blood Safety System (Haemovigilance - HV) enables to collect and analyze incidents related to donation, preparation of blood components and transfusion. The purpose is to know the complications and adverse reactions occurred during the transfusion process, to establish effective control measures that can prevent these events. HV should cover the entire transfusion chain. Among transfusion-related complications, the Hemosiderosis is an incident characterized by a very low level of reporting. We have analyzed Hemosiderosis at national state and inside our CCAA (Andalusia). Post-transfusion hemosiderosis is a frequent complication of hematologic diseases, as well as part of their treatment. It is defined as the accumulation of iron in organs and tissues in patients who are receiving regular transfusions of packed red blood cells. Malignant hematological diseases will require regularly transfusions, iron overload, therefore, will be a common side effect in these patients. It is directly related to the number of transfusions received. After transfusing 10 bags, iron is deposited in tissues, after 20, the risk of developing a secondary hemochromatosis increases.
Aims: We intend to investigate the percentage of hemosiderosis reports to the Spanish Haemovigilance system. In the European Union, there are various HV systems, to note: French Haemovigilance system: Is governmental, with a complex structure, notification of all adverse effects and mandatory reporting. English Haemovigilance system (SHOT): notification of serious adverse effects and voluntary reporting. Spanish system (HV): a simpler structure model, voluntary reporting of adverse events and reactions. Notifications are virtually nonexistent except in the French system. Although reporting of post-transfusion hemosiderosis is mandatory in the French haemovigilance network since 1994, existing data is limited.
Methods: We reviewed the transfusion incidents caused by iron overload which have been reported to the Spanish HV system and the Andalusia HV system of, comparing them with the ones notified by our hospital from 2013 to 2015 and their underlying hematologic diseases. Cases in Spanish HV system: 2007: 3. 2008: 15. 2009: 10. 2010: 10. 2011: 16. 2012: 10. 2013: 88. Andalusia: 2007-2012: 0. 2013: 71. (Figure 1).
Results: In 2013 we identified 57 cases, 32 men and 25 women, from 20 to 87 years old, average of 56, who have been transfused with packed red blood cells. The results vary from 10 CH to 73 CH transfused (average of 25 CH per patient). Postransfusional ferritin levels were over 1000 mg/l (average of 2869 mg/l). In 2014 we have notified 76 cases, 50 men and 26 women, from 24 to 83 years old) (average of 56). The number of CH transfused varied from 10 to 130 (average of 31 CH). The number of post-transfusion ferritin ranged between 1,041 and 15,190 mg/l. In 2015, 42 cases have been reported: 18 women and 24 men, between the ages of 17 and 81, with an average of 51 years old. The number of CH transfused ranged from 10 to 107 (average of 33.3 CH). Post-transfusion ferritin ranged between 1.274 and 8.858.4 mg/l. Firstly, the largest number of hyperferritinemia cases was found in patients with acute myeloid leukemia. Secondly, in patients with monoclonal gammopathies found. And thirdly, in patients with non-Hodgkin lymphoma. Other diagnoses were myelodysplastic syndrome, chronic lymphocytic leukemia etc. (Figure 2).

Figure 1.

Summary/Conclusions: The most frequent cause of hemosiderosis secondary to transfusion in our hospital has been acute leukemia, with a predominance of acute myeloid leukemia over acute lymphoblastic leukemia. It is very important to properly track ferritin levels in patients undergoing multiple transfusions in order to establish chelation therapy when necessary and to prevent the organic damage of secondary hemochromatosis. In the results published by the Andalusian HV system in 2013, we can highlight the increase of notifications

of post-transfusion hemosiderosis to the Hemovigilance system, as well as the increasing number of near-miss reported in 2013. This is valued as an improvement in the notification of such events. Communication of Hemosiderosis cases to the Hemovigilance System helps to create protocols for polytransfused patients because of hematologic diseases. Hemovigilance in Spain is, today, a fully integrated tool within the activities carried out by Transfusion Hospital Centers and Services. Among the remaining challenges we highlight: Getting a more uniform level of notification and to Advance in the optimal use of blood and blood components: safe, effective and efficient.

E1578

USE OF OCTAPLASLG IN THE TREATMENT OF THROMBOTIC MICROANGIOPATHIES (TMA)

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare and acute disorder and plasma exchange (PEX) remains the life-saving therapeutic procedure in patients with TTP and other thrombotic microangiopathic anemias (TMA). OctaplasLG (Octapharma), a virally inactivated prion reducing plasma, has replaced the Solvent-Detergent Fresh-Frozen Plasma (Octaplas®; Octapharma) to improve the safety of plasma.
Aims: Aim of this study was to assess safety of OctaplasLG administration for patients undergoing PEX.
Methods: We performed a prospective study of 90 patients, treated with PEX for TMAs from a single institution over a 36 month period, January 1st 2013-December 31st 2015. We recorded: virology on admission and in remission for all patients, allergic reactions to plasma, citrate toxicities associated with PEX, venous thrombotic events (VTE) rates and incidence of central venous access (CVA) line sepsis (which can result in exacerbation of TTP).
Results: Over the 36-month period, a total of 981 PEX procedures were performed (median 9, range 0-37). 62 patient episodes were TTP: 45 females (median age: 47 years; range: 15-89) and 17 males (median age: 49 years; range: 21-79); 51 patient episodes were De Novo presentations (43 idiopathic, 5 HIV, 1 Congenital and 2 Pancreatitis) and 11 were relapsed presentations. 17 patient episodes were HUS / aHUS, of which 12 females and 5 males. 11 patient episodes were 'Other TMAs' (8 females and 3 males). Citrate toxicity was 5.3% of PEX, (facial and limb tingling). Plasma reactions were 1.7% of PEX (hives and facial swelling) relieved by anti-histamines. No episodes of anaphylaxis and no TRALI were reported. Two cases of line associated sepsis were recorded and 11 cases of VTE were diagnosed (5 PE; day of PE (range): 5-35; platelets count (range): 35-228; 6 DVT; day of DVT (range): 6-43; platelets count (range): 86-264). Virology was checked for all patients. On admission for Hepatitis B they were subdivided into different groups: 57 patients had no evidence of infection or immunisation, 12 patients had evidence of previous immunisation and 9 patients had previous evidence of Hepatitis B infection. No viral transfer was documented following treatment. HCV Ab on admission: 89 negative, 1 positive. There was no antibody transfer post treatment. Pre PEX HIV: 5 positive, 85 negative. No seroconversion was detected post treatment.
Summary/Conclusions: Citrate toxicities are reduced in severity, likely due to shortened PEX with the Optya. Reactions with Octaplas remain minimal, with no severe anaphylaxis noted. VTE events, despite prophylactic LMWH starting when platelets are >50x10^9/L, occurred in 12% of cases, with 50% completed PEX when VTE was recorded. Adherence to our CVA line policy has resulted in a further reduction to our line related infections. In plasma high volume users, virology is important on admission to establish viral profile of the patients and in the follow-up for checking eventual infectious transmission. To date no viral transfer or antibody transfer or seroconversions were reported post treatment, likely due to safety of OctaplasLG.

E1579

DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA ASSOCIATED WITH ROMIPLOSTIN

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Background: Drug-induced immune hemolytic anemia (DIIHA) is a rare condition with an estimated incidence of about one per million patients per year. The exposure to a specific drug induces a sudden drop in circulating red blood cells (RBCs). Many drugs have been implicated in causing red cell (RCBs) destruction, with the most common being second and third generation cephalosporins. The initial serologic presentation is not fully appreciated, causing the disorder to be underdiagnosed. Romiplostim is a thrombopoietin receptor agonist approved for adult patients with chronic immune thrombocytopenic (ITP) who have been refractory to other treatments. It has also been used in some hyporegenerative thrombocytopenia cases. Here we report the case of a patient who gradually developed DIIHA with Romiplostim-related antibodies
Methods: CASE REPORT: A 67-year-old female was diagnosed with acute myeloid leukemia in February 2014. She received induction chemotherapy (QT) with Idarubicin and Cytarabine (3+7) achieving complete remission (CR) without hematological recovery. Persistent pancytopenia did not allow consolidation QT. In June 2014, she initiated erythropoietin (EPO) and Romiplostim treatment. EPO was suspended two months later and Romiplostin dose, which had come to 4 µg/kg/week was reduced to 0.5 µg/kg/week, keeping platelets count >100 X 103/µL. Subsequent bone marrow aspirations confirmed maintained complete remission. In October 2015, hemoglobin (Hb) concentration was 12.8 gr/dL (normal range 13.5-16.5 gr/dL) and platelets count 133 x 103/µL (normal range 130-400 x 103/µL). Four weeks later the patient referred asthenia. Laboratory tests showed Hb 9.7gr/dl. We ruled leukemia relapse and discontinued romiplostim. Ten days after last dose of romiplostim, laboratory analysis was notable for the following: Hb 10.2 gr/dl, reticulocytes 9.3%, LDH 513 mg/dl, bilirrubin 0.63 mg/dl and haptoglobin 117 mg/dl. Peripheral blood smear showed anisocytosis, polychromasia and spherocytes. Based on her clinical history and laboratory evaluation there was a high suspicion of a potential inmune hemolytic process. Serologic findings showed a strong positive DAT with polyspecific antiglobulin sera (3+) and monospecific anti-C3d sera (2+), being negative with monospecific anti-IgG sera. Eluate with group O RBCs was nonreactive and IAT (indirect antiglobuling test) was negative. Cold agglutinin anti-I was detected in patient´s plasma, titer 32 and thermal activity<20ºC. Patient´s plasma was strong reactive (2+) with romiplostin-treated RBCs (ID-Card LISS/Coombs, BIORAD). Eluate reacted weakly with romiplostim treated RBCs but did not react with the untreated RBCs. Both of them, plasma and eluate, were non reactive with saline-suspended untreated RBCs. After discontinuating the drug, Hb concentration and reticulocyte count were normalized. At the present time, her Hb concentration is 13.5gr/dl and DAT is negative.
Summary/Conclusions: This case highlights the importance for clinicians to maintain high index of suspicion for DIIHA in patients with unexplained hemolysis. It is also important to provide of specialized serological testing to clarify the suspected diagnosis. Romiplostim should be considered as a possible risk factor in development of immune hemolytic anemia. To our knowledge, this is the second report that implicates Romiplostim as a drug inducing DIIHA, being the other one in a patient with ITP.

E1580

ANALYSIS OF DATA CONCERNING PLATELET TRANSFUSIONS DURING A TWO-MONTH PERIOD IN A TERTIARY CARE HOSPITAL

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Background: Blood Centers should constantly evaluate their policy, get informed about the impact it has in clinical practice and eventually reinforce or modify some attitudes. Platelet transfusions are a subject of controversy concerning the importance of ABO matching, of providing single donor platelets rather than random donor etc.
Aims: The purpose of our study was to analyze data on all platelet transfusions performed in our Institution, in order to draw information about the age of the patients, the type, the blood group and the age of the platelet units issued.
We ultimately wanted to examine to what degree the current practice conformed to the principles of our Blood Center, concerning ABO and Rhesus blood group matching, administration of plateletpheresis units (SDPs) rather than random donor platelets (RDPs), issue of units as fresh as possible, avoiding outdating.
Methods: Having defined as one «transfusion event» the administration of one or more platelet units in a 24-hour period for each patient, we registered all platelet transfusion events in our Tertiary Care Hospital in the two month period May-June 2015. For each patient transfused, we registered the age (in order to separate to two categories: adults and pediatric) as well as the type (SDP or RDP) and the «age» of the units. Differences in the ABO and Rh group between the patients and the platelet units they received, were registered and analyzed.
Results: In the two-month period 258 platelet transfusions were performed, 198 in 38 adults (27 men and 11 women, aged 63±17 years) and 60 transfusions in 10 children (5 boys and 5 girls) aged 6,9±2,4 years. In total, 1482 platelet units were administered, of which 1438 RDPs and 44 SDPs. The adults were given SDPs in 9.09% of the transfusion events (18/198). In the remaining transfusion events 1285 platelet units from random donors were administered, in average 7.5±1.98 units per adult patient. The children were given SDPs in 43.33% of the transfusion events (26/60) and RDPs in the remaining transfusion events, with an average of 4.5±1.4 units per child. All SDPs were of ABO and Rh group identical to the patients', while 31,7% of RDPs were of different ABO group. The following table shows how fresh the platelet units were. In the same 2-month period, disposal of 137 out of 1619 platelet units produced, was registered: 136 (8.4%) RDPs and 1SDP (0.061%). 79/137 RDPs and 1 SDP were destroyed due to expiration, 14/137 due to positive serologic and/or molecular testing, 11/137 due to confidential unit exclusion (CUE) and 32/137 RDPs due to other reasons.

Table 1.

Day since the unit was produced	1st day	2nd day	3rd day	4th day	5th day
Units from random donors (n=1482)	475 (**32.05%**)	446 (**30.09%**)	256 (17.27%)	222 (14.98%)	83 (5.6%)
SDPs (n=44)	15 (**34.09%**)	10 (**22.72%**)	6 (13.63%)	7 (15.9%)	6 (13.63%)

Summary/Conclusions: The strategy of the Blood Center to provide platelet units of identical ABO blood group to the patient's, was accomplished 100% in the case of SDPs, but only in 68.2% of the transfusion events during which RDPs were administered. The already satisfactory percentage of «fresh» units (more than 2/3 of units administered, were less than 3 days old) can probably be further improved. It is advisable to investigate whether it would be helpful to increase the proportion of SDPs for adults, at least to the same extent as for pediatric patients. With better planning and better collaboration with the other Blood Centers, the number of units outdated could be minimized.

E1581

ASSESSING THE OUTCOMES OF PATIENTS FOLLOWING GRANULOCYTE TRANSFUSION

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Background: The use of granulocytes in transfusion medicine is not a new concept. Despite continual advances in the process and over half a century of recorded use in clinical practice, the efficacy of these blood products continues to be the subject of much debate. Authors worldwide have reported differing results regarding the outcomes of patients following such transfusions and our understanding of the clinical course of patients receiving transfusions today is largely at the same level as the original pioneers in this field.
Aims: The aim of this project was to assess the use of granulocyte transfusions in the West of Scotland in recent years and evaluate the outcomes of the patients who received these transfusions.
Methods: A retrospective single-arm case series study was carried out with the data being collected for the time period between January 2012 and October 2015.
Results: In total, 20 patients (10 [50%] women and 10 [50%] men) were included. Granulocyte transfusions were discontinued in 8 patients (40%), as there was clinical improvement. This included the patients requiring granulocytes for: severe neutropenia with a non-healing dental extraction (deceased due to end stage AML); severe neutropenia with an infected haematoma (deceased with cause unknown); neutropenic sepsis (alive); pancytopenia (alive); pneumonia (alive); peri-anal abscess (alive); necrotising fasciitis (alive); and lung fungal infection (alive). Eight patients (40%) also deteriorated and for this reason the granulocyte transfusions were stopped. This included the patients that required granulocytes for the following reasons: two patients with neutropenic pyrexia (both deceased with the cause of death being respiratory failure for one patient and a chest infection refractory to treatment for the other patient); severe neutropenia with pericarditis and a fungal infection (deceased due to neutropenic sepsis); neutropenic sepsis (deceased due to neutropenic sepsis); neutropenic sepsis with lung abscess (deceased from chest infection that was refractory to treatment); pancytopenia (deceased due to haemodynamic instability); nasal sinus fungal infection (deceased from neutropenic sepsis); and *Klebsiellaoxytoca* sepsis (deceased due to respiratory failure). Interestingly, from the patients that deteriorated the following pathogens were noted in microbiological testing: *Amoeba* (querying *Acanthamoeba*), throat and sputum *Candida*, heavy yeast growth, *Klebsiellaoxytoca*, and *Pseudomonas aeruginosa* along with *Staphylococcus hominis* in the blood. When comparing the above two groups (i.e. 8 patients showing clinical improvement *versus* 8 patients deteriorating), the median age was 40 years (IQR=47.5; minimum age=2; maximum age=64; 6 women [75%]; 2 men [25%]) for the patients that improved and 60 years (IQR=39.25; minimum age=0.92; maximum age=66; 2 women [25%]; 6 men [75%]) for the patients that deteriorated.
Summary/Conclusions: The data confirms the variable outcomes in patients receiving granulocyte transfusions. However those patients who showed clinical improvement were noted to be younger than those who deteriorated while there was also a discrepancy between the sexes with women typically having better outcomes than men. Atypical organisms were also associated with poorer outcomes suggesting a multifactorial cause for a less successful outcome. Although this is a non-randomised study the patient outcomes are encouraging considering their clinical severity. We therefore continue to use granulocyte transfusions derived from Buffy coats in these situations.

E1582

THE USE OF ERYTROCYTE-APHERESIS FOR THE TREATMENT OF HEREDITARY HEMOCHROMATOSIS: OUR EXPERIENCE

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Background: The erytrocyte- apheresis has proved advantageous for the treatment of patients with hereditary hemochromatosis (HH) and iron overload, rather than the traditional "bloodletting", allowing a less frequent treatment and more personalized. It would lead to a greater decrease in serum ferritin for single treatment procedure, without a major reduction in Hepcidin. This may be clinically relevant, being able to prevent an increase in intestinal iron uptake and a vicious circle resulting in the need for frequent treatments. The accessibility to the method, however, is hindered by the lack of access to the extracorporeal circulation, required to achieve it.
Aims: To facilitate access of the erytrocyte- apheresis to a population of subjects suffering from hemocromatosis, we made available the expertise in the field of extracorporeal circulation, the staff of a hemodialysis center. After an activity of about 10 years, we have conducted a retrospective observational study, comparing with some parameters, subjects undergoing erytrocyte-apheresis with a control group that continued to be subjected to bloodletting.
Methods: Over a period of 10 years, we treated, 37 patients with hematological diagnosis of hereditary hemochromatosis with erytrocyte- apheresis. We have selected among the patients with the same diagnosis that continued to be subjected to bloodletting, a "control" group of 38 patients with similar nosographic characteristics. The retrospective assessment has been focused on the following parameters: hematocrit, levels of blood ferritin, number of treatment procedures, length of intervals between a treatment and another. We also evaluated the costs of treatment.
Results: The mean number of treatment / year procedures was significantly lower for patients treated with erytrocyte- apheresis compared to those treated with bloodletting. The average time interval between two consecutive treatments is a result of 2.3 times longer for the erytrocyte- apheresis compared to bloodletting. The interval between two consecutive procedures of erytrocyte- apheresis has shown a tendency to a progressive increase in the longer time interval of time between a drain and the other. The average annual cost of treatment with erytrocyte- apheresis was about twice compared to those of bloodletting.
Summary/Conclusions: The erytrocyte- apheresis reduced, in our experience, significantly the number of treatment procedures rather than the bloodletting. We believe that, where there is easily available the acces to the extracorporeal circulation for cell separation, the erytrocyte- apheresis procedure could become the treatment of choice for patients suffering from hemochromatosis.

E1583

THE OUTCOME OF PREOPERATIVE TRANSFUSION GUIDELINE ON SICKLE CELL DISEASE PATIENTS AT KING FAHD HOSPITAL-JEDDAH (KSA)

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Background: We have developed a local hospital preoperative transfusion guidelines for sickle cell disease (SCD) patients to reduce the perioperative and the postoperative complications.
Aims: This study was conducted to evaluate the outcome of practice on SCD patients undergoing surgeries in our institution.
Methods: A retrospective review of 75 SCD patients undergoing surgery at King Fahd Hospital, Jeddah, Saudi Arabia was conducted between April 2005 and May 2010. The medical records were reviewed to define the perioperative risks and the postoperative complications in relation to the type of transfusion modality selected.
Results: The medical record of 75 SCD patients who undergoing surgeries were reviewed. Preoperatively, 25.3% had complete exchange transfusion (CETX), 17.3% had partial exchange transfusion (PETX), 26.7% had simple top up transfusion (STX) and 30.7% had no transfusion (NTX). The postoperative complications were 20% vaso-occlusive crises (VOC), 2.7% acute chest syndrome (ACS), and 16% had fever. There was 33.3% patients with prolonged duration of the hospital stay. There was no significant difference in the outcome of postoperative fever, VOC, ACS, and the length of hospital stay between all types of transfusion modalities. However, The correlation was highly significant between the pre-operative haemoglobin (Hb) level and postoperative fever (P=.001) and VOC (P =.002). Interestingly, SCD patients who received hydroxyurea were observed to have less postoperative complication like fever (P=.015) and vaso-occlusive crises (P=.011), while those who received prophylactic heparin in the postoperative period were found to have a reduced length of hospital stay (P=.005) and vaso-occlusive crises (P=.001).
Summary/Conclusions: The guidelines for preoperative transfusion in SCD patients was effective in reducing the postoperative morbidity and mortality. However, this guidelines establish the surgical situations where preoperative transfusion is needed and the optimum regimen for different surgical types.

E1584

FREQUENCY OF REACTIVE BLOOD DONORS IN A TERTIARY CARE HOSPITAL, KARACHI, PAKISTAN

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Background: Human Immunodeficiency Virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) are blood-borne viruses and share transmission routes among at-risk populations, specifically injection drug use and remote blood transfusions before modern donor screening for these pathogens, making co-infection common. Morbidity and mortality from infection with HCV in HIV-infected patients are increasing and have become a major challenge in the management of such patients. In recent years, number of patients infected with HBV or HCV or HIV or co-infected with either of the two viruses, has increased tremendously in Karachi population. IDUs (intravenous drug users), MSM (Men who have Sex with Men) and individuals having unsafe sex are among the people who are identified as groups at higher risk of contracting these infections than others. But these studies does not give an exact picture of prevalence and frequency of these infection in Karachi's population as focus of most of these studies were individuals already involved in behaviors (intravenous drug use and unsafe sex) regarded as high risk behaviors.
Aims: To find the frequency of different types of reactive healthy blood donors at a tertiary care hospital, Karachi, Pakistan.
Methods: The retrospective observational study carried out on both male and female healthy blood donors. Data from complete blood screening from January 2013 to December 2014 were collected and frequency of various types of reactive blood donors was sorted out to get an actual picture. All the blood products were screened for HBV and HIV Using enzyme linkedimmunosorbent assay (Elisa plate washer version 3 and Elisa plate reader stat fax 3200). HCV screening was performed on Architect 2000 SR Chemiluminescent micro plate immune assay (CMIA). Malarial parasite tested by making thick and thin smear seen under microscope. Syphilis was tested by ICT method.
Results: A total number of 6996 healthy donors were received and about 624 were found to have blood screening positive in various combination. The highest numbers of isolates was HbsAg reactive 214, HCV 213, VDRL 170, HIV 26 and 1 case of malarial parasite. More prevalent in male population. In this study there were seven donors found with HCV –VDRL co infection and five co infected with HCV and HbsAg two donors with HIV and HbsAg infected and two donors were HbsAg and VDRL reactive.
Summary/Conclusions: This study supports that HBV, HIV and syphilis prevalence is high and HIV prevalence is low in healthy blood donors.

E1585

MASSIVE TRANSFUSION IN SON ESPASES UNIVERSITY HOSPITAL FROM 2010 TO 2015: A SINGLE CENTRE EXPERIENCE

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Background: The definition of massive transfusion (MT) is the need of at least 10 packed red blood cells units (PRBCs) in 24 hours in response to a situation of uncontrolled bleeding. However there is still no consensus for the clinical management of these situations.
Aims: The aim of this study was to analyze the events of MT in our center and variables associated to mortality within 24 hours and 30 days after MT.
Methods: The records of the Transfusion Service of the Son Espases University Hospital were retrospectively reviewed for cases of patients who required at least 10 PRBCs units within 24 hours during the period of 2010-2015. Their demographic and clinical-biological data were collected.
Results: A total of 93 episodes of MT were identified between 2010 and 2015. The median age of patients was 56 years (15-88) with 69 (74%) males and 24 (26%) females. Descriptive consumption data were: total units transfused in our MT cases were 1444 PRBCs, 537 plasma units and 233 pooled platelets. Median transfusion counts were 13 (10-36) PRBCs, 6 (0-14) plasma units and 2 (0-9) pooled platelets. Median ratio PRBCs/plasma and ratio PRBCs/platelets were 2.7 (1.1-14) and 6 (1.3-23), respectively. We analyze the influence of various variables related to survival at 24 hours and 30 days of the MT. Mortality at 24 hours and 30 days of MT were respectively 28% and 38%, with no differences according to sex (p=NS) or age (p=NS). However these mortalities were significantly higher in politrauma patiens (46% and 43%) compared to aortic pathology (30% and 37%) and other causes (23% and 20%) (p=0.046 and p=0.007), for mortality at 24 hours and 30 days respectively. Variables significantly influencing mortality at 24 hours and 30 days were the number of PRBCs (p=0.008 and p=0.015) and the ratio of PRBCs/plasma transfused (p=0.003 and p=0.049), respectively. Median number of transfused PRBCs was respectively 13 (10-30) and 16 (10-36) for patients surviving and non-surviving after 24 hours and 30 days. Median PRBCs/plasma ratio was respectively 2.5 (1.1-14) and 3.9 (1.5-9) for patients surviving and non-surviving after 24 hours and respectively 2.55 (1.2-14) and 3.3 (1.1-13) for patients surviving and non-surviving after 30 days.
Summary/Conclusions: The MT is accompanied by high mortality rates and involves a high consumption of blood components. In our series, we found an increased mortality in the first 24 hours and in the first 30 days, in patients receiving higher number of PRBCs and with higher PRBCs/plasma ratios. It would be necessary to establish protocols for massive transfusion in each center to unify treatment criteria and optimize the ratio PRBCs/plasma.

E1586

CLINICAL EFFECTS OF CHRONIC RED BLOOD CELL EXCHANGE AND DIFFERENT TYPES OF RED CELL CONCENTRATES IN PATIENTS WITH SICKLE CELL DISEASE

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Background: For patients with severe SCD not eligible for hydroxyurea, two major therapeutic options are currently available: blood transfusion, and bone marrow transplantation. Either urgent or chronic red blood cell transfusion therapy, is widely used in the management of SCD but determines a progressive increase of ferritin level and is also limited by the development of antibodies to red cell antigens. The introduction of chronic red blood cell exchange and prestorage filtration to remove leucocytes and the use of techniques for multicomponent donation could be a good solutions.
Aims: Thus, the aims of our studies were to evaluate the clinical effects of the different blood components in terms of annual transfusion needs and the intervals between transfusion, moreover we evaluated the efficacy of chronic red blood cell exchange (manual or automatic with cell separator) in preventing SCD complications and limiting iron overload.
Methods: In our center we follow 78 patients affected by Sickle Cell Disease. We selected 36 patients occasionally treated with urgent red blood cell exchange because they had less than 2 complications/Year**,** and 42 patients regularly treated with chronic red blood cell exchange because they had more than 2 complications /Year with Hospital Admission. Moreover among these we selected 10 patients for fulfilling the criteria of continuous treatment at the Centre for at least 48 months with no interruptions, even sporadic and absolute transfusion dependency. All 10 patients were evaluated for a period of 4 years, during which two different systems of producing RCC were used. In the second two the patients were transfused with RCC obtained from filtering whole blood prestorage or with RCC from apheresis filtered prestorage. These products differed from those used in the preceding two years, during which the leucodepletion was obtained by bed-side filtration
Results: For all the patients we performed 782 automatic red blood cell exchanges and 4421 units of RCC were transfused. The exchange procedures were extremely well-tolerated by the patients and adverse effects were limited to symptoms of hypocalcaemia during automatic red blood cell exchange with cell separator. After every red blood cell exchange we obtained HbS level<30%. The10 patients selected received respectively a mean of 6.9 and 6.1 units of RBCs exchanged per automatic procedure, in the first two years and in the second two years. Alloantibody developed in 14 patients but only 2 clinically significant and about the observed frequency of transfusion reactions it was very low. All patients treated with chronic red blood cell exchange had an improvement of the quality of life with a reduced number of complications/year (<2/year) and good compliance and moreover patients had limited iron overload making chelating therapy easier.
Summary/Conclusions: In conclusion this study was focused on the most suitable characteristics of blood components for use in sickle cell disease patients and the choice of systematically adopting *prestorage filtration of whole blood*, enabled us to have RCC with a higher Hb concentration than standard. Moreover chronic manual or automatic red blood cell exchange as an alternative approach to simple long-term RBC transfusions give many advantages by being more rapid and tolerable as well as clinically safe and effective and minimize the development of iron overload especially when procedure was carried out with an automatic apparatus. To note that the clinical advantages for patients derived from good selection of the donor and good practices in the production of the blood components.

E1587

FREQUENCY OF RED CELL ANTIGENS AND ANTIBODIES IN THE POPULATION OF NORTH-EASTERN REGIONS OF RUSSIAN FEDERATION

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Background: Providing immunological compatibility of donor and recipient can prevent the development alloimmunization, the occurrence of hemolytic transfusion reactions and increase the effectiveness of transfusion therapy. The frequency of red blood cell (RBC) antigens and alloantibodies is different in populations from different races and geographic areas.
Aims: To study the frequency of Rhesus phenotypes and RBC alloantibodies in donors and recipients of blood components residing in north-eastern regions of the Russian Federation.
Methods: Data from 8087 donors and 2648 patients with hematological diseases were analysed in 2009-2015. Research blood samples was carried out by erythrocyte-magnetized technology (Diagast, France) and gel agglutination assay (BioRad, USA).
Results: Established following frequency of Rhesus phenotypes: CcDee -

30.35%, CCDee-19.39%, CcDEe-15.88%, ccddee-15.34%, ccDEe-13.00%, ccDEE-3.24%, ccDee-1.72%, Ccddee - 0.90%, ccddEe-0.07%, CCddee-0.05%, CcDEE-0.05%, CCDEe-0.02%, CCDEE-0%, CCddEE-0%. The incidence of RBC antibodies in blood donors is very low (0.49%) compared with the incidence in patients with hematological diseases, who received transfusion therapy (1.93%) ($p<0.01$). The most common specificities of antibodies in donors were anti-D (0.22%), in recipients - anti-E (0.3%), anti-K (0.26%), anti-D (0.23%). Differences in the frequency of anti-E and anti-K antibody in donors and recipients are significant ($p<0.01$) (table).

Table 1. Frequency and specificity of RBC alloantibodies in blood donor and patients with hematological diseases

Specificity of RBC alloantibodies	Frequency of RBC alloantibodies (%)	
	donors	patients with hematological diseases
D	0.21	0.23
DC	0.02	0.08
C	0.01	0.08
c	0	0.08
E	0.05	0.30*
C*	0.02	0.04
K	0.02	0.26*
M	0.02	0.11
Le^a	0.02	0.11
Le^b	0.02	0.08
Lu^a	0	0.08
Fy^a	0	0.04
Fy^b	0.01	0
Jk^b	0.01	0

** - The differences between the groups of donor and patients were significant (p <0.01).*

Summary/Conclusions: In the population of north-eastern regions of Russian Federation the most frequent phenotypes CcDee, CCDee, ccddee, CcDEe and anti-D, -E, -K alloantibodies. Analysis of immunohematological parameters of donors and recipients allows targeted collection, banking and storage of blood components.

LB2272

SEROLOGICAL AND MOLECULAR CHARACTERIZAZATION OF RHD VARIANTS

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Background: RhD is the most important, immunogenic and polymorphic Rh antigen, which plays a key role in transfusion medicine. Anti-D antibodies remain the leading cause of the hemolytic disease of the newborn, and antigen D compatible units are chosen when transfusions are needed. Screening tests are based on panels of monoclonal antibodies developed to identify the majority of D variants, but Rh D typing is a constant challenge in donor routine testing, since in presence of RHD blood group polymorphisms of RH partial D or weak D phenotypes may vary according to reagent and method used. In January 2015, the Lombardy transfusion system has been deeply reorganized, and screening tests performed by 27 transfusion centers have been centralized into 8 centers. In our department, we are now testing for ABD about 85,000 units per year.
Aims: The aim of the study was to describe the findings of ABD typing performed with a different system based on agglutination on solid phase technology, different from the ones previously used, based on gel-card technology.
Methods: From March 2015 to March 2016, AB0/Rh typing of blood donations were performed by solid phase technology with a completely automated system (Capture-R Ready-Screen, Immucor). Results were compared with data obtained by gel-card. Samples with negative or weak anti-D reactivity were screened for the presence of RhD variants with different anti-D sera and advanced serological kits such as ID-Partial RhD Typing (Biorad), and, furthermore, they were analyzed for DAT testing. Discrepant or inconclusive samples were selected for further investigation with molecular techniques, based on allele specific PCR for the detection of 14 RhD weak and 48 RhD partial variants (Inno-Train, Essemedical).
Results: A total of 82,000 blood donations, collected from 38,515 donors, were analyzed for AB0 and RhD blood groups. In 130 donors (0.3%) a weak or discrepant RhD typing with different anti-sera were obtained. All of them were analyzed for the presence of RhD variants by molecular approach, which identified: 117 weak D alleles (91 type 1, 7 type 2, 6 type 3, 1 type 4, 1 type 5, 10 type 11, 2 compound heterozygote type 1+4 and type 2+4) and 12 partial D alleles (9 DFR, 1 DNB, 2 DV). In 2 subjects none of the RhD variants analyzed were found. All RhD variants were identified by both gel-card and solid phase technologies, except the weak D type 11 (885G>T) variant carried by 10 subjects, which showed a completely negative results with all monoclonal gel-card antisera used, and only the D weak cells analysis on Immucor microplates revealed a weak reactivity. This RHD variant is commonly classified among the Del phenotype, since it can be only identified by adsorption and elution techniques. The RhD typing of blood component of these 10 blood donors were changed from Rh negative to Rh positive. Moreover, all the RhD variants identified showed a strong correlation between serological reactivity obtained with different anti-sera and molecular results. All RHD variants were identified in subjects with Cc or Ee phenotype, and a linkage disequilibrium between RHD variants and RHCE phenotypes were observed.
Summary/Conclusions: Solid phase methods were highly sensitive in detecting very weak RhD expression variants, such as DEL, which is important for the prevention of anti-D post-transfusion or newborn immunizations. Molecular methods help in the differentiation and definition of partial D and weak D types, providing additional information for transfusion procedures.

LB2273

HBV VACCINATION PROGRAM IN ITALIAN BLOOD DONORS: EFFECTIVENESS AND POTENTIAL THREAT OF VACCINATION FAILURES TO THE SAFETY OF BLOOD SUPPLY

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Background: In Italy, vaccination against HBV in newborns, extended to 12-year-old children, was mandated in 1991. Recombinant HBsAg of A2 genotype, aimed to protect across all genotypes, is used. However, occasional reports of infection with non-A2 HBV genotype in vaccinated blood donors presenting with occult HBV infection (OBI: HBsAg negative, HBV DNA positive) raised concerns about the broad genotype efficacy of vaccines as well as to the safety of blood supply. OBI is a potential risk factor for post-transfusion hepatitis, hepatocellular carcinoma, cirrhosis and HBV reactivation.
Aims: The aim of the study was to investigate the efficacy of HBV vaccination in Italy, where HBV D genotype is prevalent.
Methods: In March 2015 we started 12 month project among Italian blood donors vaccinated at 12 years of age (group A) or in infancy (group B). Donors were enrolled and tested for HBsAg, anti-HBc, anti-HBe, anti-HBs titre and HBV DNA. Dilution and avidity tests were performed on anti-HBc positive samples to confirm true positive results. The persistence of anti-HBs according to the time elapsed from vaccination was also evaluated.
Results: Out of 1055 enrolled blood donors, 295 were vaccinated in infancy (28%) and 760 (72%) at 12 years. No anti-HBc positive result was found in group B, whereas 5 donors (0,7%) in group A were anti-HBc positive, HBsAg and HBV DNA negative (p=0.3). Avidity testing confirmed the anti-HBc positivity in 3/5 donors (anti-HBc specificity: 99.72%), all with high avidity. None of them have detectable circulating HBV DNA. One of them, vaccinated at 13 years of age, was also anti-HBe positive. Anti-HBs titres were<10 IU/mL in 381 (36%) subjects, corresponding to 64% of donors vaccinated in infancy (n=189) and 25% of vaccinated at 12 years of age (n=192). Age at vaccination was an independent predictor of low anti-HBs titer (R2: 0.048; 95% CI: 11.14-19.24) by logistic regression analysis.
Summary/Conclusions: In Italy, HBV vaccination program in newborns seems to be effective to ensure protection against HBV infection. The prevalence of anti-HBc positivity among donors vaccinated at 12 years of age was low, but further studies are needed to clarify whether vaccinated anti-HBc positive donors are threat for transfusion safety. Vaccination in adolescence results in more prolonged immunogenicity than vaccination in infancy, reflecting a more developed immune system.

LB2274

RED CELL AND HAEMOGLOBIN ABNORMALITIES ARE POTENTIAL BARRIERS TO BLOOD DONATION IN PERSONS OF SUB-SAHARAN ETHNICITY

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Background: A substantial increase of persons affected by Sickle Cell Disease (SCD) is expected in European countries, due to migratory flows from Sub-Saharan (SSA) and other endemic areas. Blood transfusion is the essential treatment for people affected by SCD but, due to an intrinsic susceptibility, at least 1/3 of them develop red blood cell (RBC) alloimmunization, which is also favoured by the peculiar antigen patterns found in SSA. For these reasons SCD patients should receive fully matched blood starting from early childhood, and the only way to reach this objective is to include in the donor pool persons of the same ethnicity, who are also at risk of having congenital red cell and haemoglobin (Hb) defects. The conditions are potentially harmful for blood recipient due to increased risk of hemolysis and/or veno-occlusive crises after transfusion.
Aims: The aim of this study was to assess the prevalence of RBC and Hb defects in an apparently healthy population of first generation foreign citizens native of SSA areas.

Methods: In March 2014 we started a 24-months program for the recruitment of foreign citizens to become blood donors in the area of Lecco, Italy. Of 450 potentially eligible persons, 175 (65 f, 110 m), gave their informed consent to undergo clinical and behavioural pre donation assessments according to the European regulation, as well as to the glucose-6-phosphate dehydrogenase (G6PDH) concentration and Hb electrophoresis.
Results: G6PDH concentration could be determined in 169 persons, allowing us to identify 55 (33%) with reduced enzyme concentration, including 25 (15%) with severe deficiency (i.e. <5 u/gHb). Despite the X-linked transmission, 8% of females had severe deficiency. Hb electrophoresis could be assessed in 173 persons. The overall prevalence of Hb variants was 32%: 28 subjects (21% f, 12% m), were carriers of HbS; 19 (11%) had beta thalassemia trait, and 8 (5%) were HbC carriers. Coexpression of G6PDH deficiency and HbS was found in 5% of subjects.
Summary/Conclusions: Despite the clinicians expectations of an increased availability of donors from SSA to provide a better matched blood supply for SSD patients, a substantial proportion of candidate donors will be carrier of RBC and/or Hb abnormalities, representing a major contraindication to blood components preparation.

PUBLICATION ONLY

Acute lymphoblastic leukemia - Biology

PB1588

INHIBITORY EFFECTS OF QUERCETIN ON ANGIOGENESIS IN T-ALL CELL LINES AND ZEBRAFISH *IN VIVO*

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Background: Microvessel dendity was significantly increased in organs from patients with T cell acute lymphocyte Leukemia (T-ALL). Notch1 activating mutations was identified in more than 60% of T-ALL case, and has been demonstrated as one of the most important pathways in angiogenesis. Quercetin, a kind of polyphenolic bioflavonoid, has showed strong anti-tumor effects and antiangiogenesis on various cancers.
Aims: we constructed *Notch1* overexpression transgenic zebrafish, then, treated zebrafish embryos and T-ALL cell lines (A3 and Molt-4) with quercetin to explore its role in anti-angiogenesis of T-ALL.
Methods: Quercetin (>95% pure) was dissolved in <0.1% dimethylsulfoxide. Using T lymphocytes specific promoter Rag2, we introduced human intracellular domain *Notch1* (*ICN1*) gene into *Tg(fli1:EGFP)* zebrafish, tagged with RFP gene. In previously work, we vertified that these fish are closely resemble the main aspects of human ALL. Zebrafish embryos were collected and treated with various concentrations of quercetin (0, 80, 160, 240μM) dissolved in 4mL egg water and incubated in six-well plates (20 embryos per well) at 28.5°C from 14 hours post fertilization (hpf) to 86 hpf. Morphological changes and vessel density were observed and imaged with fluorescence microscope. The subintestinal vessels (SIVs) and intersegmental artery (ISV) of the zebrafish were chosen for the measurement of the length by image analyse software Image-Pro Plus 6.0. Cell line growth inhibition assay was performed at different concentrations of quercetin (0, 15, 30, 60, 90, and 120μM) with Cell Counting Kit-8. A3 and Molt-4 cells apoptosis was measured with AnnexinV-FITC/PI Apoptosis Detection Kit after treated with 30μM or 60μM of quercetin at 24, 48 and 72h. Quantitative real-time PCR and western blot analyses were used to detect the changes of angiogenic factors after dosing. All statistical analyses were carried out in Graphpad Prism 5.
Results: It showed that quercetin can significantly block the SIVs and ISV formation in *Notch1* transgenic zebrafish model. Two of the T-ALL cell lines (A3 and Molt-4) have high expression of Notch1. The IC50 at 48h for quercetin in A3 and Molt-4 were (35.17±1.46)μM and (33.24±2.35)μM, respectively. Their proliferation was significantly inhibited by quercetin in a dose- and time-dependent manner. Furthermore, the mRNA and protein levels of VEGF, DLL4, Notch1 and Hes1 were gradual inhibited with the concentration and exposure time increasing. After treatment with various concentrations (30μM and 60μM) of quercetin for 48h, the maximal apoptosis rates were (70.24±3.58)% in A3 cells, (61.05±2.37)% in Molt-4 cells, respectively. In contrast, less than 5% of untreated cells underwent apoptosis under the same conditions (Figure 1).

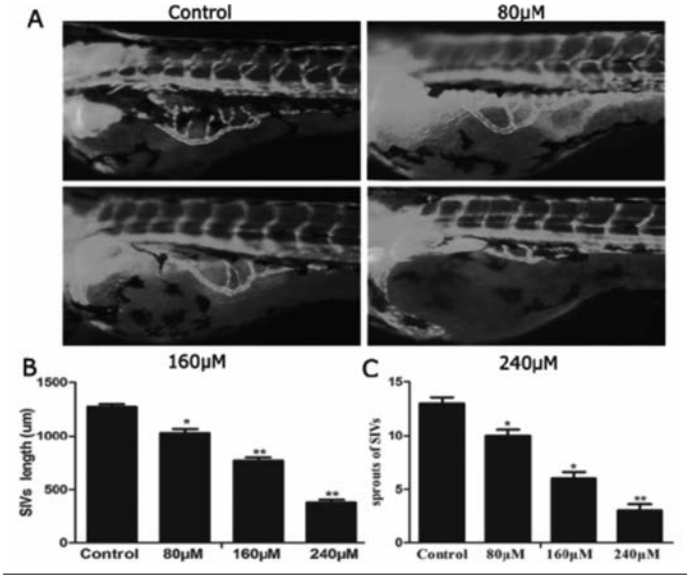

Figure 1.

Summary/Conclusions: These finding suggested that quercetin could inhibit angiogenesis by targeting VEGF regulated DLL4/Notch signaling pathway and supposed to be a potential drug candidate for T-ALL therapy.

PB1589

THE TERAPEUTIC POTENTIAL OF EMBRIONARY SIGNALING PATHWAYS INHIBITION IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Embrionary signaling pathways, such as Wingless (WNT), Hedgehog (Hh), and Notch pathways, are essential for differentiation and self-regulation of stem cells, and their deregulation have been associated with several hematological neoplasias. Acute lymphoblastic leukemia (ALL) is characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues, which can arise from the aberrant activation of embryonic signaling pathways. Therefore, these pathways may constitute new therapeutic targets for ALL treatment.
Aims: Evaluate the therapeutic potential of IWR-1, GDC-0449, and Gamma Secretase XXI inhibitor (GSXXI), inhibitors of WNT, Hh and Notch signaling pathways, respectively, in ALL *in vitro* models.
Methods: For this purpose, we used two ALL cell lines, CEM as a T cell ALL model, and KOPN-8 as a B cell ALL model. Both cell lines were cultured in absence and presence of different concentrations of IWR-1, GDC-0449 and GSXXI. Trypan blue assay was used to evaluate the effect of these inhibitors on cell viability and density. Cell death was determined by optical microscopy (May-Grunwald Giemsa staining) and by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining. FC was also used to measure levels of apoptosis modulators, BAX and BCL-2, cell cycle (PI/RNase assay) and mitochondrial membrane potential (through the fluorescent probe JC1).
Results: Our results showed that IWR-1 reduces only the viability and proliferation of KOPN-8 cells in a time and dose dependent manner (being the maximal inhibitory concentration, IC50, approximately 50 μM at 48 hours of exposure), having no effect in CEM cells. On the other hand, GDC-0449 and GSXXI reduces cell viability and proliferation in a time, dose and cell line dependent manner, being the KOPN-8 cells the most sensitive. We found that the IC50 at 48 hours to GDC-0449 was 75 μM and 150 μM and to GSXXI was 25 μM and 30 μM, for KOPN-8 and CEM cells, respectively. All inhibitors induced cell death by apoptosis, confirmed by the BAX/BCL-2 ratio, morphological analysis, and mitochondrial membrane depolarization. The analysis of cell cycle progression revealed that all inhibitors induce cell cycle arrest in G_0/G_1 phase in both cell lines, except in CEM when incubated with IWR-1
Summary/Conclusions: In conclusion, our results suggest that IWR-1, GDC-0449 and GSXXI could be a potential new targeted therapy in acute lymphoblastic leukemia; however, the therapeutic efficacy is cell type-dependent.
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PB1590

TRANSPLACENTAL CARCINOGEN EXPOSURE INCREASES THE LEVELS OF DNA DAMAGEA IN HUMAN UMBILICAL CORD BLOOD AND MOUSE C57BL/6 BONE MARROW CELLS

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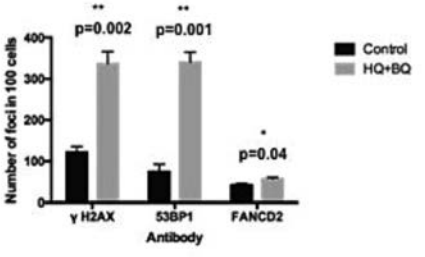
Background: Acute leukaemia is the principal subtype of paediatric cancer and, despite success in treatment, its aetiology remains unclear. Maternal exposure to radiation and benzene metabolites during foetal development has been implicated in its aetiology as the first hit in the context of a minimal two-hit model of the natural history of the disease. Although there might not be an exclusive cause, an abnormal immune response to childhood infections has been proposed as the plausible aetiology. We have previously shown that a human placental barrier responds to different types of toxic challenge and oxidative stress by secreting molecules that cause DNA damage and chromosome aberrations in different cell types distal to the barrier including human fibroblast and human embryonic stem cells.
Aims: We aimed to investigate: *a.* the aetiology of childhood leukaemia by focusing on the role of the placenta in foetal leukaemogenesis; *b.* the effect of nanoparticles (NP) as drug delivery molecules on preventing DNA lesions during pregnancy.
Methods: *In vitro*. Human umbilical cord blood (UCB) cells were exposed indirectly to 30 μM hydroquinone (HQ) and benzoquinone (BQ) across bilayered cell barriers of human trophoblast choriocarcinoma-derived cell line BeWo grown on transwell inserts (pore size, 0.4 mm). Media with no HQ and BQ was the control. *Ex vivo*. To investigate the effect of radiation-induced bystander

mechanism and pharmacological treatment on preventing DNA damages, female C57Bl/6J mice at day 12 gestation were exposed to a whole body exposure non-irradiated, 100 mGy or 1 Gy X-irradiation (AGO, United Kingdom, dose rate 0.5 Gy/min (250 kVp and 13 mA)); the placentae were removed 4 hrs post-irradiation and cultured with 3 conditions: with no drug additive, with MitoQ bound to NP (MQNP) and with blank NP (BNP) as the control for MQNP. Total bone marrow (BM) from age-matched female C57Bl/6J was exposed to conditioned media. DNA damage was measured using quantitative immunocytochemistry for a panel of DNA damage markers including γH2AX and 53BP1 (DNA double-strand break (DSB)) and FANCD2 (interstrand crosslinks (ICL)). The experiments were carried out in triplicate.
Results: Exposure of UCB to HQ and BQ across BeWo barrier increased level of DNA damage. UCB cells showed significantly more damage after 24 hrs exposure (γH2AX (p=0.002), 53BP1 (p=0.001) and FANCD2 (p=0.04), Figure 1). Exposure of murine BM cells to conditioned media with no drug additive increased DNA DSB and ICL. BM γH2AX-positive cells showed increased DNA DSB at 1 Gy (p=0.006) but the difference was not statistically significant at 100 mGy (p=0.07). BM 53BP1-positive and BM FANCD2-positive cells showed significantly more DNA damage compared to their control counterparts at both 100 mGy and 1 Gy doses (p=0.01 and p=0.001, p=0.02 and p=0.0001, respectively). MQNP prevented DNA damaging secretions in BM treated cells however this effect was more pronounced in BM 53BP1-positive and BM FANCD2-positive cells at both 100 mGy and 1 Gy doses (γH2AX (p=0.1 and p=0.06), 53BP1 (p=0.002 and p=0.02) and FANCD2 (p=0.02 and p=0.1), respectively).



Evaluation of DNA damage in human umbilical cord blood (UCB) cells following indirect transplacental exposure to carcinogens hydroquinone (HQ) and benzoquinone (BQ). Compared with the control, transplacental exposure of human UCB cells across bilayered cell barriers of BeWo cell line to HQ and BQ caused significant DNA double strand break as indicated by γH2AX and 53BP1 and interstrand cross link as indicated by FANCD2 antibodies. Bars represent the mean with S.E.M of triplicates. P values are shown above the bars.

Figure 1.

Summary/Conclusions: Our data show that transplacental exposure of human UCB and murine BM cells to carcinogens BQ/HQ and irradiation increases levels of DNA damage presumably leading to increased genome instability. Furthermore, a marked increased phosphorylation of histone H2AX, 53BP1 and FANCD2 in BM cells indicates the effect of a radiation-induced bystander mechanism. DNA damaging secretion conferred by irradiation exposure in the *ex vivo* model could be prevented by applying nanotechnology-based drug delivery molecules to the placental conditioned medium and further investigations are ongoing. These findings suggest the importance of placenta as a barrier in DNA damage signal propagation during embryo development and provide new insights into the link between placental signaling and foetal leukaemogenesis. Whether these events lead to the accumulation of further genetic aberrations in the context of a minimal two-hit model for leukaemia initiation remains to be further elucidated.

PB1591

CROSSTALK OF NOTCH1 AND NOTCH2 SIGNALING IN T-ALL CELL LINES
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Background: Notch signaling is crucial for the growth of leukemia cells. We reported the effects of γ-secretase inhibitors (GSIs), which block Notch activation, on the growth of leukemia cells. GSIs suppressed the growth through induction of apoptosis in most of cell lines. Conversely, the growth of some cell lines were promoted by GSI treatment. For example, we previously reported GSI-XXI treatment suppressed the growth of KOPT-K1 cells while it promoted that of Jurkat cells, both of which are T-lymphoblastic leukemia (T-ALL) cells with constitutive Notch activation. However, the mechanisms of this phenomenon have not been fully clarified.
Aims: In this study, we investigated the mechanisms focusing on the crosstalk between NOTCH1 and NOTCH2 signaling in Jurkat cells.
Methods: Two T-ALL cell lines (KOPT-K1 and Jurkat) were used. The cells were transfected with siRNAs targeting *NOTCH1* (siN1), *NOTCH2* (siN2) or control siRNA by using the pipette tip chamber-based electroporation system. The effects of siN1 and siN2 on the expression levels of mRNA and protein of NOTCH-related molecules were examined by quantitative RT-PCR and immunoblotting, respectively. The effects of siN1 and siN2 on short-term growth were examined using a colorimetric WST-8 assay. To examine the effects of siRNA on morphological differentiation and apoptosis, cytospin preparations were prepared from harvested cells stained with Wright stain.
Results: Transfection with siN1 and siN2 selectively suppressed the expression of *NOTCH1* and *NOTCH2* mRNA and protein, respectively. *NOTCH1* knockdown as well as *NOTCH2* knockdown suppressed the growth and induced apoptosis of KOPT-K1 while they promoted the growth of Jurkat. In Jurkat, *NOTCH1* knockdown increased the level of NOTCH2 protein. On the other hand, *NOTCH2* knockdown increased the level of NOTCH1, cleaved NOTCH1 fragment (active form of NOTCH1), HES1 and MYC protein in Jurkat. In KOPT-K1, the expression of these protein was not significantly affected by siN1 and siN2. The knockdown *NOTCH1* and *NOTCH2* did not affect the expression of mTOR, Hedgehog and Wnt signal-related proteins in both cell lines.
Summary/Conclusions: Using siRNA-mediated knockdown experiments, we found that not only NOTCH1 knockdown but also NOTCH2 knockdown affected the growth of these *NOTCH1*-mutated, *NOTCH2*-unmutated T-ALL cell lines. The growth suppression by NOTCH2 knockdown did not involve the suppression of MYC expression, which was suppressed by NOTCH1 knockdown. We also found the crosstalk between NOTCH1 and NOTCH2 in Jurkat, which suggests that Jurkat might have reciprocally compensating system between two NOTCH. It might cause the resistance to GSIs. To our knowledge, this is the first report to show the interaction between NOTCH1 and NOTCH2 expression in T-ALL cell lines.

PB1592

ACTIVATION OF IRS1/ BETA-CATENIN AXIS IN ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Acute lymphoblastic leukemia (ALL) is an aggressive cancer of immature progenitors that shows aberrant activation of signaling pathways. Insulin-like growth factor 1 (IGF1) and its receptor regulate normal cell growth and contribute to transformation and growth of malignant cells. In solid tumors, IGF1 has the ability to activate a cascade of downstream phosphorylation events including translocation of beta-catenin to the nuclei, and activation of the target gene *MYC*. In fibroblasts, IGF1 was found to induce nuclear IRS1 translocation, IRS1/beta-catenin association and *MYC* transcription. When highly expressed, MYC may acts as oncogene, contributing to the development of cancers, including hematopoietic neoplasm.
Aims: We herein aimed to investigate the IRS1/beta-catenin axis in ALL cells.
Methods: Bone marrow or peripheral blood samples were obtained from 58 patients with ALL and 13 healthy donors. T-ALL cell lines, Jurkat and MOLT-4, and B-ALL cell lines, Namalwa and Raji, were obtained from ATCC. Gene expression was measured by quantitative real-time PCR; relative expression was calculated using the expression of *beta-actin* as an endogenous control. The Mann-Whitney test was used to compare the median of gene expression among normal donors and patients with ALL. Protein expression, associations and cellular localization were evaluated by immunoprecipitation, immunoblotting and western blotting analysis, subcellular fractionation and confocal microscopy. To evaluate the effects of IGF1 upon IRS1 and beta-catenin cellular localization, cells were submitted to starvation in serum-free medium for 24 hours, followed by IGF1 stimulation (20 ng/mL) and/or IGF1R pharmacological inhibition (OSI-906; 20 μM), for 24 hours.
Results: *IRS1*, *beta-catenin*, and *MYC* relative gene expression were significantly higher in ALL patients compared to normal donors (*IRS1*, ALL: median=1.10 [minimum=0.01 - maximum=9.96] *vs* normal donors: 0.34 [0.19 - 1.49], p=0.01); (*beta-catenin*, ALL: 1.12 [0.23 - 5.18] *vs* normal donors: 0.56 [0.01 - 1.57], p=0.002); (*MYC*, ALL: 1.78 [0.06 - 28.37] *vs* normal donors: 0.33 [0:13 - 1.13], p=0.0003). A positive correlation between *beta-catenin* and *MYC* mRNA expression (p=0.002, r=0.39) and between *IRS1* and *MYC* expression (p=0.02, r=0.29) was found. In ALL cell lines (Jurkat, MOLT-4, Namalwa and Raji) high expression of IGF1R, IRS1, beta-catenin and MYC protein were observed. IRS1 and beta-catenin was found to be located in the nuclei and the cytoplasm of ALL cell lines by subcellular fractionation and western blotting analysis. The confocal microscopic analysis reveals elevated co-localization of IRS1/beta-catenin in both nuclei and cytoplasm of ALL cell lines (r≥0.69). In Jurkat cells, the constitutive protein interaction IRS1 and beta-catenin were observed; IGF1 stimulation increased nuclear translocation of IRS1 and beta-catenin. The IGF1R inhibitor OSI-906 decreased IGF1R tyrosine phosphorylation, decreased nuclear beta-catenin translocation and MYC protein expression in Jurkat cells.
Summary/Conclusions: *IRS1*, *beta-catenin* and *MYC* mRNA expression were elevated in ALL patients compared to normal controls. The IRS1/beta-catenin protein association observed in ALL cell lines, together with the data that IGF1 modulates IRS1 and beta-catenin nuclear translocation and MYC expression

indicate that the IRS1/beta-catenin axis is activated in ALL cells, which may represent an important signaling pathway involved in the pathophysiology of the disease.

PB1593

WHOLE EXOME SEQUENCING OF RELAPSED PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood responsible for a quarter of all pediatric cancers. T-cell ALL (T-ALL) makes up 10-15% of childhood ALL. Close to 80% of children diagnosed with T-ALL survive the disease if treated according to standard protocols. However, one in five T-ALL patients relapse and for this group the outcome is very dismal, less than half of the patients are cured.
Aims: We undertook exome sequencing of matched diagnostic, remission and two relapse (relapse 1 and 2) DNA samples from a pediatric T-ALL patient.
Methods: Agilent SureSelect exome capture was used and each capture library was sequenced on the HiSeq2000 platform. >98% of the targets were sequenced with at least 20x coverage.
Results: In total, 66 somatic single nucleotide variants (SNVs) were detected in the three samples. Almost half of the SNVs detected at diagnosis were also seen at relapse 1 and/or 2 (7/15, 47%). This result hence indicates that the relapse evolves from a clone present at diagnosis. *STAT5B* mutations, including N642H, were recently implicated as a putative drivers of T-ALL. We identified the N642H mutations in a homozygous state in both diagnostic and relapse samples, further validating the role of *STAT5B* in T-cell leukemogenesis. Two separate *NOTCH1* mutations were seen in minor subclones at diagnosis. Interestingly, these mutations were not seen in the relapse samples, however relapse 2 acquired a *FBXW7* mutation which could render the leukemic cells renewed aberrant *NOTCH1* activity. The vast majority of identified SNVs (51/66, 77%) were selectively seen in the relapsed samples. Notably, some relapse-specific mutations were detected in genes responsible for drug resistance; *ABCA2* and *NT5C2*.
Summary/Conclusions: To elucidate genes involved in relapsed T-ALL could hopefully result in new targeted treatments.

PB1594

INCOMPLETE IGH GENE REARRANGEMENTS (DH-JH) AS A MINIMAL RESIDUAL DISEASE TARGET IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

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Background: Monitoring of minimal residual disease (MRD) is an important prognostic tool in acute lymphoblastic leukemia (ALL) patients. Currently, one of the most widely used techniques to detect and quantify MRD is allele-specific real-time polymerase chain reaction (qPCR) based on clonal rearrangement of immunoglobulin heavy chain (IGH) or T-cell receptor genes. These molecular targets can be detected in approximately 90% of adult B-cell precursor ALL at initial diagnosis. In patients, where it is not possible to detect a clonal complete IGH rearrangement, additional MRD targets, such as immunoglobulin light chain (IGL) or incomplete IGH (DH-JH) rearrangements, could be also used. The first step in the recombination process of the IGH locus is a rearrangement of DH and JH segments which represents one of the earliest events in B-cell development. Incomplete DH-JH joints were observed in 20 – 25% of B-cell precursor ALLs. Therefore, in adult ALL patients without clonal complete IGH rearrangement, incomplete DH-JH rearrangements could be potentially used as MRD target.
Aims: The goal of our work was the application of clonal incomplete DH-JH rearrangements as MRD targets in routine laboratory practice.
Methods: Since 2010, we have performed clonal complete IGH rearrangement analysis in 45 patients with ALL suspicion in effort to obtain suitable MRD target. In 18 patients, where it was not possible to detect clonal complete IGH marker, screening by the multiplex PCR using BIOMED-2 primer sets for the detection of clonal DH-JH rearrangement was made as the next step. After sequencing of the clonal DH-JH rearrangement, allele-specific oligonucleotides were designed for each MRD target on the basis of the sequence data of the junctional regions. Tests for MRD were conducted by qPCR using TaqMan technology (Rotor-Gene Q, Qiagen).
Results: Clonal incomplete DH-JH rearrangements were detected and sequenced in 11 out of 18 ALL patients at initial diagnosis. Two out of 11 patients had another available molecular target for MRD monitoring (BCR/ABL, MLL/ENL) and for additional 3 patients MRD analysis was not requested. The qPCR assays were developed for 6 patients who were subsequently monitored for MRD. From February 2011 to January 2016 we examined 59 samples from bone marrow (n=57) or peripheral blood (n=2). The assay detection sensitivity achieved the threshold of 10^{-4} to 10^{-5} (1 leukemic cell in 10 000 cells to 1 leukemic cell in 100 000 cells). The MRD levels of residual leukemic cells correlated with clinical outcome. The clonal DH-JH markers found in these patients and used for the MRD analysis were the only option for residual leukemic cells monitoring with sensitivity of at least four orders of magnitude.
Summary/Conclusions: Clonal incomplete DH-JH gene rearrangement targets are applicable for routine MRD testing of ALL patients as a reliable sensitive method for MRD assessment and should be considered as a complementary target for PCR-based clonality assessment and MRD monitoring.

PB1595

WHOLE EXOME SEQUENCING (WES) IN PHILADELPHIA NEGATIVE (PH-) ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) EXTRAMEDULLARY RELAPSES IDENTIFIED COMMON JAK2 MUTATIONS

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Background: Acute lymphoblastic leukemia (ALL) is a complex disease with multiple factors related both to the disease itself and host biology that significantly affect outcome. Appropriate risk stratification and a better understanding of the genomic landscape underlying this disease have led to improvement in overall survival in children. Even with these advances, outcome remains unsatisfactory in adults, mainly in the Philadelphia chromosome negative subgroup. Here we report a 22-year-old man with pre-B ALL who was negative for the recurrent known molecular rearrangements (*E2A-PBX, TEL, AML1- MLL-AF4*). The patient (pt) received different therapeutic regimens and allogenic stem cell transplantation but he did not achieve a remission. Fourteen months after diagnosis (dx), leukemia extramedullary relapsed at the breast and later at lymph node in the groin (CD34+, TdT+,PAX5+, CD19+, CD22+, CD3-).
Aims: to dissect the genetic landscape in order to predict treatment failure and identify targets amenable to inhibition by targeted therapies we compared the genomic profile of 3 different tissues.
Methods: Whole exome sequencing (WES) was performed on dx bone marrow (BM), breast (BR) and lymph node (LN) samples at the time of relapse using the Illumina Hiseq2000 platform. Matched samples of primary tumour and germline DNA from buccal swab were also analyzed. The post-transplantation BR and LN samples were further matched with donor peripheral blood DNA. MuTect and Varscan tools to call mutations (Single Nucleotide Variants=SNVs and/or INDELs) were used. 3 sample SNP array analysis were also performed.
Results: WES analysis from 3 samples identified 522 point mutations and 35 indels that occur in 474 genes (Table 1). The number of total mutations at the time of relapse is higher both in BR and LN samples compare to dx BM. The majority of mutations occurred on genes localized on chr1 (74); the mean number of mutations/chr is 24 (2-74). BR and LN sample have 57 mutations in common (5 in BM and BR, 2 in BM and LN). Mutect identified 26 mutations in both relapsed samples. They include a mutation in *FAAH* gene, which is also found fused with *NSUN4* at the dx (RNASeq). This analysis revealed some other fused genes that were detected mutated with WES. Nine genes are confirmed to be mutated in all 3 samples: 7 nonsynonymous SNVs (*ACHE, COL4A2, LOC554223, NPHS1, SLC36A1, TMEM89* and *JAK2*), 1 stopgain mutation (*NTNG2*) and 1 indel (*HELZ2*). Excluding *JAK2*, no direct relations between these genes and leukemia were reported so far. The *JAK2* mutation S1032Y were detected in all 3 samples, instead the R683G one in the BM and BR. *CD8* is and presents 2 mutations in both BM and BR. SNP array analysis also reveals that this gene was deleted in heterozygosity in the BM and LN. One copy of *EBF1, INPP4B, ZCCHC7* genes are deleted in all 3 samples (SNP array). *EBF1* is described to be associated to ALL. So far, we confirmed *ZCCHC7* is fused with *PAX5* in BM and BR, as previously described (Roberts KG, 2012).

Table 1.

Sample	N.Low Frequency SNVs (Mutect)	N. Other SNVs (Varscan)	INDELs (Varscan)	Tot
BM	35	64	5	**104**
BR	26	225	20	**271**
LN	26	146	10	**182**
Tot	**87**	**435**	**35**	**557**

Summary/Conclusions: The Ph- ALL extramedullary relapses show higher genetic instability, with similar low frequency mutation profile. WES suggest

that *JAK2* alterations are driver mutations. This finding could be helpful for a targeted therapy. Supported by: ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.

PB1596

NOTCH1 INHIBITION REGULATES EVOLUTIONARY CONSERVED MIRNAS IN T-CELL LYMPHOBLASTIC LEUKEMIA

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Background: Activating mutations in the NOTCH1 ligand-activated transcription factor oncogene are found in over 60% of human T-cell lymphoblastic leukemia (T-ALL), where they result in high levels of NOTCH1 signaling. Among multiple functions of oncogenic NOTCH1, there are evidences of the role of non-coding RNAs in NOTCH-induced leukemia. MicroRNAs (miRNAs) are short, single-stranded RNA molecules of approximately 22 nucleotides in length that regulate gene expression by directing their target mRNAs for degradation or translational repression. Dual translocations, that simultaneously affect the 17-92 cluster and *NOTCH1,* highlight the oncogenic importance of this interaction in T-ALL. Moreover, miR-19 was found to play a crucial role in promoting leukemogenesis in NOTCH1-induced T-ALL. In addition, miR-451 and miR-223 have been described to play an important role downstream NOTCH1 in T-ALL cells both in mouse and human models. Beside these relevant discoveries, data regarding the miRNAs that are significantly regulated following NOTCH1 inhibition are still ill-defined.
Aims: In this study we analyzed NOTH1-regulated miRNAs following NOTCH1 inhibition in T-ALL cells in view of future therapies that may combine NOTCH1 inhibition with microRNA based therapy. Specifically we pursued the following aims: - To generate microarray profiles of NOTCH1-regulated microRNAs from T-ALL cells using a mouse model of NOTCH1-induced leukaemia; - To combine analysis of NOTCH1-regulated microRNAs and gene expression profiling following *in vivo* inhibition of NOTCH1 signalling pathway; -To identify and validate evolutionary conserved differentially regulated miRNAs.
Methods: We thus used a mouse model of NOTCH1-induced leukemia, that carries a NOTCH1 mutation recurrently found in human T-ALL patients (L1601P/DPEST). NOTCH1 inhibition was performed *in vivo* using a gamma secretase inhibitor (DBZ) and the treatment schedule was sufficient to determine a significant reduction of cleaved-NOTCH1. miRNAs profiling was performed using a mouse array (8X60K release 19.0; Agilent) that detected 1,247 mouse miRNAs. In parallel, gene expression analysis was performed using SurePrint G3 Mouse Gene Expression v2 array (Agilent) for the identification of about 27000 genes and 4578 lncRNAs.
Results: MYC and NOTCH signature resulted strongly down-regulated following NOTCH1 inhibition by Gene Set Enriched Analysis (GSEA) demonstrating the efficacy of our experimental model. From the miRNAs prolife, we obtained 27 up-regulated and 22 down-regulated miRNAs following NOTCH1 inhibition. Notably, among the NOTCH1 down-regulated miRNAs, we found the miR-17-92 cluster, previously reported highly expressed in T-ALL samples. Their regulation was also confirmed in another mouse model of NOTCH1-induced T-ALL and in human T-leukemia cells. Notably, we identified miR-34a-5p, miR-22a-3p, miR-199a-5p and miR-29a-3p significantly up-regulated following NOTCH1-inhibition suggesting a putative role as tumor suppressors in NOTCH1-driven leukemia. In particular, miR-22a-3p resulted significantly up-regulated following NOTCH1 inhibition both in mouse and human T-ALL cells.
Summary/Conclusions: In conclusion, the cluster 17-92 resulted to have a prominent role amongst the down-regulated miRNAs both in mouse and human T-ALL cells. Importantly, we identified novel up-regulated miRNAs downstream NOTCH1 inhibition. In particular, miRNAs 22a-3p resulted significantly up-regulated in human T-ALLs upon GSIs treatment. Further bioinformatics and functional analysis will be performed to elucidate the role of these novel miRNAs in NOTCH1-induced leukemia.

PB1597

ACUTE LYMPHOBLASTIC LEUKEMIA: A REPORT OF TWO CASES WITH THE RARE E1A3 BCR/ABL FUSION PROTEIN

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Background: The Philadelphia chromosome is the result of a reciprocal translocation between chromosome 22 and chromosome 9 that juxtaposes the breakpoint cluster region (BCR) to the Abelson tyrosine kinase protein. Several variants exist depending of the breakpoint in the BCR gene, being the more known ones p210, p230 and p190 bcr-abl which is the most frequently associated with ALL. Other rare variants exists (e1a3, b2a3, e6a2) the e1a3 is created by the fusion of BCR exon 1 to ABL exon 3. The molecular prognostic of these rare variant forms is still unclear.
Aims: We report two cases of adult B-ALL Ph+bearing the rare BCR/ABL fusion protein e1a3. It is important that our lab is able to detect these variants of unclear prognosis.
Methods: The first patient is a 43 years old man who presented this blood test: leukocytes 136,00x10 3/uL, Hemoglobin 11,70gr/dL, platelets 39,70x10 3/uL. In the peripheral blood smear 93% of small-medium sized lymphoblasts. The bone marrow aspirate confirmed the infiltration of 90% lymphoblasts; the immunophenotype was: CD19+CD10+CD34+TdT+DR+CD79 alfa+CD20-Cytoplasm IgM negative CD33+. The cytogenetic analysis of the BMA revealed the karyotype: 46,XY,del(9)(p22)t(9;22)(q34;q11),del(20)(q13) which confirm the diagnosis of B-ALL Ph+.Patient underwent induction chemotherapy plus imatinib reaching a morphological CR with persistence of Ph+. Currently is candidate to an allogenic SCT as consolidation. The second patient is a 65 year old man whose blood test revealed: leukocytes 223,00 10 3/uL, hemoglobin 11,60 gr/dL and platelets 118 10 3/uL. In the peripheral blood smear: 80% lymphoblasts confirmed by the immunophenotype CD19+CD34+ DR+CD10+CD22+low intensity CD20- TdT+Cytoplasmatic IgM negative CD33+. The BMA confirmed an infiltration of 25% lymphoblasts. The cytogenetic analysis showed the karyotype: 46,XY,t(9;22)(q34;q11). He started induction therapy plus imatib; because of refractory disease is currently ongoing reinduction. He's not candidate to SCT because of his un-fit status.
In both cases the molecular analysis detected the presence of the BCR-ABL e1a3 variant. Our lab procedure isolates patient RNA from peripheral blood and is subjected to a two round multiplex RT-PCR reaction. In order to avoid RNA quality and/or handling errors, we included an internal positive control in which a 690-bp segment of the ubiquitously expressed transcription factor E2A mRNA was amplified. The primers and PCR conditions used in the first and second round of the nested PCR reaction are described by Pallisgaard *et al.* We identified an atypical amplification band of approximately 100 bp. In order to confirm the presence of a BCR-ABL transcript this band was extracted from the agarose gel, purified and then analyzed by DNA sequencing. cDNA sequence confirmed the presence of the e1a3 BCR-ABL transcripts.
Results: Reviewing the literature we found 20 cases bearing the e1a3 fusion proteins already described: 14 patients with B-ALL, 5 with CML and 1 patient first diagnosed with CML who experienced a B-ALL type blast crisis. Among the B-ALL patients, 8 are reported dead. It should be studied if this variant correlates to a worse prognosis, which is currently unknown (Figure 1).

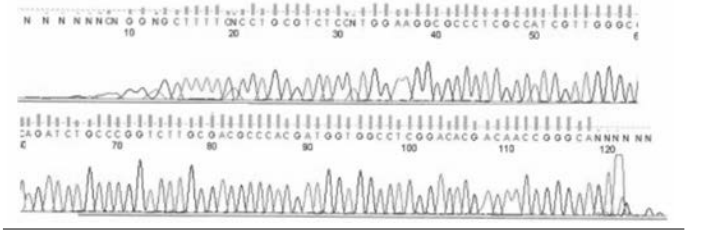

Figure 1.

Summary/Conclusions: We add to the literature two new cases of patients with B-ALL bearing the rare BCR/ABL fusion protein underlining the importance of detecting it; this variant could be missed in the laboratory testing giving a false negative; its role is unclear but seems to have a worse prognosis. More studies have to be done in order to understand the prognosis of this rare variant.

PB1598

ABERRANT DNA METHYLATION-INDUCED GENE INACTIVATION IS ASSOCIATED WITH THE T-CELL LEUKEMIAS DIAGNOSIS AND/OR THERAPY

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Background: Aberrant hypermethylation of tumor suppressor genes is known to play an important role in the development of many tumors, and aberrant DNA hypermethylation was recently identified in hematologic malignancies, where it is thought to hold relevance in leukemogenesis.
Aims: These observations led us to focus on our comprehensive study to examine the prevalence of aberrant promoter methylation in a selected panel of genes that could be potentially involved in T-cell leukemia. In addition to identifying new biomarkers, exploration of methylation patterns could be used to guide T-cell leukemia therapies.
Methods: Here, we used pyrosequencing (a sensitive, easy and effective real-time sequencing-by-synthesis technique) to assess changes in the methylation levels of individual genes between normal peripheral blood (in which they are not normally methylated) and two T-cell keukemia cell lines (in which they are hypermethylated).
Results: We report that there are differences in the DNA methylation patterns seen in normal peripheral blood and two T-cell leukemia cell lines. We identify nine genes (CLEC4E, CR1, DBC1, EPO, HAL-DOA, IGF2, IL12B, ITGA1, and LMX1B) that are significantly hypermethylated in T-cell leukemias cell lines,

and suggest that aberrant hypermethylation of these normally unmethylated genes may induce their transcriptional and expressional silencing. Furthermore, we observed that the expression levels of DNMT1 and DNMT3a were significantly decreased by 5-aza-2'-deoxycytidine (5-Aza-dC), which is a demethylation agent known to deplete DNA methyltransferases (DNMTs) in leukemia cancer cells and restore the expression levels of their target genes in Jurkat cells.
Summary/Conclusions: Together, our results show that aberrant hypermethylation is an important molecular mechanism in the progression of T-cell leukemias, and thus could prove useful as a prognostic and/or diagnostic marker. Moreover, 5-Aza-dC might be a promising candidate for the treatment of T-cell leukemia.

PB1599

EVALUATION OF WNT AND HEDGEHOG SIGNALING PATHWAYS IN PEDIATRIC B ACUTE LYMPHOBLASTIC LEUKEMIA - A PRELIMINARY STUDY

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Background: Deregulation of signaling pathways such as Wingless (Wnt) and Hedghehog (Hh) that participate in normal hematopoietic stem-cell self-renewal, differentiation and proliferation have been implied in the leukemogenic process.
Aims: Our goal was to establish the activation patterns of Wnt and Hh signaling pathways in pediatric B-ALL patients in order to identify novel prognostic markers and new targets for therapeutic strategies.
Methods: We studied 9 bone marrow samples from pediatric patients with B-ALL, 8 at diagnosis and 1 at relapse (average age 10,2y, 4F:5M). Seven B-ALL had no recurrent genetic alterations (group 1, n=6), including the relapsed patient (group 2, n=1), and 2 B-ALL had a t(4;11) (MLL-AF4) (group 3, n=2). Three bone marrow samples without neoplastic disease were used as controls (average age 9y, 3F). All samples were submitted to a gene expression array that included 84 genes for each signaling pathway, Wnt (RT2 Profiler PCR Array, Qiagen) and Hh (Hedgehog Signaling Pathway, Biorad). Results were analyzed by RT2 Profiler PCR Array Data Analysis v3.5 (Qiagen) and considered statistically significant when $p \leq 0,05$.
Results: In B-ALL patients Wnt signaling pathway genes were tendentiously downregulated. Group 1, comparing with control group, showed downregulation of *WNT* (*WNT1/2B/5B/6/7A/7B/10A/10B/16*) ($p \leq 0,05$) and *FZD* (*FZD4/5/7/9*) ($p \leq 0,05$) family genes, Wnt negative regulators (*DKK3,KREMEN1,SFRP4*) ($p \leq 0,05$) and cell cycle target genes (*CCND1,RHOU*)($p \leq 0,05$). Group 3, when compared with group 1 revealed upregulation of WNT (*WNT1,WNT10B*) ($p \leq 0,05$), FZD (*FZD3,FZD5,FZD9*) ($p \leq 0,05$), negatives regulators (*WIF1, KREMEN1*) ($p \leq 0,05$)and target genes *WISP1* and *FOSL1* ($p \leq 0,05$). B-ALL patients at relapse, when compared with the other groups, showed a tendency for Wnt upregulation, including *LEF-1*.
Hh signaling pathway results were more heterogeneous. Group 1, comparing to controls, presented a downregulation of some ligands (*SHH*) and receptors genes (*PTCHD1,BOC,LRP2*) ($p<0,05$) and upregulation of others (*CDON,PTCHD2,RAB23)* ($p<0,05$), with downregulation of some important Hh regulators and target genes (*GLI2,ZIC2,FGF9,OTX2,GREM1,SFRP1, VEGFA*)($p<0,05$) and upregulation of others (*CSNK1E, BCL2,*$p<0,05$). Both, group 3 and the B-ALL patient at relapse, showed a tendency for downregulation of Hh ligands, target genes and transcription factors, when compared to group 1.
Summary/Conclusions: Our results, although very preliminary, suggest that B-ALL pediatric patients with no recurrent genetic alterations tend to present downregulation of Wnt signaling pathway genes and abnormal expression of Hh signaling pathway. Present results suggest that these pathways may provide novel prognostic markers and therapeutic targets, and are currently being validated in a larger patient cohort.

PB1600

SERUM PROFILE OF CYTOKINES, CYTOKINE RECEPTORS AND ADHESION MOLECULES IN PATIENTS WITH NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA AND IN HEALTHY SUBJECTS

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Background: Cytokines and adhesion molecules have been studied as markers of immune system activation in various diseases including hematological malignancies. The knowledge gained from multiple cytokine and adhesion molecule analysis could allow better diagnosis and disease management.
Aims: The aim of our study was to evaluate serum profile of cytokines, cytokine receptors and adhesion molecules in patients with newly diagnosed acute lymphoblastic leukemia (ALL) and in healthy subjects. Correlations between analytes were evaluated separately in both groups.
Methods: Serum samples of 30 newly diagnosed ALL patients (median age 46, range 22–75 years, 20 males) and 15 healthy subjects (median age 41, range 25–58 years, 11 males) were analyzed. We evaluated serum levels of 31 analytes, specifically 21 cytokines, 4 soluble cytokine receptors, 5 soluble adhesion molecules and Matrix Metalloproteinase-9. From cytokines, we measured Interleukins (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-23), Epidermal Growth Factor (EGF), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interferon-γ (IFN-γ), Macrophage Inflammatory Protein-1α (MIP-1α), Monocyte Chemotactic Protein-1 (MCP-1), Tumour Necrosis Factor-α (TNF-α), Vascular Endothelial Growth Factor (VEGF) and soluble receptors for IL-2 (sIL-2Rα), IL-6 (sIL-6R), TNF-α type I and II (sTNFR-1,2). From soluble adhesion molecules, we measured E-Selectin (E-SEL), L-Selectin (L-SEL), P-Selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1). All analytes were measured by biochip array technology on Evidence Investigator analyzer (Randox). Statistical evaluation was done by a professional statistician using software R 3.2.3 (R Core Team 2015). Probability values (p) <0.05 were considered statistically significant.
Results: Comparing cytokine and adhesion molecule levels in newly diagnosed ALL and healthy subjects, we found significant increase in ALL in serum levels of IL-6, IL-8, IL-15, MIP-1α, MCP-1, sIL-2Rα, sIL-6R, sTNFR-1, sTNFR-2, L-SEL, ICAM-1 and VCAM-1 ($p<0.01$). Furthermore, we found in ALL significant decrease in serum levels of IL-3, IL-4 and GM-CSF ($p<0.01$). Serum levels of other evaluated analytes were without significant differences. In the group of ALL patients, we found statistically significant correlations between sTNFR-1 and sTNFR-2 ($r=0.805$; $p<0.0001$), IL-1α and IL-4 ($r=0.700$; $p=0.008$), sTNFR-2 and MIP-1α ($r=0.657$; $p=0.037$), sTNFR-2 and VCAM-1 ($r=0.652$; $p=0.044$). In the control group of healthy subjects, we found statistically significant correlations between EGF and IL-7 ($r=0.876$; $p=0.009$), EGF and IL-8 ($r=0.856$; $p=0.022$). Other correlations between analytes did not reach statistical significance.
Summary/Conclusions: Our results show that serum levels of some cytokines, cytokine receptors and adhesion molecules are significantly altered in patients with newly diagnosed ALL, reflecting activity of the disease. We found statistically significant correlations between some analytes within ALL and control group. Further studies are needed to establish if the alterations observed in the levels of these molecules could be used as a clinically relevant biomarker for ALL.
The work was supported by a long-term organisation development plan 1011 (FMHS).

PB1601

WILM'S TUMOUR GENE (WT1) EXPRESSION IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The Wilms Tumor gene (WT1) encodes a transcription factor involved in kidney development and malignancy. WT1 expression in a subpopulation of early CD34+cells has suggested its involvement in hematopoiesis. Wilms' tumor gene 1 (WT1) is overexpressed in the majority of adult acute leukemias and has been identified as an independent adverse prognostic factor. Acute lymphoblastic leukemia (ALL) is the most common childhood cancer representing 23% of pediatric cancers. Previous reports show controversial results regarding utility of WT1 expression as a prognostic and MRD marker in childhood ALL.
Aims: The aim of this work was to study the impact of WT-1 gene expression at diagnosis in a group of Egyptian children with ALL relating it to prognosis, also determine the efficacy of using this marker in minimal residual disease monitoring as compared to standard immunophenotyping methods.
Methods: This study was conducted on 70 children with newly diagnosed as ALL, assessment of WT-1 gene was done by real time PCR in BM samples at diagnosis and at day 28 of treatment**.**
Results: Positive WT-1 gene expression was positive in 48 cases (68.6%) and negative expression in 22 cases (31.4%). There was statistically significant difference between positive and negative WT-1 gene expression groups regarding immunophenotyping, where all cases that showed aberrant expression of CD13 and CD33 were positive for WT1($p=0.028$). There was significant correlation between WT1 expression at diagnosis and response to induction chemotherapy, 12/48 cases positive for WT-1 gene expression, showed MRD at d 28 while in negative WT1 group none were positive for MRD ($p=0.029$). There was a correlation between WT1 level expression at day 28 and MRD as detected by immunophenotyping. ($p=0.021$).
Summary/Conclusions: WT-1 gene expression is important prognostic factor related to response to therapy as defined by d28 MRD. WT1 level can be used as MRD marker by itself for follow up and comparable to standard immunophenotypic methods.

PB1602

PROGNOSTIC IMPACT OF ABERRANT SURFACE MARKERS EXPRESSION IN T- ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Immunophenotyping is very important in diagnosis, and risk stratification of T-ALL with MRD proven to be the strongest prognostic indicator. Few small-scale studies have reported aberrant CD markers (CD10-CD34-CD13/CD33) in T-ALL with much conflict about its prognostic impact.
Aims: To study aberrant expression of CD10, CD34 and myeloid associated antigens (CD13/CD33) in T-ALL patients and to investigate its correlation with clinical and biologic features, treatment response (MRD) and overall survival.
Methods: This study included 62 newly diagnosed T-ALL patients from the National Cancer Institute (NCI) in Egypt during the time period from September 2012 to December 2014. All patients had undergone full history, thorough clinical examination, complete blood count (CBC), and BM aspiration. Immunophenotypic markers and minimal residual disease (MRD) at fixed time points in BM aspirate samples were studied by four-color flowcytometry EPICS XL (Coulter Corporation, Hialeah, FL, USA).
Results: Patients were classified into three phenotypes based on their immunophenotyping characteristics (CD3 cytoplasmic/surface, CD1a, CD4/CD8 expression): early T (16/62, 25.8%), T-intermediate (39/62, 63%), and T- late (7/62, 11.2%). Comparisons of different phenotypes regarding clinical factors (Age, Sex, initial mediastinal mass), hematological (Hemoglobin, leucocytes counts (TLC), Platelet counts, initial BM blast%), aberrant CD markers (CD10, CD34, CD13/CD33), MRD (Day 15, 33 and 56) and overall survival revealed significant variations regarding: TLC where the highest was T-late phenotype (P=0.014), MRD D33 (P=0.039) and MRD D56 (P=0.007) where T-late phenotype shows a better outcome. CD10 was expressed on 17/62 (27.4%), CD13 and/or CD33 on 10/62 (16.1%), CD34 on 15/62 (24.2%) of cases and co-expression of CD10 and CD34 on 5/62 (8%). Significant associations were found aberrant markers and different phenotypic groups where CD10+CD34+phenotype and CD13/CD33 occur almost exclusively in early-T groups (P=0.007 and 0.002 respectively). CD10 shows borderline significant better MRD outcome at D33 (P=0.057) and also slightly better overall survival. CD34 shows significantly worse MRD D33 (P=0.045) but no correlation with OS. Though all five patients with CD10+CD34+phenotype shows MRD<0.01 by D33 and then after, no significant correlation was found with MRD nor OS; which maybe be explained by small patient number. CD13/CD33 expression shows significant correlation with worse MRD D33 (P=0.017) with worse -but not significant- OS (Table 1).

Table 1. Clinical, biological and prognostic criteria of T-ALL phenotypes.

Positive in CD expression: >20% of blasts are positive for this CD marker, (*n); number of the patient' samples recruited at this specific time point

	T-ALL (n=62)			P value
	T-early (25.8%, n=16)	T-intermediate (63%, n=39)	T-late (11.2%, n=7)	
Age (years) Median, (range)	18, (2-60)	20, (3-42)	15(3-17)	ns
Sex (M:F ratio)	2.2:1	3.33:1	1:1.33	ns
Mediastinal mass (Positive/negative)	(2/14)	(5/34)	(2/5)	ns
Hemoglobin gm/dl (Mean+ SD)	8.7+2.44	9.99+ 2.88	8.47+ 3.22	ns
Platelet count ($X10^9$/L) Median, range	51 (3-410)	54 (6-380)	76 (17-225)	ns
Leucocyte count ($X10^9$/L) Median, range	72.2 (2.2-553)	51.6 (1.1-761.3)	263 (13-660)	P=0.014
Aberrant expression: (Positive/negative)				
1. CD10	4/12 (25%)	8/31(20%)	5/2(71%)	P=0.02
2. CD34	9/7 (56%)	4/35 (10%)	2/5 (29%)	P=0.001
3. CD10+CD34+	4/12 (25%)	0/39 (0%)	1/6 (14%)	P=007
4. CD13/CD33	7/9 (44%)	3/36 (7.7%)	0/7 (0%)	P=0.002
MRD (Positive/negative)				
D15 (n*=35)	4/2 (66.7%)	12/8(66.7%)	3/2(66.7%)	ns
D33 (n*=23)	3/2(60%)	4/9 (30.8%)	0/5 (0%)	P=0.039
D56 (n*=17)	4/1 (80%)	1/8(11.1%)	0/3(0%)	P=0.007
1 year OS	81.2%	82%	100%	ns

Summary/Conclusions: Immunophenotypic groups and aberrant expression (CD10, CD34, CD10+CD34 and CD13/CD33) have an important prognostic value in T-ALL, which is evident by their effect on MRD D33, D56 and OS.

PB1603

BOTANICAL ALKYL HYDROQUINONE HQ17(3) INDUCES ENDOPLASMIC-RETICULUM STRESS AND AUTOPHAGY BEFORE THE ONSET OF CYTOTOXICITY ON THE T(9;22) PHILADELPHIA CHROMOSOME POSITIVE SUP-B15 ALL CELLS

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Background: Acute lymphoblastic leukemia with Philadelphia chromosome (Ph^+-ALL)(t(9;22)*BCR-ABL*) is a very high risk (VHR) hematological neoplasm. Constitutively active BCR-ABL oncoprotein together with multiple genetic lesions contribute to a very aggressive clinical cause that tyrosine kinase inhibitors (TKIs) combined with multi-agent chemotherapy fail to convey long-term disease control. Searching for agents selective to leukemias and investigating the molecule mechanisms involved in the inhibitory effects on leukemic cells will help to develop new anti-leukemic therapeutics for the Ph^+-ALL. HQ17(3) [10'(Z),13'(E),15'(E)-heptadecatrienyl-hydroquinone], a natural product isolated from the sap of *Rhus succedanea*, exhibited very effective cytotoxic effect on the TKI-refractory Ph^+-ALL SUP-B15 (IC_{50}:1.9 μM) and other ALL cell lines, but spared normal peripheral blood mononuclear cells. Combination of HQ17(3) and Imatinib has synergistic cytotoxic effect on SUP-B15 cells.
Aims: To investigate the characters of, and the molecular pathways involved in the HQ17(3)-induced cytotoxic effects in Ph^+-ALL SUP-B15 cells.
Methods: HQ17(3)-treated and control SUP-B15 cells were stained and analyzed by flow cytometry: membrane lipid disturbance was analyzed by Annexin V/PI stain, DNA fragmentation was defined as sub-G1 fraction of cellular DNA content after the PI staining, mitochondrial membrane potential (MMP) loss were stained by DiOC6(3). Pan-caspase inhibitor (zVAD-fmk), receptor interacting protein 1 (RIP1) inhibitor (necrostatin-1, Nec-1), iron-chelator (deferroxamine, DFO), 3-methyladenine (3MA) or chloroquine (CQ) (autophagy inhibitors), and lysosomal protease inhibitors were used in combination with HQ17(3) in some experiments. Acridine orange stain and confocal microscopy are used to visualize the changes of lysosomes in the presence of HQ17(3). Autophagic flow in response to HQ17(3) was revealed by accumulation of LC3B-II visualized by western blot analysis and aggregation of ectopically expressed GFP-LC3 indicator.
Results: Introduction of HQ17(3) induced extensive cell death within 24 hours characterized by losing plasma membrane integrity (PI^+) concomitant with PS exposure (Annexin V^+), which was not prevented by zVAD-fmk and/or Nec-1. Cell death displayed MMP loss and profound nuclear DNA fragmentation that could be attenuated by ROS scavengers. Acidic vesicles were significantly increased 4 hours after treatment of HQ17(3) then diminished when cell death was evident. Lysosomotropic DFO abolished the HQ17(3)-induced acidic vesicles and subsequent cell death. Application of AEBSF (serine protease inh.) and/or pepstatin/CA074-Me (cathepsin D/B inh.) did not rescue cells from death. Autophagy markers were enhanced in HQ17(3)-treated cells. Autophagy inhibitors (3MA and CQ) showed a modest protective effect. Further, HQ17(3) treatment gave rise to early and sustained AKT and mTOR activation and induced ER stress as shown unfolded protein response markers eIF2a phosphorylation and upregulated ER chaperon Grp78.
Summary/Conclusions: Naturally-derived HQ17(3) displayed significant cytotoxicity on Ph^+-ALL SUP-B15 cells. HQ17(3) leads to ER stress and induces iron-dependent autophagy followed by necrotic-like, caspase-independent cell demise that is different from RIP1-mediated necroptosis or lysosomal protease-mediated cell death. These results suggest that agents selectively induce or sustain ROS in leukemic cells may induce ER stress and autophagy-associated cell death, and would potentially augment the treatment for VHR-ALL with t(9;22) translocation.

PB1604

COMPARATIVE ANALYSIS OF CLONAL IG AND TCR GENE REARRANGEMENTS IN ADULT PH-NEGATIVE ALL AT THE DIAGNOSIS AND AT THE RELAPSE

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Background: Clonal rearrangements of immunoglobulin (IG) and T-cell receptor (TCR) genes are the targets for minimal residual disease (MRD) assessment. The level of the MRD is an independent prognostic factor and its monitoring is important to identify patients with high risk of relapse. One of the pitfalls of MRD detection by means of IG and TCR gene rearrangements in ALL is clonal evolution possibly caused by V(D)J recombinase activity. Several studies have showed that the pattern of IG and TCR gene rearrangements in ALL patients may change during the course of disease. In relapse some gene rearrangements may be lost while new gene rearrangements may occur.

Change of clonal rearrangements, during tumor progression may lead to the loss of a target for MRD studies and false-negative results.
Aims: Studying the phenomenon of clonal heterogeneity in adult patients with ALL at the diagnosis and relapse of disease.
Methods: We have determined T- and B-cell clonality in six adults with Ph-negative ALL at the time of diagnosis and at the relapse. B-cell clonality was assessed by immunoglobulin heavy chain gene rearrangements IGH (VH – JH) and light kappa chain gene rearrangements IGK (Vk – Jk/Vk – KDE/Intron RSS – KDE). T-cell clonality was assessed by T-cell receptor gamma chain TCRG (VG – JG), beta chain TCRB (VB – JB /DB – JB) and delta chain TCRD (VD – JD/DD2 – JD/ VD – DD3/ DD2 – DD3) gene rearrangements. Multiplex BIOMED-2 primer system for PCR and Gene-Scan analysis have been used.
Results: In two patients with B-cell ALL one of clonal rearrangement presented at the diagnosis was lost at the relapse of disease while new rearrangements emerged. In one patient with a diagnosis of early T-ALL clonal rearrangements of the genes TCRD, TCRB and TCRG, identified at the diagnosis were exactly the same in relapse. In one patient all the rearrangements identified at the diagnosis were preserved at the relapse and several new rearrangements appeared. In one case five rearrangements disappeared at the relapse of disease out of seven identified at the diagnosis (Figure 1). In one patient only one clonal rearrangement was revealed at the diagnosis of B-cell ALL and at the relapse of the disease this rearrangement was preserved, but there were a few new. To analyze initial ALL samples for possible presence of minor clones that lately we have found in relapse we used appropriate family-specific primers to V and J-region instead of multiplex primer mixtures. Despite increased to 10^{-2}-10^{-3}sensitivity no detectable cell populations of these subclones were found.

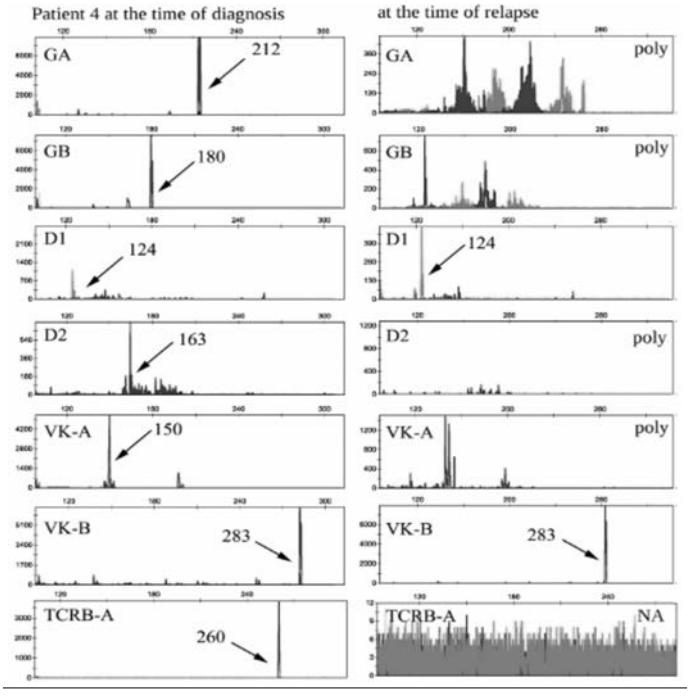

Figure 1.

Summary/Conclusions: Mismatch in clonal rearrangements at the diagnosis and at the relapse of disease was identified in five of six (83%) patients, indicating clonal instability during the course of treatment. Clonal evolution and diversity of clonal IG and TCR gene rearrangements could be one of the features of tumor progression. Also it could be speculated that many clonal IG and TCR gene rearrangements may present at different amounts at the diagnosis, however less abundant clones could be "invisible" due to the limitation of measuring sensitivity. Later major clones may disappear under the influence of chemotherapy while others could gain proliferation capacity. Study of the clonal evolution and heterogeneity in ALL and their impact on treatment efficacy should help to develop new prognostic factors and therapeutic strategies.
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PB1605

POTENTIAL GOOD TARGET FOR IMMUNOTHERAPY – PRAME ANTIGEN HAS SELF-ACTIVATING ABILITY

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Background: According to literature, PRAME (preferentially expressed antigen in melanoma) protein may be exposed on extracellular membrane. Interestingly, that PRAME is structural homolog of TLR2 protein. TLR2 activates NF-kB signaling pathway and NF-κB activates an expression of TLR2. As a result, TLR2-mediated activation of expression TLR2 is possible event. If PRAME is similar to TLR2, it's possible to suggest PRAME-mediated activation of its own expression. PRAME protein is expressed in a wide range of cancers. In addition, this protein is non-active in cells of somatic tissues and is highly immunogenic. If PRAME is expressed in a tumor cell, it's possible to increase an expression level of PRAME; this can lead to higher immunogenicity of tumor cells. This method could be a powerful in context of specific anti-PRAME immunotherapy.
Aims: To show that intracellular PRAME protein expression level is increased in case of binding anti-PRAME antibody with cell membrane.
Methods: We created a mouse monoclonal antibody 5D3F2, specifically recognizing PRAME antigen. Tree cell lines were used for incubation with anti-PRAME antibody: acute monocytic leukemia cell line THP-1, acute myeloid leukemia cell line NOMO-1 and disseminated melanoma cell line mel IBR. Expression level of PRAME gene was evaluated by RQ-PCR. Cell lines THP-1 and NOMO-1 were incubated in RPMI 1640 with addition of anti-PRAME antibody. Antibody concentration was 10 ug/ml, and amount of cells counted 10000/ml. Samples of cells was analyzed after 1, 2 and 4 hours and after 1, 2 and 3 days of incubation. Number of PRAME-expressing cells was determined by flow cytometry using a FITC-labeled antibodies 5D3F2. All experiments were repeated 3 times.
Results: PRAME gene expression level was 1,67% in THP-1; 0,46% in NOMO-1 and 963% in mel IBR relative of ABL expression level before experiment. According to flow cytometry data, number of PRAME-expressing cells THP-1 and NOMO-1 before experiment was 11,4% and 3,6%, respectively. Intensity of PRAME-expressing cells in THP-1 fluorescence after incubation with antibody 5D3F2 was 101,21% (after 1 hour), 75,79% (2 hour), 88,45% (4 hour), 421,28% (1 day), 433,33% (2 day) and 820,37% (3 day). Fluorescence intensity of PRAME-expressing cells in NOMO-1 after incubation with antibody 5D3F2 was 93,88% (1 hour), 87,07% (2 hour), 74,67% (4 hour), 175,45% (1 day), 562,67% (2 day) and 146,33% (3 day).
Summary/Conclusions: PRAME protein is expressed on THP-1 and NOMO-1 cell surface. Antibody 5D3F2 binds with cell membrane and it could explain decrease of fluorescence intensity in first hours. FITC-labeled 5D3F2 show this reduction because many PRAME epitopes are closed by non-labeled 5D3F2. But after 1 and more days number of PRAME epitopes on cell surface grows, and flow cytometry reaction displays it. This finding suggests that it is possible to develop an anti-PRAME monoclonal antibody for therapy PRAME-positive disease. Moreover, PRAME expression level may be increased during therapy with these antibodies. It can be useful, because PRAME-expressing cell becomes more immunogenic after binding with a potential therapeutic anti-PRAME antibody. We did not investigate the role of NF-kB, but it will be our next challenge.

PB1606

ABERRANT IMMUNOPHENOTYPES IN B-ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Recently immunophenotyping has a critical role for diagnosis and classification of leukemia and lymphoma. Immunophenotyping is used increasingly for identification of some subtypes, treatment follow-up of minimal residual disease (MRD) and even for prognostic purposes. Also, myeloid and T cell aberrant antigen expressions help to distinguish B acute lymphoblastic leukemia (B-ALL) from hematogones.
Aims: The aim of this study was to evaluate myeloid and T cell aberrant expressions in B-ALL patients who had been referred to our flow cytometry center.
Methods: We retrospectively analyzed the flow cytometry data of newly diagnosed 417 B-ALL samples which were collected in our laboratory between the years 2002-2016. We used a large panel of monoclonal antibodies against lymphoid, myeloid and precursors antigens.
Results: Aberrant antigens were detected in 143/417 (34.29%) of B-ALL patients. Expression of myeloid antigens was a common aberrancy in B-ALL. The leukemic lymphoblasts exhibited aberrant expression of 4 myeloid antigens and 5 T-cell antigens. The most frequently expressed antigen was CD33 (74/143), followed by CD13 (57/143), MPO (2/143), and CD11b (1/143). Dual expression of CD13 and CD33 was seen in 24 patients. Expression of T-cell aberrant antigens was observed in only 8 patients. CD2 was positive in 3 patients, CD5 was positive in 2 patients, and CD3, CD7, CD56 were positive separately in 3 patients.
Summary/Conclusions: Aberrant antigen expression is important in MRD follow-up, also it is reported to be associated with prognosis and cytogenetic abnormalities. In literature, adult B-ALL cases expressing CD13 and CD33 have been reported to be related to BCR-ABL positivity. MPO aberrant antigen expressions are very rare and BCR-ABL has been reported mostly negative in MPO positive patients. We have detected MPO aberrant antigen expression in 2 patients, none of them has BCR-ABL positivity. Expression of T-cell antigens

has rarely been reported in B-ALL, and the significance of this aberrant antigen expression is unclear. Several studies showed that T-cell aberrant expression leads higher relapse risk and it is associated with adverse cytogenetic abnormalities.

PB1607

THE EXPRESSION LEVEL OF HLS5 IS CORRELATED WITH THE CLINICAL OUTCOMES OF ACUTE LYMPHOID LEUKEMIA (ALL) PATIENTS

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Background: HLS5 belongs to the RBCC (Ring-finger, B-box, coiled-coil) family, a family of tumor suppressive genes including Pm, Tif1-α,Herf and Rfp, it has been reported to regulate erythroid differentiation, to be activated during the conversion of J2E erythroleukemic cells to monocytoid cells, and assumed to be a tumor supressor. However, the role of HLS5 in acute lymphoblastic leukemia has never been explored.
Aims: To identify the expression of HLS5 and the relationship to the clinical outcom to ALL.
Methods: Bone marrow of 63 *de novo* ALL patients were collected, the mononucleated cells separated, RNA extracted and the HLS5 expression level detected using real-time quantitative PCR. SPSS 16.0 used for data analysis.
Results: The high HLS5 expression level (the higher 25% of all the patients) is correlated with longer overall survival and event free survival (both $p<0.05$), but the correlations between HLS5 expression level and blast count, WBC, Hb level, platelet count, IK6 status and Ph chromosome status are not significant.
Summary/Conclusions: The expression level of HLS5 in *de novo* ALL patients may associate with the clinical outcome, but the exact mechanism needs further investigation.

Acute lymphoblastic leukemia - Clinical

PB1608

COMPARISON OF THE PROGNOSTIC SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE AND THE CONVENTIONAL PROGNOSTIC FACTORS IN THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS

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Background: During the past couple of decades the response rates, the leukemia free survival and the 5 year overall survival (OS) in adult patients with acute lymphoblastic leukaemia (ALL) have improved significantly. There is an ongoing trend to improve further OS through better risk assessment. The risk stratification in different treatment protocols is usually based on groups of conventional prognostic risk factors, such as – white blood cells count (WBC) at diagnosis, immunophenotype, cytogenetic and molecular abnormalities, achievement of CR and time to achievement of CR, age with some variations among the different study groups. To improve the results of risk stratification through the conventional risk factors, the evaluation of the level of minimal residual disease (MRD) and its use for treatment decision making have been introduced. Several studies have already established the contributory role of MRD levels for the prediction of relapse and continuous complete remission (CCR) in the ALL patients. Despite the growing involvement of MRD evaluation in the risk stratification of adult ALL patients, there is a continuing need to better address the specific role MRD examination should play among other ALL prognostic factors and to better establish the best time point for ALL MRD evaluation.
Aims: With the present retrospective assessment we aimed to compare the impact of minimal residual disease and a group of conventional prognostic factors on the overall survival of adult ALL patients.
Methods: We analyzed retrospectively 81 patients with adult ALL, that were treated in the National Specialized Hospital for Active Treatment of Hematology Diseases (NSHATHD) between 1.1.2009 and 1.1.2016, of them 53 were CR achievers. The minimal residual disease (MRD) levels of the patients were measured through 8-color flow cytometry and through semiquantitative RT-PCR, following the BIOMED 1 program protocol for the patients with a MLL-AF4 and BCR-ABL fusion transcripts chromosome. MRD negativity or molecular complete remission (molCR) was defined as a level of less than 10^{-4} ALL cells or lack of expression of fusion transcripts.
Results: MRD negativity had a statistically significant impact on OS 70.3% *vs* 10.7% for the MRD negativity non-achievers. The conventional risk factors did not seem to convey greater prognostic significance with differences between conventional risk groups in terms of OS not reaching statistical signifcance and the multivariate analysis demonstrating significance only of MRD status, WBC and PSECOG with hazard ratios of 5.241, 3.070, 2.061, respectively. Allogeneic hematopoietic stem cells transplantation significantly improved OS in the patients who did not achieve MRD negativity with 3-year overall survival of 38.9% *vs* 11.7%.
Summary/Conclusions: The impact of molCR status on survival will probably lead to new risk stratification strategies based to a greater extent or even exclusively on the MRD status. The optimal time for MRD risk stratification remains to be established.

PB1609

PROGNOSTIC IMPACT OF CD20 EXPRESSION IN ADULTS WITH PHILADELPHIA CHROMOSOME NEGATIVE PRECURSOR B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Prognostic influence of immunophenotypic markers in acute lymphoblastic leukemia (ALL) is well recognized. The prognostic significance of CD20 expression in precursor B-cell acute lymphoblastic leukemia (ALL) has been investigated first in childhood and than in adult with conflicting results.
Aims: The aim of this study was to determine the prognostic impact of CD20 expression on outcome in adult patients with Philadelphia chromosome negative (Ph-) precursor B-cell ALL. The second goal was to estimate, in comparison to the results, is there a need to incorporate monoclonal anti CD20 antibody (Rituximab) in routine treatment of these patients, where it is not available, to improve their outcome.
Methods: This single center study involved 122 patients with *de novo* Ph- precursor B-cell ALL diagnosed in period 2003 – 2015, with follow-up period of 72

months. For CD20 antigen expression, detected by flow-cytometry, the cut-off value ≥20% was taken as positive (CD20+). As risk factors for rate of complete remission (CR) and overall survival (OS) in months in this cohort of patients the following were evaluated: age, leukocytosis (<30x10^9/L *vs* ≥30x10^9/L) and CD20 expression. In period of 2003 – 2006, the patients were treated with regimen LALA 94, while in period 2007-2015 they were treated with protocol Hyper-CVAD. The applying of monoclonal anti CD20 antibody (Rituximab) with chemotherapy was no available.

Results: The mean age of the patients was 42 years (range 19-77). The CD20+was recorded in 41 (33.6%) of patients. The estimated risk factors had no influence to achievement of CR. However, the univariate analysis showed that age ≥55 years (p=0.035) and CD20+(p=0.042) had significant impact on poor OS. Multivariate analysis indicated CD20+as the most important risk factor for poor OS: p=0.001, HR=3.853(95% CI=1.755-8.461.

Summary/Conclusions: This study showed the negative impact of CD20 expression in Ph- precursor ALL on outcome of these patients. Also, we point out the importance of incorporation monoclonal anti CD20 antibody (Rituximab) in combine with chemotherapy in routine treatment of these patients to improve its outcome.

PB1610

CLINICOPATHOLOGIC CHARACTERISTICS ASSOCIATED WITH NATURAL KILLER CELL ACTIVITY BY MEASUREMENT OF INTERFERON-GAMMA IN HEMATOLOGIC MALIGNANCIES

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Background: Natural killer (NK) cells play a key role in innate immune responses and an important component of anti-cancer defenses. Evaluation of NK cell activity could be invaluable to estimate the status and the outcome of cancers. Defects in NK cell cytotoxicity have been reported in hematologic malignancies. Conventional methods such as 51Cr release assay and CD107a degranulation assay may be useful to determine NK cell activity, but they require complex experimental setups and are not appropriate for routine laboratory test. Recently, a commercialized kit for measurement of NK cell activity was developed, which measure released interferon-gamma secreted by *ex vivo* stimulated NK cells in whole blood.

Aims: The purpose of this study was to investigate clinicopathologic characteristics associated with NK cell activity by measurement of interferon-gamma.

Methods: The study group included 134 patients (61 at diagnosis and 73 at follow up) diagnosed as having hematologic malignancies between Mar 2015 and Jan 2016. The NK cell activity was measured by commercialized kit (NK Vue assay, ATgen, Sungnam, Korea), which is a quantitative sandwich ELISA to measure released interferon-gamma secreted by NK cells in whole blood collected in PROMOCA tube (an engineered recombinant cytokine that specifically activates NK cells). NK cell activity was expressed as ratio which patient's result was divided by upper normal level. NK cell activity was analyzed using 294 specimens obtained from patients at diagnosis or follow up.

Results: The median ratio value of NK cell activity was 1.27 (range: 0.03-8.0). Comparing with NK cell activity of 61 patients at diagnosis (12 AML, 5 ALL, 3 CML, 2 MDS/MPN, 11 MM, 25 lymphoma, 3 solid cancer), ALL patients had significantly decreased NK cell activity (median: 0.16, range: 0.16-0.26) than lymphoma patients (median: 1.05, range: 0.03-2.35, *P*=0.0196). No significant differences were found among other hematologic malignancies at diagnosis.

Among the total patients including at diagnosis and follow up, NK cell activity was positively correlated with percentage of lymphocytes (r=0.346, *P*<0.0001), whereas NK cell activity showed weak negative associations with total WBC counts (r=-0.126, *P*=0.0324), neutrophil counts (r=-0.155, *P*=0.0083) and NLR (neutrophil/lymphocyte ratio) (r=-0.227, *P*<0.0001). Of particular, NK cell activity had positive correlations with percentage of lymphocytes in patients with AML (r=0.693, *P*=0.0125), lymphoma (r=0.502, *P*<0.0001) and multiple myeloma (r=0.427, *P*=0.0011). The regression analysis between NK cell activity and lymphocyte subsets identified by flow cytometry (CD56+NK cell, CD3+T-cell, CD4+T-cell and CD19+B-cell) did not show significant associations except for CD8+T cell counts (r=0.159, *P*=0.0083). The CRP didn't show a significant association with NK cell activity(r=0.126, *P*=0.6076).

Summary/Conclusions: This study showed that the NK cell activity was significantly decreased in ALL patients, comparing with that of lymphoma patients and NK cell activity was not correlated with NK cell counts identified by flow cytometry. NK cell assay by measurement of interferon-gamma secreted by *ex vivo* stimulated NK cells in whole blood, could be useful to evaluate and monitor the NK cell activity in hematologic malignancies.

PB1611

MINIMAL RESIDUAL DISEASE: COMPARISON BETWEEN TWO METHODS IN A CLINICAL DIAGNOSTIC LABORATORY.

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Background: Flow cytometry and molecular techniques are the most important methods for monitoring minimal residual disease (MRD) in leukemia.

Evaluation of specific molecular markers represents the most sensitive and specific analysis in MRD leukemia, however the existence a small number of markers which in few cases are valid to follow the course of the disease is a limiting factor. Because of this, there is the necessity to identify new widely represented markers in AML and that could predict relapse

On the other hand, immunophenotype associated to leukemia (LAIP) have been proved to be very useful in the detection of MRD and it can be used up to 85% of patients.

Aims: The aim of this study was to compare the concordance of MRD measured by flow cytometry with standardized molecular techniques including NPM1, Inv16 and AML1-ETO. Furthermore, we also analyzed the behavior of WT1 at this respect in order to determine whether overexpression of WT1 could be a new MRD marker.

Methods: A total of 35 samples of bone marrow from patients with AML in different stages of the disease were assessed. We analyzed NPM1, Inv16 and AML1-ETO by using the gold standard quantitative PCR techniques and these results were compared first with the results obtained by flow cytometry, and then with WT1 levels measured through non standardized quantitative PCR.

In order to analyze antigen expression by flow cytometry, we used a combination of eight monoclonal antibodies, according to the panels and procedures suggested by EuroFlow Group. We analyzed about 1,000,000 events to asses MRD with a sensitivity of 10^{-4}, so MRD was registered as positive when we detected at least 100 events with LAIP, (MRD=0.01%).

Results: Our results revealed:- High concordance between MRD by Flow Cytometry and standardized molecular techniques, with a sensitivity of 88% and a specificity of 72% (Table 1). However, when we compared standardized *vs* non standardized techniques, WT1 showed a sensitivity of 100% and specificity of 47% (Table 2).

Table 1.

MRD	Molecular Markers +	Molecular Markers -	
FC +	15	2	17
FC -	5	13	18
	20	15	35

Table 2.

MRD	Molecular Markers +	Molecular Markers -	
WT1 +	4	0	4
WT1 -	11	10	21
	15	10	25

Summary/Conclusions: 1. Flow cytometry is a useful tool for detecting MRD in AML. However, it is important to establish cutoffs to predict relapse in the different stages of the disease.2. The results regarding WT1 may reflect the lack of standardization in the quantification of the marker, although it shows high specificity for the presence of disease.

PB1612

SEASONAL CLUSTERING OF ADULT ACUTE LEUKEMIAS IN A SUB-TROPICAL AREA OF BRAZIL

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Background: Seasonal peaks in the diagnosis of acute leukemias have been reported in different countries all over the world. These peaks may be caused by temporal changes in environmental factors (pollutant agents, allergens, pathogens) or by the known seasonal changes in the human immune system. Particularly, childhood acute lymphoblastic leukemia (ALL) has been studied, but results are inconsistent. Winter peaks have been found in Sweden, Finland, Iran, and South Africa, but a summer peak has been described in England. Brazil is a large country with latitudes ranging from the equator to sub-tropical areas. The Tropic of Capricorn passes near the city of São Paulo. So, the state of São Paulo has a hot and rainy summer, and a dry winter with days of low temperatures in the night.

Aims: We examined the seasonal trends in adult acute leukemia in our region analyzing the relation between the number of newly diagnosed cases of ALL and AML per month and maximum and minimum temperatures, as well as pluvimetric data during the last 25 years.

Methods: We examined the monthly number of newly diagnosed cases of AML and ALL (age <18 years) and its relation to monthly maximal, minimal and median temperatures as well as the amount of rainfall.

Results: During this period, 1303 AMLs and 236 ALLs entered the study. Concerning ALL, there was a significant positive correlation between the absolute minimum monthly temperature, the pluviometric index of the month of diagnosis, as well as these features in the 3 previous months. In a multiple regression analysis, only the pluvimetric index of the month before diagnosis entered into the final model (r=0.177; p=0.001). Concerning AML, there was a significant positive correlation (r=0.12; p=0.01) only with the absolute maximum temperature of the month of diagnosis. None of the other variables examined were found to be significant.
Summary/Conclusions: We observed a seasonal variation in the diagnosis of adult ALL related to temperature and especially to rainfall in the months preceding the diagnosis. This may be associated with a higher incidence of environmental allergens or infections in the more humid months of the year, leading to the outbreak of the disease, as has also been postulated for childhood and adult ALL in other countries. Concerning AML, a clear relation was found only with actual high temperature peaks. Perhaps, the temperature stress could provoke decompensation of an already existing bone marrow failure making the patients seek for medical assistance. This does not exclude that other environmental factors related to the pathogenesis of AML may act with different intensities in different climatic conditions.

PB1613

PROGNOSIS OF CHILDREN WITH ACUTE HYPERLEUKOCYTIC LYMPHOBLASTIC LEUKEMIA TREATED WITH MODIFIED ST JUDE TOTAL XV PROTOCOL

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Background: Acute leukemias with high white blood count (greater than or equal to 100,000/mm3) have a poor prognosis with an increased risk of early morbidity and mortality.
Aims: In this study we aim to analyse outcome of all complications due to hyperleucocytosis including tumor lysis syndrome, hyperuricemia, renal failure, disseminated intravascular coagulation and treatment modalities of these complications during remission induction beside long term outcome.
Methods: Between January 2008 to December 2015, 206 children with ALL were diagnosed at Hacettepe University Faculty of Medicine, Ihsan Dogramaci Children's Hospital. However 33 were excluded from the study (infant leukemia (n=11), relapse ALL referred to our center and/or initialy treatment was given by another center (n=6), secondary ALL(n=1), mature B cell ALL(n=7), early death (n=7), lost to follow up(n=1)). 173 remained in the study and 20 out of 173 were diagnosed as hyperleukocytic high risk acute ALL.
Results: There were 8 female,12 male with a median age of 5 years old (between 1 to16,7 years). Mean initial WBC conunt is $206x10^3$/µl ($119\text{-}462x10^{-3}$/µl). 9 were T cell immunophenotype however 8 were Calla+B cell and 3 were Calla-B cell phenotype. Initial CNS involvement was 33% among these hyperleukocytic ALL patients. Translocation (9;22) was found to be positive in 2 and they received allogeneic hematopoeitic stem cell transplantation. MRD status by flowcytometer was done on the 15th day of remission induction and all except one was found to be under <0.1%. Relapse was observed 3 out of 20 (15%) in this group(combined relapse BM+CNS(n=1), isolated CNS(n=1), isolated bone marrow (n=1)). However all patients including 3 HSCT patients and relapse patients were in complete remission and alive.
Summary/Conclusions: In our study hyperleukocytic high risk All patients did show slightly improved survival when compared with the literature.

PB1614

THE POTENTIAL EFFECT OF CATHEPSIN B ON THE CLINICAL COURSE OF CHILDHOOD ALL

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Background: Cathepsins belong to the ubiquitous family of lysosomal cysteine proteases responsible for the conversion and degradation of intracellular proteins. In many types of cancer increased overexpression of cathepsin B (CTSB) was observed, in some solid tumors, overexpression of cathepsin B and L is considered a negative prognostic factor. Published pilot data showing a relationship between an increased expression of cathepsin B and L (CTSB, CTSL) and a worse outcome in acute myeloblastic leukemia.
Aims: The aim of this study was to evaluate the potential significance of CTSB expression in childhood ALL.
Methods: CTSB expression was evaluated in bone marrow cells collected at the time of diagnosis in 32 children aged 1.4 to 17.9 years, mean 7.16, 17 boys with newly diagnosed acute lymphoblastic leukemia who were treated at a single center according the protocol ALL IC 2002. In the bone marrow samples taken at the diagnosis RNA was isolated, in a further step of reverse transcription reactions were carried out using a commercially available kit (Applied Biosystems, USA). Determination of the expression was performed by real-time PCR.
Results: For the further analysis the expression above the upper quartile was defined as high. It was observed that the children with high CTSB expression were diagnosed at a younger age (median 6.51 *vs* 2.41 years, p=0.047). There was no correlation between the level of expression of CTSB in leukemia cells and WBC at baseline (p=0.31). The CTSB expression level in leukemia cells had no effect on the activity of L-asparaginase after the first dose (median of 89.42 IU/L *vs* 93 IU/L, p=0.226), early response (status bone marrow 15th day of induction, p=0.256), the response to steroid therapy (p=0.53) and the status of the CNS (p=0.37).
Summary/Conclusions: In summary, this pilot study may suggest the potential importance of CTSB in acute lymphoblastic leukemia in children. Further analysis in a larger study group is needed.

PB1615

HEPATIC VENOOCCLUSIVE DISEASE DURING TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA MANAGED WITH DEFIBROTIDE

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Background: Hepatic venoocclusive disease (VOD) which is the non-thrombotic obliteration of small intrahepatic veins, presents with the clinical triad of jaundice, hepatomegaly, and/or upper right quadrant pain and ascites. Definitive diagnostic tests are not available.
Aims: VOD is a common complication of cytoreductive therapy used for hematopoietic stem cell transplantation (HSCT). However, it may develop during conventional-dose treatment of acute lymphoblastic leukemia (ALL), and even more rarely, during induction treatment.
Methods: We describe four patients in whom VOD developed during induction treatment for ALL.
Results: All of the four cases, three male and one female, were diagnosed with B-cell ALL. One of them developed VOD at the end of his first protocol phase-1 (P1F1), day 31 of his treatment and presented with hepatomegaly, direct hyperbilirubinemia and increased liver transaminases. The patient's ultrasonography of the liver with doppler studies showed minimal ascites and reversal of flow in the portal veins which suggests the presence of VOD. Other three cases developed VOD at second cytarabin (ARA-C) block of the protocol 2 phase 2 (P2F2) and presented with abdominal distension, weight gain, hepatomegaly, ascites, direct hyperbilirubinemia, severe thrombocytopenia, anemia, increase of liver transaminases and abnormalities of coagulation. Defibrotide was started at a dose 25 mg/kg/day and VOD recovered both with clinical and laboratory findings at all patients.
Summary/Conclusions: Hepatic venoocclusive disease not only develops after HSCT, but also may develop during treatment for ALL. All patients with elevated liver enzymes, ascites, hepatomegaly, hyperbilirubinemia and severe thrombocytopenia must be evaluated for VOD.

PB1616

STUDY OF BONE DENSITY AND OSTEOCALCIN LEVEL IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Leukemias are the most common malignant neoplasms in childhood, with acute lymphoblastic leukemia (ALL) accounting for 75% of all childhood leukemias and 30% of all childhood cancers. An increasing number of children survive ALL, approaching approximately 80% over the last 2 decades, as a result of improved and more intense chemotherapy. Musculoskeletal abnormalities are well recognized in children with ALL at diagnosis, during treatment, and persist as long-term sequelae after treatment. Osteotoxic chemotherapy, steroid exposure, poor nutrition, low vitamin D, and poor muscle mass contribute to the development or worsening of bone pathology during therapy that may result in osteoporosis, fracture, and osteonecrosis. Risk-directed therapy has substantially improved treatment outcomes for pediatric ALL, with 5-year survival rates approximating 80% . Given success of this magnitude, it is important to address the management and prevention of bone toxicity. The current study evaluated the bone density and alterations in biochemical mineral status in children with ALL at time of diagnosis, during chemotherapy and after finishing chemotherapy.
Aims: To study Bone density and serum osteocalcin level in children with acute lymphoblastic leukemia.
Methods: Serum osteocalcin and DXA scan were evaluated in 45 children with ALL,15 at diagnosis and before starting chemotherapy, 15 during chemotherapy and 15 after completing their treatment regimen.
Results: From the total 45 patients, 23 (51.1%) had low osteocalcin level and

36 (80%) had low bone mineral density (48% had osteopenia and 32% had osteoporosis).
Summary/Conclusions: Musculoskeletal abnormalities are present in children with ALL at diagnosis, during treatment, and persist as long-term sequelae after treatment.

PB1617

EARLY IDENTIFICATION OF CALM-AF10 MUTATION BY MOLECULAR PROFILING HELPS TO RISK STRATIFY HIGH RISK T-ALL FOR EARLY VUD ALLOGENEIC BONE MARROW TRANSPLANT

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Background: The t(10;11)(p12;q23) and t(10;11)(p12;q14) translocations, which encode the clathrin-assembly protein-like lymphoid myeloid leukemia gene (PICALM)- MLLT10 (formerly CALM-AF10), are recurrent chromosomal rearrangements found in some patients with T-cell acute lymphoblastic leukemia (T-ALL), and are associated with poor outcomes to standard chemotherapy. PICALM-MLLT10-positive cases often have positive expression of CD13, CD33 or CD34, but negative expression of the T-lineage markers CD5, TdT, cCD3 and CD7.
Aims: Mutational analysis on RT-PCR in conjunction with flow cytometry, fluorescent *in situ* hybridization (FISH) and metaphase karyotyping, increases the sensitivity for detecting primary and secondary cytogenetic abnormalities in patients with high-risk T-ALL.
Methods: We report a 34-year-old lady with T-ALL who presented with a mediastinal mass. Blast cells in the bone marrow aspirate demonstrated strong expression of CD7, CD13, CD56, HLA-DR, cytoplasmic CD3, and equivocal expression of CD99. No other T-cell-associated antigens were detectable (including CD1a, CD2, surface CD3, CD4, CD5, CD8, CD16, CD57, CD34 and TdT). The T-Lymphoblastic Leukaemia FISH panel was negative. Chromosomal analysis showed 46,XX,t(10;11)(p13;q14)[5]/46,XX, with balanced reciprocal translocation between the short arm of chromosome 10 and the long arm of chromosome 11. Genome molecular profiling showed recurrent translocation PICALM-MLLT10 [CALM-AF10];t(10;11)(p13;q14) with no disruption of the MLL (11q23) locus, confirming the negative MLL FISH result. The patient achieved complete remission following phase-two induction on the UKALL14 protocol and elected to have a volunteer unrelated donor (VUD) myeloablative allogeneic bone marrow transplant. She remains in complete remission 14 months post-transplant.
Results: The positive outcome for our patient, following allogeneic transplant, reflects similar reports from the UKALLXII/ECOG2993 trial for patients with the MLLT10 [CALM-AF10];t(10;11)(p13;q14) mutation. The use of mutational analysis on RT-PCR early in the diagnostic process, in this case, allowed prompt detection of a high-risk cytogenetic abnormality and more accurate risk stratification that guided the decision to proceed to allogeneic transplant.
Summary/Conclusions: Early identification of high risk cytogenetic abnormality incorporating mutational analysis using RT -PCR along with immunophenotype, FISH and karyotype help to risk stratify patients for allogeneic bone marrow transplant resulting in successful outcome in patients with in high risk T-ALL.

PB1618

THE INCIDENCE OF ACUTE LEUKEMIA IN SOME REGIONS OF THE RUSSIAN FEDERATION

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Background: The incidence of acute leukemia (AL) in US and European countries is about 5 per 100000 according data from cancer registries. Russian Federal Cancer Register (RFCR) reported AL incidence rate across the country as about 3 per 100000.
Aims: To evaluate the age-gender specific incidence, profile and other epidemiologic characteristics AL in Russia regions.
Methods: Russian Hematology Society in 2013 initiated the AL population based registry study in which participated 5 regions of Russia Federation. The primary goal was to pre-registry patients for clinical studies, and secondary - to evaluate the epidemiology of AL. 5 regions were included into analysis of epidemiology statistics. The criteria were the fullness and reliability of AL cases registration. Also, only regions with one big hematological center accumulating all AL cases were chosen. All new cases were recorded on-line into special Web based data capture system.
Results: The registry was started at 1st April of 2013 and is ongoing. 216 new ALL cases from Kirov, Ryazan, Kaluga, Tambov regions and Mordovia republic (4,7 M of population) were included into analysis. The distribution by AL subtypes was following: AML - 131 (60,6%), ALL - 40 (18,5%), APL - 8(3,7%), other - 37 (17,1%). Median age was for AML - 59 (17-85), ALL - 38 (16-80), APL 55 (38-79) years. Gender female/male proportions 121\95.
The incidence rate estimates by region are listed in Table1.

Table.1 Incidence of adult AL in 5 Russian regions.

Region	Adult population (10^6)			Registered cases			Duration	Incidence		
	All	M	F	All	M	F	(y)	All	M	F
Kirov	1.15	0.52	0.63	47	22	25	2.14	1.90	1.98	1.84
Mordovia	0.72	0.33	0.40	52	29	23	2.06	3.49	4.31	2.81
Ryazan	1.01	0.45	0.56	45	17	28	1.90	2.34	1.98	2.63
Kaluga	0.88	0.39	0.48	33	15	18	2.03	1.84	1.86	1.83
Tambov	0.95	0.43	0.52	39	12	27	1.98	2.06	1.41	2.60

The frequencies of AL cases in age strata are distributed as following: 5 (2,3%) - in 15-19 age group; 39 (18%) - in 20-39 age group; 78 (36,1%) - in 40-59 age group; 89 (41,2%) - in 60-79 age group; 5 (2,3%) - in 80-99 age group.
Summary/Conclusions: AL patients registered in the Russian Federation are younger then AL patients of European registries (AML -58years *vs* 71years; ALL - 38 years *vs* 54years, respectively). The significant percentage of unidentified variants AL (17.1%) is a significant problem. We should emphasize that the recorded incidence of AL in our study is quite low. It's close of morbidity estimations of RFCR, but notably lower than European and American registers. This discrepancy can be explained by significantly reduced registration activity and primary diagnostics of AL in the older age groups.

PB1619

PRIMING REGIMENTS BASED ON IDARUBICIN, OR ACLACINOMYCIN OR PIRARUBICIN COMBINING WITH CYTARABINE AND G-CSF FOR REINDUCTION OF REFRACTORY/RELAPSED ALL

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Background: Acute lymphoblastic leukemia (ALL) is one of the most common leukemias with complete remission(CR) rate between 70% and 90%. While the refractory/relapsed ALL is more difficult to treat with poor prognosis. CAG regiment was used for for 6 refractory/relapsed T-cell ALL patients and received high CR rate. So how about CAG regiment for B-cell ALL? What is the situation after the expansion of the cases ? Whether the response rate, adverse reactions would be improved if we use other anthracyclines, such as idarubicin and pirarubicin instead of aclacinomycin for the refractory/relapsed ALL? In our study, the IAG, CAG or PAG regiments were used for refractory/relapsed acute lymphoblasticleukemia respectively The response rate and adverse reactions were elevated.
Aims: To compare the therapeutic effects and adverse reactions of the chemotherapies which were based on idarubicin, aclacinomycin or pirarubicin combining cytarabine and granulocyte-colony stimulating factor as IAG, CAG, and PAG regimens respectively, for refractory/relapsed acute lymphoblastic leukemia.
Methods: The priming reinduction regiment was used to 43 cases with refractory/relapsed acute lymphoblastic leukemia (ALL). IAG, CAG and PAG regiments were non-randomly used to 13, 18 and 12 cases respectively. The clinical effects and adverse reactions of three regiments were evaluated..The factors which might affect the clinical effects were evaluated according to different ages, immunophenotypings and white cell counts.
Results: he overall response rate (ORR) of the priming regiments was 62.8%, with 46.5%(20) of complete remission (CR), 9.6% (7) of partial remissions (PR) and 37.2% (16)of non-remission (NR). 7 (53.8%) CR and 2 (15.4%) PR were observed in the IGA group with the ORR at 69.2%. In the CAG group, there were 8 CR cases (44.4%) and 3 PR cases (16.6%) with the ORR at 61%. While in the PAG group 5 CR cases (41.7%) and 2 PR cases (16.6%) with the ORR at 58.3% were observed. There was no statistical difference of ORRs among the three groups (P=0.837); no statistical difference of the ORRs between the patients over and under 60 years old.(60.0% *vs* 54.3%; P=0.272); no statistical difference was showed of the ORRs between the T-cell ALL and B-cell ALL patients (68.4% *vs* 54.1%; P=0.542) and between hyperleukocytic group and

non- hyperleukocytic group (50% *vs* 64.9%; P=0.808). Major adverse reactions, including bone marrow suppression, infection, gastrointestinal reaction and liver function impairment were tolerable after anti-infection, blood cell increasing and transfusion therapy. 6 patients survived and 12 died in the CAG group, 5 cases survived and 8 died in the IAG group, and in the TAG group, the numbers were 7 and 5. No statistical difference of survival time was found when compared in pairs or simultaneously for the three groups.
Summary/Conclusions: Preliminary results show that the priming chemotherapy regimens based on small doses of different kinds of anthracyclines were effective on refractory/relapsed ALL patients with good tolerance, minor non-hematological toxicity and side-effects.

PB1620

TEL-AML1 POSITIVE CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA-SINGLE CENTRE EXPERIENCE
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Background: The reciprocal translocation t(12;21)(p13;q22) is the most frequent chromosomal rearrangement in childhood B-cell precursor acute lymphoblastic leukaemia (ALL), which results in a chimeric transcription factor TEL-AML1 (ETV6-RUNX1) and is associated with favorable prognosis.
Aims: We performed a retrospective analysis of B-cell ALL patients who were treated in the last five years in our department in order to investigate the incidence, the clinical characteristics and cytogenetic features of TEL-AML1 positive patients.
Methods: Pretreatment bone marrow (BM) samples were successfully analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) for the molecular detection of the TEL/AML1 fusion gene. BM samples were also cultured and analyzed by standard cytogenetic methods. The immunophenotype was determined using a panel of monoclonal antibodies (MoAbs) with flow cytometry.
Results: A total of 51 patients with ALL displayed G-banded karyotypes and were informative for molecular analysis and were included for analysis. All patients were treated according to ALLIC-BFM 2009 protocol. On the basis of EGIL classification, the immunophenotype analysis permitted the characterization of 32 cases (62.7%) as pre-B ALL, 10 (19.6%) as common ALL and 9 (17.7%) as pro-B ALL. The TEL-AML1 fusion gene was identified in 11 patients of which 7 (63.6%) had pre-B, 3 (27.3%) pro-B ALL and 1 (90.1%) common ALL. The incidence of TEL-AML1 fusion gene was 21.6% (11/51 patients). The TEL-AML1 positive patients were studied with regard to their gender and it revealed that 7 were females and 4 males with median age of 4.16 (range:3.41-13.16 years). The median leucocyte count was $8.3x10^9$/L (range $2.6\text{-}67x10^9$/L) According to the presenting features, 91% of the TEL/AML1-positive cases were enrolled in the IR group and 9% in the SR. Moreover, all TEL/AML1-positive cases lacked evidence for BCR/ABL and MLL/AF4 fusion mRNAs. No structural chromosomal changes were noted in TEL-AML1 positive children. Hyperdiploidy of 47-48 chromosomes was encountered in 18.18% (2/11) of the children with TEL-AML1 rearrangement; however, none of TEL-AML1 positive patients had hyperdiploidy of more than 50 chromosomes. All cases were prednisone good responders and they were all very early good responders (minimal residual disease <10-3 at day 15. All patients are in continuous complete remission with event-free and overall survival of 100%.
Summary/Conclusions: Conclusions: The TEL-AML1 fusion gene is a common genetic anomaly in childhood ALL patients and our results are consistent with literature. Further investigation with a larger sample size and for a longer time is warranted.

PB1621

MATRIX METALLOPROTEINASES 2 & 9 AND TISSUE INHIBITOR OF MATRIX METALLOPROTEINASE 1 AS MAJOR PLAYERS IN CLINICAL COURSE OF EGYPTIAN PEDIATERIC ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS
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Background: Acute lymphoblastic leukemia (ALL) is the most frequent acute leukemia affecting pediatric patients. Gelatinase B/matrix metalloproteinase - 9 (MMP9) as well as MMP-2 (gelatinase-A) and their inhibtor Tissue inhibitor of matrix metalloproteinases (TIMP)-1 play a crucial role modulating the biology of the cancer stem cell niche.
Aims: Study the impact of expression of MMP2, MMP9 and TIMP1, as well as, MMP2/TIMP1 and MMP9/TIMP1 ratios on different facets of disease progression in pediatric ALL in terms of laboratory and clinical parameters having prognostic significance, disease-free and overall survival .
Methods: Bone marrow samples were drawn from 53 Egyptian pediatric ALL patients presented to Pediatric Oncology Department, National Cancer Institute, Cairo University. An informed consent in accordance with the Declaration of Helsinki was obtained. Intracytoplasmic MMP-9 FITC, MMP-2 PE and TIMP-1 FITC was done. MoAbs were supplied from R&D system(614 McKinley Place NE, Minneapolis, MN 55413) analysis was done within 24 hours of sampling. Sample analysis was done by multicolor flow cytometry (Coulter Epics XL, Hialeah, USA). Gating strategy was applied using dim CD45/side scatter. Data analysis was done on Winlist 6 (Verity Software House, Topsham, ME).
Results: The study included 53 pediatric ALL patients, 34 males and 19 females, age ranged from 1-18 year with a median of 6 years. A significantly higher total leukocyte count (TLC) among TIMP-1 positive ALL cases and a borderline significantly higher TLC among MMP9 positive ones (P=0.03 and 0.06, respectively) were found. As regards clinical parameters, hepatomegaly was higher among MMP-9 positive cases (P=0.03). Regarding the relation of MMP2 and MMP9 in to their inhibitor; MMP2/TIMP1 ratio was significantly correlated to MMP9/TIMP1 ratio *(p<0.001)*.
ROC curve using MMP2/TIMP2 ratio as greater than or equal to 0.02 will detect MRD D42 response (negative or <0.01) with a sensitivity of 73.7%, specificity of 72.7%, positive predictive value 82.4% and negative predictive value of 61.5%. A low MMP2/TIMP1 ratio correlated significantly with presence of splenomegaly (*p<0.02)*. Patients with MMP2/TIMP1 ratio <2.0% had shorter overall survival with mean 48±3.4 months as compared to those with ratios ≥2.0% *(p=0.04)*.
Summary/Conclusions: MMP2, MMP9, TIMP1expression as well as, MMP2/TIMP1 and MMP9/TIMP1 ratios had influential implication on disease progression and clinical course on pediatric acute lymphoblastic leukemia.

PB1622

OUTCOME OF ADULT PHILADELPHIA ACUTE LYMPHOBLASTIC LEUKEMIA IN THE IMATINIB ERA IN THE SOUTH OF TUNISIA
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Background: The Philadelphia chromosome (Ph), t(9;22), is seen in about 20 to 30% of adults diagnosed with acute lymphoblastic leukemia (ALL). It has been associated with poorer prognosis. Tyrosine kinase inhibitors (TKIs) targeting the *BCR-ABL* oncogenic protein from this translocation have been incorporated into treatment regimens used to treat patients with Ph-positive ALL. In this study we analyzed the clinical, biological features and therapeutic results of patients with Ph ALL treated with chemotheray and Imatinib.
Aims: Determinate the outcome of Philadelphia acute lymphoblastic leukemia in the era of Imatinib in the South of Tunisia
Methods: From January 2007 to March 2015, 10 patients aged 21 to 55 years diagnosis with *de novo* Philadelphia chromosome–positive acute lymphoblastic leukemia, in the department of clinical hematology of Hedi Chaker Hospital, and treated according to the GRAAPH protocol. Cytogenetic analysis and Bcr-Abl transcript was performed for all patients. Imatinib was associated with chemotherapy since 2007 at a dose of 600-800 mg/day. Hematopoietic stem cells transplantation (SCT) was indicated in patients aged under 45 years in complete remission and have a sibling related donor.
Results: Ten patients with *de novo* Ph ALL were treated with the GRAAPH protocol. The median age at the diagnosis was 44 years (21 to 55 years), 6 patients were aged under 45 years. Sex ratio (M/F) was 0,43. The median WBC was 28G/L (3,4 to 104 G/L). Nine patients had a transcript Bcr-Abl positive and one patient had only the t (9,22) without transcript Bcr-Abl. Eight patients (80%) achieved complete remission (CR). Among the 4 patients in CR and with indication to allogeneic SCT, only 3 patients were allograft. Among them, 2 patients are alive in good molecular response after a follow up of 4 and 2 years and the other patient died in post allogeneic transplant with acute graft *versus* host disease (GVHD). The 5 patients who were not allograft, 2 are alive in good molecular response after a follow up of 4 and 6 years and the other 3 patients died with disease progression .
Summary/Conclusions: In the pre-imatinib era, treatment outcomes of adult patients with Philadelphia chromosome (Ph)-positive acute lymphoblastic leukemia (ALL) were dismal. However, imatinib, in combination with conventional chemotherapy, has dramatically changed outcomes, producing approximately 95% complete remission, 80% in our study and 50% overall survival with or without allogeneic SCT.

PB1623

NK-CELL LYMPHOBLASTIC LEUKAEMIA/LYMPHOMA: CLINICAL AND LABORATORY FEATURES, AND PROGNOSIS
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Background: NK-cell lymphoblastic leukemia/lymphoma (NK-LL) is a rare type of acute leukemias. According to the WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues NK-LL is reviewed in chapter "Acute leukaemias of ambiguous lineage".
Aims: To determine clinical and laboratory features and prognosis of NK-LL.
Methods: From 2000 to 2014 only in 1 of 161 (0.6%) patients treated in the department of Hematology of N.N. Blokhin Russian Cancer Research Center was diagnosed NK-LL. Over the same period a diagnosis of NK-LL has been set additionally 3 patients in the Laboratory of Immunology Hematopoietic of N.N. Blokhin Russian Cancer Research Center more. The disease was diagnosed in accordance to the 2008 WHO classification. The differential diagnosis was carried out with other CD56-positive tumors – AML-M0, blastic plasmacytoid dendritic cell neoplasm, T-ALL, and neoplasms of mature NK cells. In the group NK-LL were 4 patients: 3 men and 1 woman. The median age was 52.5 years (ranged from 29 to 82 years).
Results: All patients stated the total bone marrow blast metaplasia (>70%), common extramedullary lesion: generalized lymphadenopathy, hepatosplenomegaly, lesions of skin, tonsils, mediastinal, central nervous system (CNS) by type neuroleukemia. Blasts cytochemical reaction on myeloperoxidase, lipids and nonspecific esterase were negative. In all cases the blast cells were strong positive to CD56 (69.8-99.1%) and the T-associated antigen CD7 (66.2 - 92%), and negative to myeloid, T- and B-lymphoid antigens. PCR identified the lack of gene rearrangement chain T-cell receptor in one patient. Cytogenetic study was not performed. Induction therapy of patients with NK-LL carried out mainly by the program of treatment of acute lymphoblastic leukemia (ALL). CR was achieved in 2 patients, however, proved to be of short-term (1 and 7 months.). The longest remission duration (20 mo.) was obtained using a combined regimen RACOP for the treatment of relapse. Overall survival in the group did not exceed 3 years (1-29 mo.).
Summary/Conclusions: The analysis indicates the low efficiency of current regimens NK-LL. Success in treatment of patients depends on prompt and accurate diagnosis, as well as the development of new therapeutic approaches.

Acute myeloid leukemia - Biology

PB1624

TECHNICAL VALIDATION OF A NEXT-GENERATION SEQUENCING PANEL FOR ACUTE MYELOID LEUKEMIA DIAGNOSIS

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Background: Recurrently mutated genes have been described for acute myeloid leukemia (AML). However, only a few (*NPM1*, *CEBPA*, *FLT3*) have prognostic and clinical implications and are systematically analysed, usually by individual assays. The development of high throughput techniques as next-generation sequencing (NGS) allows a parallel detection of these markers and enables the inclusion of novel molecular targets.
Aims: Our aim is to validate the clinical applicability to routine laboratories of a hotspot NGS panel that includes recurrently validated mutated genes and other potentially actionable targets.
Methods: We included 130 *de novo* AML samples previously characterized for *FLT3*-D835, *NPM1*-T288, *DNMT3A*-R882, and *CEBPA* mutations by conventional molecular biology techniques (CMBT). We tested the Ion Ampliseq AML community panel (Life Technologies) (IAACP) which requires 40 ng of DNA per sample and includes hotspots of *ASXL1, BRAF, CBL, FLT3, IDH1, IDH2, JAK 2, KIT, KRAS, NRAS, PTPN11, RUNX1* and *WT1;* and the entire coding sequence of *CEBPA, DNMT3A, GATA2, TET2 and TP53*. The design does not include the *FLT3*-ITD region. Libraries were sequenced in the Ion PGM (n=12) and the Ion Proton platforms (n=118). For comparison, 3 patients were sequenced in both platforms. To determine if the hotspot approach is adequate, a subset of 25 patients was also analyzed with a custom panel (Sure Design Tool (Agilent)) that included the complete coding sequence of the genes included in the IAACP except from *CBL* and *GATA2*. Libraries were prepared with 250 ng of DNA per sample and sequenced in an Illumina platform.
Results: The IAACP sequenced in the Proton platform yielded a mean depth of 5916 reads, mean uniformity was 92.19% and mean on-target reads 89.05%. Two amplicons (RUNX1_239.66909 and ASXL1_235.1.76662) showed a strong strand bias (x5) towards the forward strand systematically. The PGM platform yielded a mean depth of 2500 reads, mean uniformity of 91.86% and mean on-target reads of 93.39%. GATA2_47.1.16110 and DNMT3A_23.66955 amplicons showed a strong bias towards the forward strand (100X) and the reverse strand (42X), respectively. For both platforms we observed intra-run differences regarding read depth and strand bias, however, none of the 239 amplicons showed a mean depth lower that 250X (Figure 1). Patients sequenced in both platforms yielded the same mutations. IAACP found 100% of the previously known mutations by CMBT plus 11 extra mutations that were negative by CMBT. These mutations were reconfirmed by Sanger sequencing. Among the 25 patients analysed with the custom panel, 53 variants were found. Fifty (95%) were also detected with the IAACP. The remaining 3 variants (5%) were located outside the hotspot regions in genes with poor clinical significance. Additionally, the IAACP detected 13 variants in the overlapping regions not found by the custom panel.

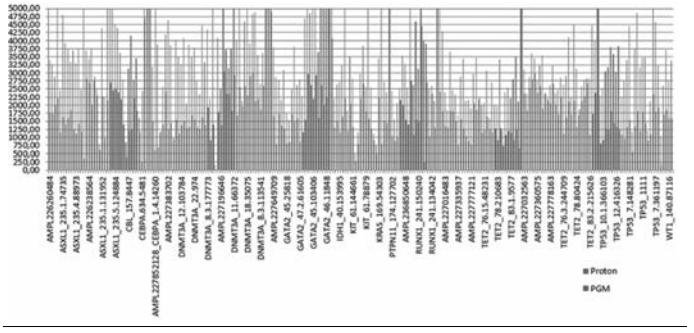

Figure 1.

Summary/Conclusions: In conclusion, the IAACP is reliable and reproducible for routine diagnosis. It allows detection of known important mutations with increased sensibility compared with CMBT, and also detection of complementary mutations with potential clinical implications. The main limitation of its design is the absence of amplicons covering *FLT3*-ITD region. The hotspot approach yields a higher mean read depth than the whole coding sequence analysis without significant losses and it requires less input DNA. Moreover, analysis of strand bias and low coverage regions is mandatory to assess sequencing quality and to perform extra assays if necessary.
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PB1625

MUTATIONAL ANALYSIS OF ACUTE MYELOID LEUKEMIA WITH A NEXT-GENERATION SEQUENCING PANEL

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Background: In the last years numerous recurrently mutated genes have been described for acute myeloid leukemia (AML). Although only *CEBPA*, *NPM1* and *FLT3-ITD* have been incorporated to risk stratification algorithms, other genes like *RUNX1*, *ASXL1, DNMT3A* or *TP53* are currently being evaluated and could be soon incorporated. Furthermore, *FLT3*, *IDH1/2* or *KRAS* are being tested as potential therapeutic targets. Next generation sequencing (NGS) assays allows a parallel detection of these genes.
Aims: The aim of this study is to characterize the mutational status of a series of "*de novo*" AML patients using a NGS panel that includes the hotspots of most recurrently mutated genes and other potentially actionable targets.
Methods: We studied 130 AML patients diagnosed from June 1999 to June 2014 in a single centre. Inclusion criteria were age <65 years, diagnosed with non-promyelocytic AML, treated according PETHEMA AML clinical protocols and with available DNA sample at diagnosis. Ion Ampliseq AML community (IAAC) panel was employed for mutation detection. This panel includes hotspots of *ASXL1 (*exon 12*), BRAF* (V600E)*, CBL* (exons 8-9)*, FLT3* (codons 676, 830-850)*, IDH1* (exon 4)*, IDH2* (exon 4)*, JAK 2* (exon 14*), KIT* (exons 8, 10, 11 and 17)*, KRAS* (exons 2-4)*, NRAS* (exons 2-4)*, PTPN11* (exons 3,7,8,13)*, RUNX1* (exons 3-8) and *WT1* (exons 7 and 9)*,* and the entire coding sequence of *CEBPA, DNMT3A, GATA, TET2 and TP53*. Libraries were amplified with Ion Torrent Ampliseq 2.0 beta and sequenced in the Ion PGM or Proton platforms. Variant annotation was carried out with the Ion Reporter Software (Life Technologies). Polymorphisms, synonymous, low depth read mutations (<100X) and low read% variants (<5%) were filtered out. Moreover, regions with intrinsic strand bias or low coverage were curated with the Integrative Genomics Viewer (IGV). *FLT3*-ITD mutations, which are not included in the panel design, were detected by capillary electrophoresis (Thiede 2002 Blood).
Results: Regarding cytogenetics, 9 patients showed favourable risk, 82 with intermediate risk (70 normal karyotype) and 23 unfavourable risk. For the remaining 6 patients cytogenetics could not be assessed. The IAAC panel found 322 variants in 125 patients. In brief, 59 *NPM1* mutations, 49 in *DNMT3A* (30/49 mutations (61,22%) were the R882 mutation), 36 in *TET2*, 31 in *CEBPA* (13 patients showed biallelic mutations), 21 in *RUNX1*, 18 in *NRAS*, 16 in *IDH2*, 15 in *FLT3*, 15 in *TP53*, 11 in *GATA2*, 11 in *PTPN11*, 11 in *ASXL1*, 8 in *WT1*, 7 in *IDH1*, 7 in *KRAS*, 4 in *KIT*, 2 in *CBL* and 1 mutation in *BRAF* (Figure 1). Moreover, 31 *FLT3*-ITD mutations were detected by conventional techniques. The average of mutations per patient was 2.71 (range 0-8). Only 5 (3.8%) remained wild type. *NPM1*, *DNMT3A* and *FLT3* (including *FLT3*-ITD) mutations were significantly enriched in the intermediate risk group (p<0.001 p=0.002, p=0.004, respectively), while *TP53* mutations aggregated in the unfavourable karyotype group (p<0.001). *NPM1* mutations significantly concurred with *DNMT3A* and *FLT3* mutations (p<0.0001, p<0.0001, respectively), and these three genes are mutually exclusive with *TP53* (p<0.001; p=0.005, p=0.011) and *RUNX1* (p=0.001, p=0.018, p=0.047) mutations. *NPM1* and *FLT3* were also mutually exclusive with *GATA2* (p=0.010 and p=0.016). *NPM1* and *CEBPA* were mutually exclusive (p=0.036), as well as *FLT3* with *NRAS* (p=0.014), and *ASXL1* with *NRAS* (p=0.038). *NPM1* and *PTPN11* mutations showed concurrence (p=0.012) (Figure 2).
Summary/Conclusions: IAAC panel detects mutations with validated prognostic relevance (*NPM1* and *CEBPA*) and other mutations with probable diagnostic or prognostic value and/or potential therapeutic targets are also studied and identified in one assay. The parallel study of numerous mutations allows the identification of recurrence and exclusivity patterns.

PB1626

MYELOID-SPECIFIC EXPRESSION OF SOX4 INDUCES EXPANDED MYELOPOIESIS WITH RETARDED CELL DIFFERENTIATION - A NOVEL ZEBRAFISH LEUKEMIA MODEL

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Background: SOX4 belongs to a family of Sox (SRY-related HMG-box) transcription factors, including Sox4, Sox11, and Sox12. Abnormal expression of SOX4 is related to malignant transformation and cancer metastasis. Overexpression of SOX4 is associated with clonal dominance of hematopoietic stem cells, repopulation advantage of various stem/progenitor cells, block in differentiation of myeloid progenitor cells, and induction of myeloid leukemia. Recently, the expression of SOX4, as a direct target of C/EBPα, is reported to inversely correlate with C/EBPα activity. Downregulation of SOX4 attenuates self-renewal capability of leukemic cells and restored their normal differentiation process. SOX4 overexpression resulting from C/EBPα inactivation contributes to the development of leukemia with a distinct leukemia-initiating cell (LIC) phenotype. In addition, several fusion proteins resulting from chromosomal aberration, such as *MOZ-TIF2*, *AML1-ETO*, *UNP98-HOXA9*, were closely linked to SOX4 upregulation, further suggesting the significance of SOX4 in leukemogenesis. Zebrafish is a popular animal model in biomedical researches. Here, we add to its strength for leukemia research by providing a novel transgenic SOX4 zebrafish model.
Aims: To establish SOX4 transgenic zebrafish research model.
Methods: By using Multisite Gateway system® and Tol2 transposon technology, we established a construct including *spi1* promoter, human *SOX4* gene and *EGFP*. This construct was then injected into zebrafish embryos. After a series of fluorescent selection, stable transgenic *SOX4* zebrafish - Tg(CG-*spi1-SOX4-EGFP*)were generated. The expression of hematopoiesis-related transcription factors were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR) or whole-mount *in situ* hybridization (WISH) technique. The histological pictures of kidney marrow (KM) were examined by light microscope
Results: We found that *SOX4* could be detected at 20 hpf (0.2~1.7x10^3 copies), and then increased gradually during development. At 5 dpf, level of *SOX4* was up to 2~10x10^3 copies. However, there were no significant differences in the expression of hematopoiesis-related transcription factors, such as *csf1r*, *cebpa*, *l-plastin*, *mpeg1*, *mpo*, *runx1* and *c-myb* in spite of the expression of *SOX4*. These results indicated that the *SOX4* transgenic zebrafish had normal hematopoiesis in the larval stage. However, in comparison with age-matched AB-wild type fish, the KM of 5-month old transgenic zebrafish Tg(CG-*spi1-SOX4-EGFP*) had a greater number of myeloid progenitors (9.24±2.75% *vs* 5.27±0.74%; p<0.05); those of 9-month old had a greater number of myeloid progenitors (18.00±8.78% *vs* 5.73±0.64, p<0.01), less number of mature erythroid cells (19.55±5.98% *vs* 27.93±6.03%, p<0.05) and greater M:E ratio (10.39±7.81 *vs* 3.72:1±0.53, p<0.05); those of 12-month old had significant difference in all blood components (17.61±8.07% *vs* 7.00±1.10% in myeloid progenitors; 62.06±7.82% *vs* 42.07±3.18% in myelomonocyte/neutrophil; 4.11±0.72% *vs* 16.20±1.12% in lymphocytes; 5.44±1.45% *vs* 10.06±2.55% in immature erythroid cells;10.78±3.40% *vs* 4.61±0.64% in mature erythroid cells and M:E ratio of15.77±5.34 *vs* 4.61±0.64, p<0.05 for all above).
Summary/Conclusions: These results indicate that *SOX4* transgenic zebrafish develops a gradual expansion of myeloid cells, which fail to differentiate, and ultimately transform into myeloid leukemia.

PB1627

Abstract withdrawn.

PB1628

A COMBINED APPROACH TO DETECT RARE FUSION EVENTS IN ACUTE MYELOID LEUKEMIA

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Background: A complex network of events characterizes Acute Myeloid Leukemia (AML), including point mutations, epigenetic modification, copy-number alterations, gene-expression alterations and fusion events. A recent study revealed that 45% of AML patients carry one fusion gene and 50% of detected fusions were novel and not recurrent events (TCGA, N Engl J Med; 2013). However, the leukemogenic potential of these fusions and their prognostic role are still unknown.
Aims: The aim of the study was to identify rare gene fusions having a causative role in leukemogenesis and which may be a target for personalized therapy in AML cases with rare chromosomal translocations.
Methods: RNAseq was performed on 4 AML patients (#59810, #20, #84 and #21, Hiseq1000 Illumina) and deFuse and Chimerascan was used to detect fusions. Putative chimeras were prioritized according to cytogenetic analysis. In addition, we exploited Pegasus and Oncofuse to select biologically relevant fusions cryptic at cytogenetic analysis. Selected fusions were validated with Sanger sequencing.
Results: The reliability of our bioinformatic analysis was confirmed thanks to the detection of the CBFβ-MYH11 transcript in the sample #84 associated to the inv(16). We detected two in-frame fusion genes in sample #20: CPD-PXT1

which appeared as the reciprocal fusion product of t(6;17) translocation, and SAV1-GYPB, which remained cryptic at cytogenetic analysis. The first chimera involved a metallocarboxypeptidase and a partner whose biological function is unknown. The second chimera involved the oncogene SAV1, a central player of the Hippo pathway, which controls the proliferation and promotes the apoptosis. The fusion event causes the loss of the protein-protein interaction domain of SAV1, which is fundamental for its stability. Sample #21 carried an in-frame fusion transcript involving the genes *OAZ1* and *MAFK*. The first gene is involved in the polyamine synthesis, while the latter one is a transcriptional regulator. The putative fusion protein was formed by the *cis*-acting elements sensitive to polyamine levels of *OAZ1* and the bZIP domain of *MAFK*. We detected two fusion transcripts in sample #59810: ZEB2-BCL11B, associated with translocation t(2;14), and CNOT2-WT1, associated with t(11;12). In particular, three different isoforms of the in-frame chimera ZEB2-BCL11B were expressed: the full length (Isoform 1), the Isoform 2 with one exon of *BCL11B* spliced and the Isoform 3 with 2 exons of *BCL11B* spliced. Gene expression profiling showed an upregulation of *ZEB2* and *BCL11B* transcripts in the patient's blasts, compared to 53 AML samples with no chromosomal aberrations in the 14q32 region. On the contrary, CNOT2-WT1 was an out-of-frame gene fusion and the reciprocal of the fusion revealed the intron retention of *CNOT2*.

Summary/Conclusions: Our data suggest that fusion events are frequent in AML and different approaches, including G-banding, molecular biology, bioinformatics and statistics, need to be integrated in order to better understand AML pathogenesis. The lack of recurrence in the landscape of AML gene fusions confers heterogeneity to the pathogenesis of the disease and we are further investigating the pathogenetic potential of the identified translocations, in order to identify common patterns of oncogenic events, which may be exploited to stratify patients and tailor personalized therapies.

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PB1629

PDCD1 EXPRESSION AND SNP ANALYSES IN AML, MDS AND S-AML PATIENTS

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Background: One of the potential mechanism responsible for evading cytotoxic T lymphocytes by tumor cells might be the programmed death-1 receptor (PD-1) signaling pathway. PD-1 and its ligand PD-L1 play a key role in tumor immune escape and the formation of tumor microenvironment, promoting tumor development. Recent findings showed elevated expression of PD-1 and PD-L1 on CD34+cells in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) patients suggesting that deregulation of PD-1/PD-L1 axis may contribute the MDS pathogenesis. Moreover, in patients treated with epigenetic therapy PD-L1 and PD-1 expression was upregulated indicating that induction of PD-1 and PD-1 ligands may be involved in hypomethylating agents (HMAs) resistance. *PDCD1* polymorphisms are associated with susceptibility to several types of cancer.

Aims: We aim to characterize the *PDCD1* expression and six *PDCD1* polymorphisms in AML, MDS and s-AML patients samples as well as determine whether expression of PD-1 in those patients is driven by the genetic background.

Methods: *PDCD1* mRNA expression was assessed in bone marrow mononuclear cells (BMMC) of 62 MDS, 54 AML and 8 s-AML patients samples using qRT-PCR method. Six SNP for PD-1 that demonstrate relevant associations with a higher risk of developing autoimmune diseases were assessed in AML, MDS and s-AML patients as well as 100 healthy volunteers (HVs): PD-1.1 (rs36084323), PD-1.3 (rs11568821), PD-1.5 (rs2227981), PD-1.6 (rs10204525), PD -1.7 (rs41386349), PD-1.9 (rs2227982). Moreover, immunohistochemical (IHC) stainings of 12 AML and 8 MDS bone marrow smears for PD-1 protein were performed.

Results: We observed significant decreased *PDCD1* expression in AML group compared to HVs and MDS, (median: 0.0001 *vs* 0.0003, p<0.001 and 0.0001 *vs* 0.0003, p<0.001) but no differences in MDS and s-AML groups compared to HVs. IHC stainings showed PD-1 expression on blast cells in 6/12 AML and 6/8 MDS cases ranging from 1-92%. AML patients were stratified into three genetic groups according to prognosis: favorable risk, intermediate I risk and intermediate II/adverse risk and *PDCD1* expression was compared between these groups. No significant differences were found between these groups. MDS patients were divided according to IPSS scoring systems into two groups: first with IPSS ranging from 0.0 to 0.5 and second with IPSS from 1 to 3. No significant difference were observed between these groups in case of *PDCD1* expression (median: 0.0004 *vs* 0.0002, p=0.11). We observed significant differences in *PDCD1* expression level regarding to PD-1.1.5 polymorphism. Moreover, analysis of a PD-1.1.3 polymorphism in HVs and MDS groups revealed that genotype GG was associated with nearly fivefold lower risk of disease (OR=4.93, p=0.009). We observed significant differences in OS in AML patients in case of presence of certain genotypes of PD-1.1.6. Genotype AA was significant associated with higher risk of shorter OS compared to the rest of the genotypes (58 *vs* 333 days, HR=35; p=0.0188).

Summary/Conclusions: We found significant differences in *PDCD1* expression in AML and MDS patients that might indicate deregulation of a signal transduction through the PD-1/PD-L1 axis. By IHC stainings we were able to determine PD-1 expression on blast cells in 6/12 AML and 6/8 MDS bone marrow smears. Moreover, SNP analysis in AML patients revealed potential prognostic impact of PD-1.1.6 polymorphism.

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PB1630

MOLECULAR CYTOGENETIC ANALYSIS OF COMPLEX KARYOTYPES IN PATIENTS WITH MDS AND AML

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Background: Cytogenetic findings at diagnosis are among the most important independent prognostic factors in patients with MDS and AML. Complex karyotype involving three or more chromosome abnormalities is associated with poor prognosis and disease progression. Precise identification of chromosome abnormalities in complex karyotype by conventional cytogenetic analysis (CCA) is limited due to low resolution of this method. Molecular cytogenetic techniques such as fluorescence *in situ* hybridization on interphase cells nuclei (FISH), multicolor fluorescence *in situ* hybridization on chromosomes (mFISH) and multicolor banding (mBAND) have significantly higher sensitivity and allow to identify complex chromosome abnormalities, marker chromosomes, submicroscopic deletions and specify chromosomes breakpoints.

Aims: The aim of our study is to characterize complex karyotypes in patients with MDS and AML using combination of molecular cytogenetic techniques.

Methods: Over a 2-year period CCA of bone marrow samples was performed in 234 patients with MDS and 83 patients with AML at diagnosis. Complex karyotypes were revealed in 20 patients (9 males and 11 females, median age 55 years), 11 patients with MDS (4.7%) and 9 patients with AML (10.8%). mFISH (24XCyte, MetaSystems) was performed in all 20 patients. Based on the results of CCA and mFISH we analyzed abnormal chromosomes using FISH with locus-specific and centromere DNA probes (LSI EGR1/D5S23, D5S721 Dual Color Probe Set in 12 cases, TP53/CEP 17 FISH Probe Kit in 10 cases, D7S522/CEP 7 FISH Probe Kit in 9 cases, CEP 8 SpectrumOrange DNA Probe Kit, ATM/CEP 11 FISH Probe Kit, LSI MLL Dual Color, Break Apart Rearrangement, D20S108 FISH Probe Kit, 1p36 Microdeletion Region Probe – LSI p58 (1p36)/TelVysion 1p/LSI 1q25 in each case one, ABBOTT), as well as mBAND probe (XCyte mBAND 5, XCyte mBAND 7, XCyte mBAND 15, XCyte mBAND 17, MetaSystems).

Results: CCA revealed an average of 4 karyotype abnormalities (from 3 to 14). Structural chromosome rearrangements were found in 20 cases: random translocations in 5 cases (25%), all of them were defined as simple reciprocal translocations, additional material of unknown origin in 12 cases (60%), marker chromosomes in 11 cases (55%). Non-random structural rearrangements were found in 3 patients: t(6;9) – 1, del5q31 -1 and del7q31 - 1 case. Most frequent numerical abnormalities were typical for MDS and AML trisomy 8 – 5 cases (25%), monosomy 5 - 8 (40%), 7 - 7 (35%) and 17 - 3 cases (15%). Molecular cytogenetic analysis revealed additional chromosome aberrations and/or additional chromosome breakpoints in 17 of 20 cases (85%). Translocations were found in 18 cases (90%): 9 (50%) – simple reciprocal translocations, 4 (22%) – complex translocations involving 3 chromosomes, 3 (16,5%) - complex translocations involving more than 3 chromosomes. In karyotype of 17 patients (85%) we found abnormalities of chromosomes 5 (60%), 7 (50%), 11 (20%) and 17 (45%). Except one case, chromosome breakpoints were identified in typical regions of known or potential genes: 5q31, 7q31, 11q23 and 17p13. Deletions 5q and 7q were confirmed in both cases. Monosomy 7 was confirmed in two of seven cases only. In all other cases with monosomy 5, 7, and 17 on the results of conventional analysis mFISH revealed fragments of these chromosomes involved in translocations. Based on the results of FISH all of these translocations, except one case, were combined by deletion of loci 5q31, 7q31 and 17p13. All marker chromosomes and chromosomes with additional material of unknown origin were recognized as complex translocations or derivative chromosomes with breakpoints in both arms.

Summary/Conclusions: In our study applying of molecular cytogenetic methods allowed to identify all numerical and structural abnormalities, unrecognized at CCA, clarify chromosome breakpoints and determine markers chromosomes origin. True monosomy 7 was confirmed in 10% only, and for chromosomes 5 and 17 true monosomy is not found. Combination of conventional and molecular cytogenetic techniques (FISH, mFISH and mBAND) is necessary for precise characteristic of complex karyotypes in MDS and AML and determination of exact breakpoints loci of potential oncogenes and tumor suppressor genes.

PB1631

GENOMIC CHARACTERIZATION OF PATIENTS WITH LOW-RISK ACUTE MYELOID LEUKEMIA (AML)

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Background: Not all acute myeloid leukemia (AML) patients classified at diagnosis as cytogenetic and/or molecular favorable risk display a good outcome. In fact, up to 40% of patients with core binding factor (CBF) AML will relapse and not all may be cured. In the same way, a half of NPM1 mutated patients have a relapse during the first 3 years after diagnosis and only biallelic disruption of *CEBPA* (biCEBPA) is required for a favorable outcome that not avoid a relapse rate of 44%. However, mutations responsible for poor outcome in these previously low-risk classified subsets have not been clearly defined.
Aims: 1) To determine the mutational profile of favorable risk AML: CBF-AML, NPM1-mutated AML lacking FLT3-ITD and biCEBPA AML. 2) To identify mutations at diagnosis responsible for poor outcome by comparing patients who experiment relapse *vs* patients who do not relapse.

Table 1.

Variable	n median (range)
n	**46**
Sex (Female/Male)	20/26
Age, years	55 (14 - 79)
Leukocytes, 10^9/L	30 (1 - 213)
Blasts BM, %	60 (13 - 96)
Cytogenetic risk	
Low	**18**
inv(16)	11
t(8;21)	7
Intermediate	**28**
Normal karyotype	28
FLT3-ITD, n	**0**
Other mutations	
NPM1	26
biCEBPA	2
Response to induction treatment	
Complete remission (CR), n	**46**
Time to 1st CR (months)	1 (0.5 -2.7)
First relapse, n	**17**
Time to 1st relapse (months)	8.7 (2.6 - 59)
Stem cell transplantation in 1st CR	**24**
Autologous	9
Allogeneic	15

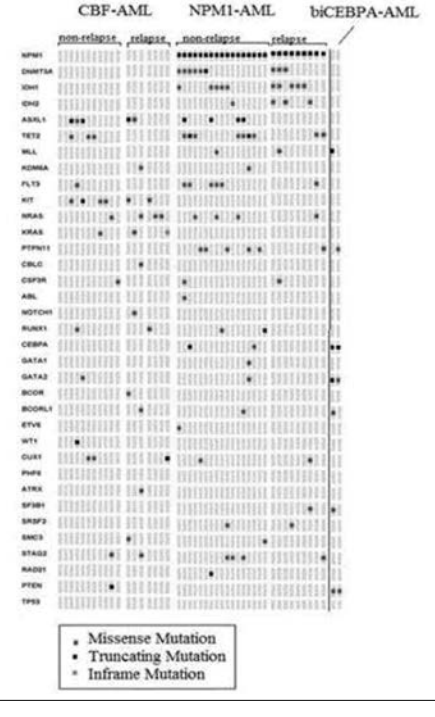

Figure 1.

Methods: A cohort of 46 patients with favorable-risk AML (median age 55 years) (PETHEMA AML-99-2010) (Table 1). FLT3-ITD mutations were analyzed by fluorescent PCR and capillary electrophoresis. None of the patients harbored FLT3-ITD. A total of 54 genes were targeted by 568 amplicons that ranged from 225 to 275 bp. The combined coverage was 141 kb in sequence length. Amplicon libraries were prepared by TruSight Myeloid sequencing panel (Illumina, CA) and paired-end sequencing runs were performed on a MiSeq (Illumina) genome sequencer. Minimum depth for reliable analysis was fixed in 100x. Sequences obtained were analyzed with the Variant Studio v2.1 software (Illumina).
Results: We found 144 mutations in 44 of 46 patients of the global series (3.3 (0-8 mutations/patient) with a mean read depth of 8230x. Only 2 patients remained wild-type for all analyzed genes. Figure 1 displays the mutational distribution of the patients who suffered relapse and patients who do not relapse in the three groups studied. The CBF group (n=18) showed a high frequency of KIT mutations (33%) that were not present in the other low-risk groups. Seven patients harbored NRAS/KRAS mutations (39%), showing a great involvement of RAS pathway in this subset. Other relevant genes affected were ASXL1 and CUX1 (28% and 17%, respectively). The NPM1 mutated group (n=26) presented a high incidence of DNMT3A, IDH1 and IDH2 mutations, as described previously (35%, 38% and 15%, respectively). Moreover, a 23% of these patients carried mutations in the phosphatase PTPN11 and a 15% in the cohesin complex gene STAG2. The two biCEBPA mutated patients showed altered variants in the transcriptional regulator GATA2. Regarding the prognostic value of these alterations, we found that patients with a better course of the disease had a lower frequency of mutations in NRAS/KRAS genes. Therefore, we analyzed the prognostic value of these variants in the CBF group and it was correlated with a trend to a shorter relapse free survival (3 years, 86% *vs* 42% p=0.125). With respect to KIT mutations we did not find significant differences in their clinical adverse impact since 5 of the 6 mutated patients were in first complete remission probably because they underwent stem cell transplantation. Concerning the NPM1 group, mutations were similar in patients with a good outcome and patients who relapse.
Summary/Conclusions: This technology is able to find altered variants with high accuracy in AML patients. Although based on small numbers of patients, we observed a clear alteration of the RAS pathway in CBF patients, which suggests the need of further studies about new alternatives to standard chemotherapy in NRAS/KRAS mutated patients.

PB1632

CLINICAL RELEVANCE OF RECURRENT ALLELE-SPECIFIC RECOMBINATION EXPRESSING THE WNT10BIVS1 ALLELE VARIANT IN ACUTE MYELOID LEUKEMIA

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Background: The WNT/β-catenin pathway play a critical function in the regulation of cell proliferation, differentiation, and apoptosis, playing a major role in fueling stem cell activity and sustaining tissue regeneration in a dose dependent manner in several adult stem cell niches including bone marrow. Previous results obtained by our research team provided direct evidences that WNT/β-catenin signaling is diffusely activated in the $AC133^+$acute myeloid leukemia (AML) cells. The mRNA *in situ* detection analysis revealed that WNT10B results to be expressed in leukemic blasts as well as in stromal-like cells, suggesting an autocrine/paracrine mechanism of Wnt signaling induction in the leukemic bone marrow microenvironment. Conversely, the activation of Wnt signaling, marked by expression of the dephosphorylated β-catenins, is restricted only to a smaller subpopulation of $AC133^{bright}$ cells.
Aims: In the present study, focusing our attention on the major locus associated in hematopoiesis to the regenerative function, we provide evidences for a recurrent rearrangement involving the WNT10B locus within intron 1 (IVS1). Moreover, we demonstrated the consequent expression of a non-physiological transcript variant, $WNT10B^{IVS1}$, retaining 77 nucleotide of IVS1 and lacking exon1, and we analyzed the significance in AML.
Methods: In order to provide accurate quantification of mRNA levels of WNT10B and the related $WNT10B^{IVS1}$ transcript variant and analyze the clinical relevance of their expression, we carried out the gene expression analysis by Droplet Digital™ PCR on mononucleated cells derived from n=125 AML patients [*de novo*, N=118 (intermediate-adverse risk N=70; favorable risk N=48); therapy-related, N=7, informed consent was obtained]. Analyzing patients according to the three main scoring systems, formerly the Medical Research Council (MRC), European LeukemiaNet (ELN), and National Comprehensive Cancer Network (NCCN), we were able to distinguish groups of patients at different outcomes with a statistically significant wise.
Results: We observed that canonical WNT10B mRNA was highly expressed in all *de novo* AML patients here examined, while $WNT10B^{IVS1}$ mRNA transcript levels resulted undetectable in patients classified with favorable-risk (p <0.001).

Furthermore, we demonstrated absence of both WNT10B and WNT10B^{IVS1} expression in therapy-related patients (p <0.005). Moreover, we have also evaluated the effects of transient *wnt10b* overexpression on early hematopoiesis in the zebrafish model and therefore, to this aim *wnt10b* synthetic mRNA was microinjected in the zebrafish zygote to mimic, *in vivo,* the Wnt signaling overexpression we had previously observed in AC133^{bright} AML cells. Interestingly, an increase of erythromyeloid progenitors, and a simultaneous reduction in the number of circulating neutrophils is detected.
Summary/Conclusions: The results presented here provide compelling evidence that regeneration-associated Wnt signaling exceeds the homeostatic range in the majority of human AML cases. Taking these issues into consideration, we revealed that it is possible to recognize three distinct WNT10B/ WNT10B^{IVS1} patterns, suggesting a potential role of WNT10B^{IVS1} transcript as a marker for the intermediate-adverse risk AML patients. These findings, if confirmed in a larger population of patients, may help to refine diagnostic or prognostic criteria for previously described neoplasms, and to introduce newly recognized disease entities possibly characterized by distinct causative pathogenic mechanisms.

PB1633

ANALYSIS OF CLONAL IMMUNOGLOBULIN AND T-CELL RECEPTOR GENE REARRANGEMENTS IN ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML), the most common form of leukemia, carries a high mortality rate and economic burden. In 2015, approximately 39,130 individuals were diagnosed in the United States and Europe with roughly half that number dying from the disease (1,2). With the advent of more sensitive molecular assays, the complex architecture and functional heterogeneity of AML has become appreciated though not yet fully elucidated. Fundamentally, understanding the hematopoietic stem cell (HSC) self-renewal and differentiation model will aid in this goal. Initial hierarchical hematopoietic models focused on the lymphoid and myeloid lineage groupings to be segregated. Recently studies suggest that lineage commitment of hematopoietic progenitors may be both multidirectional and reversible with changes in lineage caused by both intrinsic and environmental factors (3). Though AML is classified as a myeloid neoplasm, we were interested in determining the prevalence of clonal rearrangements within the immunoglobulin heavy (*IGH*) and light chain (*IGK*), T-cell receptor gamma (*TRG*) loci in AML patient samples.
Aims: Assess the frequency of *IGH*, *IGK*, and *TRG* rearrangements in AML patient samples.
Methods: DNA was extracted by QIAcube from a random sampling of 200 AML anonymized patient residual peripheral blood (PB) or bone marrow (BM) specimens. DNA was quantified by NanoDrop and normalized. Each DNA sample was tested for 6 different PCR master mixes: IdentiClone *IGH* Tubes A, B, C, which target the framework (FR) 1, 2, and 3 regions, respectively; *IGK* Tube A, *IGK* Tube B, and *TRG* 2.0. Amplicon products were analyzed using the ABI 3500 XL instrument. Based on the florescent signals, clonal (positive) or polyclonal (negative) were assessed per instructions of use accompanying the IdentiClone assay.
Results: The IdentiClone *IGH* assay identified 23 (12%), 14 (7%) and 16 (8%) clonal positive samples for FR 1, 2 and 3, respectively. Combining all three *IGH* tubes increased the clonal detection rate to 28 (14%) with 172 (86%) samples determined to be negative. The IdentiClone *IGK* assays identified 39 (20%) and 29 (15%) clonal positive samples for Tube A and Tube B, respectively. Combining the two *IGK* tubes increased clonal detection to 56 (28%), with 143 (72%) determined to be negative. Combining all 5 *IGH+IGK* tubes, 65 (33%) clonal positive samples and 135 (68%) clonal negative samples were identified. The *TRG* 2.0 assay detected 85 (43%) clonal positive samples and 114 (57%) clonal negative samples. Overall, using 6 tubes of PCR MM across *IGH*, *IGK* and *TRG* assays, 113 (57%) samples were identified as clonal positive and 87 (44%) samples were identified as clonal negative.
Summary/Conclusions: Over 50% of AML samples demonstrated at least one clonal IG or *TCR* gene rearrangement. Although clonal rearrangements occur in individuals over 60 years of age, clonal rearrangement assays combined with outcome data for AML patients may provide clinical utility and further elucidate functional heterogeneity.

PB1634

GPC4, THE GENE OF SIMPSON-GOLABI-BEHMEL SYNDROME, IS AMPLIFIED ON EXON 9 AND REVEAL A POSSIBLE PATHOGENETIC ROLE FOR APL PROGRESSION OR RELAPSE.

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Background: Cytogenetical and molecular analysis of acute promyelocytic leukemia (APL) have improved the knowledge about the genomic mechanism at the origin of this pathology identifying t(15;17) and the fusion gene PML-RARa. New targeted therapies were introduced in treatment of APL, as all-trans retinoic acid (ATRA) and arsenic trioxide. Even though these therapies, a 10% of patients (pts) still relapse. Single Nucleotide Polymorphism (SNP) microarray can detect cytogenetic lesions mostly involving structural alterations with losses or gains of chromosomic material. These abnormalities could be predictive of response and can help define therapeutic strategies. SNP microarray can also detect copy-neutral loss of heterozygosity (CN-LOH) or Uniparental Disomy (UPD), relevant to induce oncogene duplication, tumor suppressor inhibition and epigenetic reprogramming.
Aims: To improve conventional cytogenetic analysis and identify new genomic abnormalities that underlie the pathogenesis of APL, we perform SNP array-based genotyping.
Methods: We performed SNP 6.0 and Cytoscan HD Array (Affymetrix) in 23 APL (21 *de novo* and 2 relapsed) and then analyzed by Nexus Copy Number (BioDiscovery, v.7.5) and R-Bioconductor. This method reveal Copy Number Alteration (CNA) and CN-LOH that standard cytogenetic analysis could not detect. Briefly, CNA were filtered for variants already annotated in Database of Genomic Variants (DGV). Furthermore, CNA less than 1 kb were removed. Significant (p<0.05) genes in which CNA events occur were summarized per patients. Moreover, commons CNV regions among patients were detected.All the pts in our cohort were treated at the hematological Institute of Bologna. Each patient was affected by newly diagnosed or relapsed APL, in accordance with WHO 2008 classification. All of them were treated with ATRA-IDA schedule of therapy.
Results: By Nexus Copy Number, we found that the relapsed pts presented more CNA than the newly diagnosis, in particular 3 out of 23 pts (a newly diagnosis and 2 relapses), showed interesting alteration on GPC4 (exon 9) and in the q26.2region of GPC3. We found that this group of 3 pts presented a minimal common region of amplification of GPC4 (chr X: 132,432,100-132,435,915) with a significant p value (p<0.05).Moreover, GPC3 q26.2 region presented significant abnormalities with more copy number gain and copy number loss, which may play a role in the pathogenesis of APL. Also UPD were found for both GPC4 (5/23 pts) and GPC3 (6/23 pts) genes, with a p value of 0.0264 for GPC4 and 0.009 for GPC3.
Summary/Conclusions: GPC4 and GPC3 are coding genes for glypicans, heparan sulfate proteoglycans which have a role in the control of cell growth and cell division. Alteration of these genes are described as first events in the pathogenesis of Simpson-Golabi-Behmel syndrome, characterized by abnormal cellular proliferation and augmented cancer risk. Our suggestion is that these two genes may play a role in the improvement of pathogenesis and resistance of APL pts. Further studies will be necessary to confirm and validate these data in order to understand the role of these genes in APL.
Aknowledgement: ELN, AIL, AIRC, PRIN, progetto Regione-Università 2010-12(L. Bolondi), FP7 NGS-PTL project

PB1635

PROGNOSTIC IMPACT OF COMPLEX KARYOTYPE ON OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA WITH CHROMOSOME 5 AND/OR 7 ABNORMALITIES

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Background: Chromosome 5 and 7 abnormalities often occur in acute myeloid leukemia (AML) both as a single chromosomal aberration and a part of the complex karyotype (CK), and constitute a high-risk group. Breems *et al.*, 2008 have identified a monosomal karyotype (MK), defined by the presence of at least 1 autosomal monosomy and 1 structural chromosomal abnormality or at least 2 autosomal monosomies, which associated with dismal outcome in AML patients. However, whether or not the prognostic impact of MK and CK remains relevant for patients who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) is still unclear.
Aims: To evaluate the prognostic impact of chromosome 5 and 7 abnormalities both as a single chromosome aberration and a part of the complex or monosomal karyotype on outcome of allogeneic hematopoietic stem cell transplantation in AML patients.
Methods: In this study, outcomes in 44 AML patients with 5q-, 7q-/monosomy 7, who were transplanted in our University between 2008 and 2015, were analyzed. All patients and transplant characteristics are listed in Table 1.
Results: CK and MK were identified in 19 (43%) and 8 (18%) cases, respectively. The median follow-up was 525 (46-2186) days. Overall survival (OS) was 39% (95% CI 22-55) and event free survival (EFS) was 20% (95% CI 8-35) at 3 years estimated with Kaplan-Meier method. In univariate analysis, prognostic factors associated with increased OS and EFS were age (>18 *vs* <18 years; p=0.01, p=0.05, respectively), the disease status at transplant (complete remission *vs* active diease; p=0.10, p=0.01, respectively), complex karyotype (CK- *vs* CK+; p=0.05, p=0.002, respectively), and stem cells source (bone

marrow *vs* other source; p=0.03 for OS only); monosomal karyotype (MK- *vs* MK+; p=0.009 for EFS only). Other factors including variant of AML, patient sex, donor type, conditioning regimen, number of transplanted CD34+cells, and type of chromosome abnormality were not associated with survival. In multivariate analysis, age (HR-3.67; P=0.01), the disease status at allo-HSCT (HR-2.64; P=0.03), the stem cell source (HR-3.04; P=0.02), and complex karyotype (HR-2.48; P=0.03) remained statistically significant for OS. Moreover, age (HR-2.63; P=0.01), the disease status at transplant (HR-2.63; P=0.01) and complex karyotype (HR-3.29; P=0.002) were independent predictors of EFS (Figure 1).

Table 1.

Number of patients	44 (100%)
AML	
De novo AML	27 (61%)
Secondary AML	17 (39%)
Patient sex, n (%)	
Male	22 (50%)
Female	22 (50%)
Age at HSCT, median, (range) years	31.2 (1.2-67)
Age group	
≤18 yo	15 (34%)
≥18 yo	29 (66%)
Cytogenetics, n (%)	
5q	15 (34%)
-7/7q-	24 (55%)
5q- together with -7/7q-	5 (11%)
Complex karyotype -	25 (57%)
Complex karyotype+	19 (43%)
Monosomal karyotype –	36 (82%)
Monosomal karyotype+	8 (18%)
Clinical stage at HSCT, n (%)	
CR1≥	13 (30%)
CR2	7 (16%)
Active disease	24 (54%)
HSC source, n (%)	
Bone marrow	24 (55%)
Peripheral blood	15 (34%)
Both	5 (11%)
Conditioning regimen, n (%)	
MA	10 (23%)
Non-MA	34 (77%)
Donor type, n (%)	
HLA-id sibling	13 (30%)
Matched unrelated	20 (45%)
Haploidentical	11 (25%)
Number of transplanted CD34+ cells, mediana (range) x10^6/kg	6.3 (1.6-17.9)

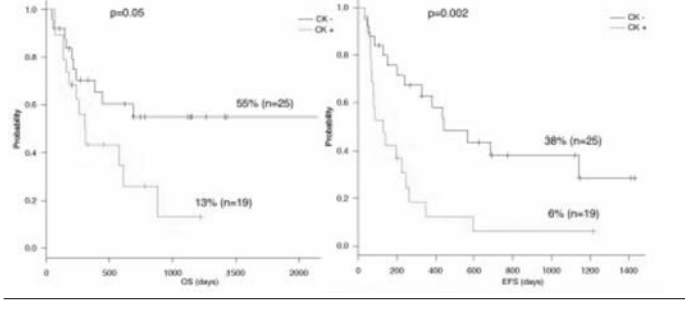

Figure 1.

Summary/Conclusions: The results indicate, that chromosome 5 and 7 abnormalities as a part of the complex karyotype but not monosomal karyotype is high-risk factor in AML patients undergoing allo-HSCT.

PB1636

Abstract withdrawn.

PB1637

MUTATIONAL PROFILE BY NEXT-GENERATION SEQUENCING IN ACUTE MYELOID LEUKEMIA (AML): A SINGLE CENTER EXPERIENCE

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Background: AML is a clonal disorder of hematopoietic stem and progenitor cells caused by acquired and occasionally inherited genetic alterations. Cytogenetic aberrations together with gene mutations are powerful prognostic markers and the mutational profile can be incorporated in the decision making processes to treatment.
Aims: A real-life cohort of AML patients from Sardinia (Italy) was analyzed to evaluate the usefulness of a next-generation sequencing (NGS) gene panel for the detection of mutations associated with AML in a context of routine diagnostic molecular workup.
Methods: Thirty AML patients, 15 males and 15 females with median age of 60 years (range 18-88 years) were enrolled for the study. DNAs were screened with IonAmpliseq AML panel (ThermoFisher) on IonPGM platform. The panel includes hotspots mutations of the genes: *ASXL1* (exon 12), *BRAF* (V600E),*CBL*(exons 8-9), *FLT3*(codons 676,830-850), *IDH1*(exon 4), *IDH2*(exon 4), *JAK2* (exon 14), *KIT* (exons 8, 10, 11 and 17), *KRAS* (exons 2-4), *NRAS* (exons 2-4), *PTPN11* (exons 3,7,8,13), *RUNX1* (exons3-8), *WT1* (exons 7,9), and the entire coding sequence of *CEBPA*, *DNMT3A*, *GATA*, *TET2* and *TP53* genes.The panel design does not include the *FLT3*-ITD gene region. Genomic libraries were prepared using Ion AmpliSeq™2.0 (ThermoFisher) chemistry and library clonal amplification was performed by emulsion PCR using Ion PGM™Template HiQ OT2 Reagents. Combination of 5 libraries on an Ion 318™Chip resulted in >2500X average coverage depth with >97% of the target bases covered at 500X. Genomic variants were filtered and analysed through AmpliseqAML single 5.0 workflow.
Results: The mean count of sequencing reads obtained per sample was 0.73 million and the mean sequencing depth was over 2500X with 91% uniformity. Seventy-five mutations in 19 genes were detected in 31 samples with an average of 2.3 mutations per sample. *TP53* mutations were found in 4 patients of whom one with unfavorable cytogenetics.The AML panel detected 15 mutations (20%) in *NPM1*, 6 (8%) *TET2,* 10 (13%) *DNMT3A*, 6 (8%) *CEBPA*, 5 (7%)*TP53*, 3 (4%) *RUNX1*,4 (5%) *FLT3*, 4 (5%) *IDH2*, 2 (3%) *ASXL1*, 5 (7%) *GATA2*, 6 (8%) *PTPN11* and 3 (4%) *KIT* genes. Only one patient remained wild-type for these genes. Based on mechanism of action, genes involved in signal transduction and DNA methylation were the most frequently mutated, accounting for the 39% and 32% of detected mutations, respectively. The AML gene panel identified 100% of previously identified mutations by conventional molecular biology techniques in *FLT3*(TKD), *NPM1*, *IDH1-2* and *CEBPA* genes. Mutational profiles obtained by NGS allowed to refine cytogenetic classification in the normal karyotype subgroup shifting a large proportion of patients (24 out of 30) from the intermediate to poor risk category. In one patient we were able to assess clonal dominance over the course of the disease treatment. A female 50 y.o. diagnosed with AML-M2 presented at diagnosis *IDH2* p.R172K mutation, *DNMT3A* p.F640fs*11 and *CEBPA* p.P196_P197insHP at frequencies of 42%, 49% and 20%, respectively. Monitoring of mutation levels in two consecutive bone marrow samples during treatment according to AML1310 protocol, demonstrated treatment associated clonal responses. *IDH2* mutation had a frequency of 7.3% after 4 weeks of therapy and resulted absent after 8 weeks (not detected over 6500 reads), while *DNMT3A* and *CEBPA* mutations were present at the levels of 30% and 47%, respectively. Longitudinal monitoring of three mutations indicated the presence of independent clones behaving discordantly during therapy.
Summary/Conclusions: A targeted multi gene panel analysis can provide in a short turnaround time a pretreatment mutational profile as well as the MRD status helping decision of intensity and type of induction therapy and modulation of post remission strategies.

PB1638

MRD MONITORING AND DETECTION OF MOLECULAR RELAPSE IN PEDIATRIC AML USING STANDARDIZED QPCR ASSAYS

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Background: Acute myeloid leukemia (AML) is one of the most life threatening malignancies in children and adolescents. In current multi-center treatment protocols, the remission status of a patient is still determined morphologically and/or by flow cytometric as the percentage of AML blasts in the bone marrow. Although introduced in ALL for more than 10 years, no AML study group so far has been able to systematically perform real-time quantitative polymerase chain reaction (qPCR) as one of the most senstive methods to detect the presence of leukemic cells down to levels of 1:10^6.
Aims: The aim of the present study was to develop the methodology for monitoring the residual levels of AML blasts over time for patients with defined genetic aberrations enrolled in the AML-BFM 2012 clinical trial or in the study registry. These aberrations included t(8;21), t(9;11), inv(16) and NPM1 Mutation A. All patients were treated according to the AML-BFM study protocols.
Methods: We performed qPCR on mRNA isolated from bone marrow biopsies

and peripheral blood samples of pediatric AML patients. Bone marrow specimen were collected at initial diagnosis and after therapy blocks as indicated in the protocol. If possible, peripheral blood was collected every 4 weeks during maintenance therapy. We collaborated with a diagnostic laboratory in Denmark to develop and optimize standard operation procedures (SOPs) and to further standardize the MRD-monitoring methodology. Mutation-specific TaqMan probes were used and validation was performed by exchanging and analysing samples and data in parallel in both laboratories using the ΔΔCt method. *ABL proto-oncogene 1*(*ABL*) and *beta-2-microglobulin* (*B2M*) were selected as reference genes for amplification.
Results: Out of 159 patients with newly diagnosed pediatric AML from 06/2014 to 02/2016, 44 patients had genetic aberrations that could be detected and monitored using qPCR with specific primers. This included 12 patients with t(8;21), 16 patients with t(9;11), 12 patients with inv(16) and 4 patients with NPM1 mutation A. For 36 patients, we were able to analyze samples from at least 3 time points. We were able to reach sensitivity levels from 10^{-3} to 10^{-6} depending on both the quality of the bone marrow specimen as well as the specific assay.
Summary/Conclusions: We demonstrated here that qPCR for four defined genetic aberrations during and after therapy is a useful tool to prospectively monitor the AML blast levels. Due to the high sensitivity of this methodology it is possible to detect a molecular relapse at an earlier time point compared to standard morphological or flow cytometric analysis. We also showed that the successful standardization of the qPCR allowed to directly compare the MRD results from different laboratories.

PB1639

AMPLICON BASED PANEL TARGETED RESEQUENCING WITH THE TRUSIGHT MYELOID PANEL IN 100 PEDIATRIC AML PATIENTS
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Background: Acute myeloid leukemia (AML) is one of the most threatening malignancies in children and adolescents. The accumulation of mutations in leukemia stem cells (LSC) is believed to lead to the development of leukemia. Cyto- and molecular genetics already identified several aberrations which are relevant in leukemogenesis, prognosis and therapy. Nevertheless, the molecular landscape and clonal evolution of AML and its clinical relevance, especially for pediatric patients, is not yet well described. Next Generation Sequencing as an emerging sequencing technology provides the possibility to generate sequence data of high quality and detect genetic aberrations in a minimum of time.
Aims: The aim of this study was to apply amplicon based panel targeted resequencing by using the TruSight Myeloid Panel (Illumina) on a MiSeq Dx System (Illumina) to analyse 100 children with suspicion of AML at the time of initial diagnosis to detect the genetic variants.
Methods: All the patients analysed in this study were screened in order confirm or exclude the diagnosis of AML. With the confirmation of AML, the patients were treated according to the AML-BFM therapy protocols. Next Generation sequencing (NGS) with the TruSight Myeloid panel on a MiseqDX was performed. The sequencing panel is designed to identify somatic mutations associated to myeloid malignancies in 54 genes. Validation was initially performed using data for 10 patients obtained from Sanger-Sequencing from 7 diagnostically relevant mutations and compared to the data obtained from NGS. The genes actually relevant for prognosis and treatment stratification are *CEBPA, FLT3, GATA1, KIT, NRAS, NPM1* and *WT1*.
Results: Every variant detected with Sanger Sequencing could be recovered in the NGS data. In 4 patients we could even detect additional mutations. Variants were detected and analysed using two different analysis software (Variant studio *vs* Sophia DDM) and the results were compared. Almost all variants were detectable in both software, although great insertions and deletions are detectable only by Sophia DDM. We could detect *CEBPA* mutations in 16 patients, *FLT3* ITD in 10 patients, *FLT3* TKD mutations in 2 patients, *GATA1* mutations in 6 patients, *KIT* mutations in 25 patients, *NPM1* mutations in 7 patients, *NRAS* mutations in 14 patients and *WT1* mutations in 24 patients. In 26 patients, we could not detect any mutation in these 7 genes. 23 patients had two different mutations at the same time and even three mutations could be detected in 4 patients. The analysis of variants detected in the 47 other genes covered in the panel is still ongoing.
Summary/Conclusions: Amplicon based panel targeted resequencing with the TruSight Myeloid panel provides the possibility to detect mutations in 54 genes associated to myeloid malignancies within 3 days after the sample arrived at our lab. The aim now is to adapt the report of the clinical findings to the detected variants, especially for variants in genes that were not yet diagnostically relevant. The confirmation of pathogenicity of variants in a broad range of genes could promote the possibilities of personalized targeted therapy.

PB1640

EMBRYONIC STEM CELLS ANTIGEN (ESCA) EXPRESSION IN ACUTE MYELOID LEUKEMIA CELLS
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Background: Acute Myeloid Leukemia (AML) is characterized by the expansion and resistance to apoptosis of poorly differentiated myeloid cells. This disease occurs when genetic and epigenetic processes transform an immature hematopoietic stem or progenitor cell, which reacquire self-renewing properties, and retain an undifferentiated state. Long term propagation of the disease is mainly due to a subset of proliferative, undifferentiated population, termed leukemic stem cells (LSC), which give rise to the leukemic clone. The frequency of LSC in the bulk of leukemic cells is highly variable. Several studies have suggested that LSC belongs to the $CD34^+CD38^-$ compartment and represent the malignant counterpart of normal hematopoietic stem cells (HSC). LSC can be identified by aberrant expression or down regulation of differentiation markers expressed by normal HSC. Self-renewal and lack of differentiation are also features of stem cells and has been associated with the expression of genes including those known as "Yamanaka's factors". The products of these genes are surface receptors (SSEA1 and SSEA3) and transcription factors (OCT¾, SOX2, and NANOG). There are some data concerning the abnormal expression of these proteins in solid tumors, but very few data are available in AML.
Aims: The aim of our study was to evaluate the expression of 5 ESCA (SSEA1, SSEA3, NANOG, OCT¾ and SOX2) in leukemic cell lines, and in hematopoietic stem cell (HSC) subsets ($CD34^+CD38^-$ and $CD34^+CD38^+$) from normal bone marrows (NBM) and in 106 samples from AML patients.
Methods: The expression of 5 ESCA was assessed by Multicolor Flow Cytometry and was confirmed by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).
Results: We observed a high expression of ESCA in AML cell lines (Mean Fluorescence Intensity Ratio MFIR>10, Cycle threshold Ct<40). Their expression was evaluated in $CD34^+$, as well as in the remaining $CD45^{+low}$ "blasts" after basophils and dendritic cells removal. We observed an up-regulation of the transcription factors OCT¾ and SOX2 with 2-fold higher expression ($p<0.05$) in AML cells as compared to normal cells. These results were associated with the up-regulation of SSEA3. Conversely, SSEA1 protein was down-regulated in LSC (1.7-fold higher expression in normal cells compared to leukemic cells, $p<0.05$). The expression of OCT¾, SOX2 and SSEA3 was higher in $CD34^+CD38^-$ than in CD34+CD38+subsets in HSC as well as in leukemic cells. The level of SSEA1 and NANOG were higher in more differentiated ($CD34^+CD38^+$) cells. Significant correlations were observed with recurrent molecular abnormalities. The expression of OCT¾ and SOX2 was lower in promyelocytic leukemia (APL) ($p<0.005$). SOX2 was also lower in AML with RUNX1-RUNX1T1 rearrangements (but not in AML with CBFβ-MYH11). Instead SSEA1 levels were higher in AML with CBF rearrangements ($p<0.001$), but not in APL. There was no correlation with other biological characteristics (WBC, other genetics subtypes). We also evaluated the prognostic value of ESCA expression in the 69 patients who received an intensive treatment. The rate of complete remission was not influenced by the level of expression of ESCA. There was a trend ($p=0.06$) for better overall and leukemia-free survival for patients with high OCT¾ and SOX2 levels, that was unexpected because of the inverse correlation with favourable genetic subtypes.
Summary/Conclusions: In conclusion, these results prompt us to evaluate the potential role of ESCA in leukemogenesis and to test the relevance of these markers for better LSC identification. Prognostic value should be assessed in larger series by multivariate analysis.

PB1641

ASSOCIATION BETWEEN POLYMORPHISM OF FOLATE AND METHIONINE METABOLISM GENES AND ABERRANT DNA METHYLATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROME
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Background: Aberrant DNA methylation of tumor-supressor genes may be involved in the development of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Polymorphism of folate and methionine metabolism (FMM) related genes can influence the methylation processes and, therefore, operated on both AML and MDS development.
Aims: The aim of this study was to assess the association between polymorphism of FMM related genes and aberrant methylation of promoter regions of several tumor supressor genes in AML and MDS patients.
Methods: Fifty-seven patients with AML (20 men and 37 women, mean age 51.0 yrs) and 51 patients with MDS (24 men and 27 women, mean age 62.4 yrs) were genotyped for the MTHFR C677T, MTHFR A1298C, MTR A2756G, MTRR A66G and MTHFD G1958A polymorphisms by PCR-RFLP technique. The differences in allele and genotype frequencies were assessed by Fisher's exact test with computation of odds ratios (OR), their 95% confidence intervals (CI) and p-values. Methylation-specific PCR was used to study the methylation status of promoter regions of *SOX7*, *p15^{INK4b}*, SFRP1, SFRP4 and SFRP5 genes.

Results: Thirty patients AML (10 men and 20 women) and 21 patients MDS (9 men and 12 women) had aberrant methylation of 0-2 genes (0-2 MG group AML and MDS respectively). Twenty seven patients AML (10 men and 17 women) and 30 patients MDS (15 men and 15 women) had aberrant methylation of 3-5 genes (3-5 MG group AML and MDS respectively). In MDS group, hypermethylation of all 5 genes was found in 5 men and was not found in female patients (20.8% *vs* 0.0%, respectively, OR=15.5, 95%CI: 0.8-297.0, p=0.02). At the same time, aberrant methylation of all 5 genes was found in 8 AML patients (4 men and 4 women, 20.0% *vs* 10.8%, respectively, OR=1.9, 95%CI: 0.4-8.2, p=0.45). The presence of the MTHFR 1298C allele was increased in male patients AML from the group 0-2 MG when compared to male MDS patients in the 0-2 MG group (73% *vs* 43%, respectively, OR=3.7, 95%CI: 1.1-11.9, p=0.042). The presence of the MTHFR 677T allele was increased in female AML patients from the group 3-5 MG when compared to female AML patients in the 0-2 MG group (76.5% *vs* 35.0%, respectively, OR=6.0, 95%CI: 1.4-25.7, p=0.02), but was not different between women from 3-5 MG and 0-2 MG groups MDS patients (46.7% *vs* 33.4%, respectively, OR=1.8, 95%CI: 0.4-8.4, p=0.7). It is interesting, that positivity for the MTHFR 677T allele was significantly increased in male MDS patients from the group 0-2 MG when compared to male 3-5 MG group (100.0% *vs* 46.7%, respectively, OR=21.5, 95%CI: 1.2-437, p=0.01). In male patients with MDS, the MTHFD 1958 AA genotype was more frequently seen in the group 3-5 MG than in the group 0-2 MG (33.0% *vs* 0.0%, respectively, OR=9.9, 95%CI: 0.5-205.0, p=0.12). This genotype was also overrepresented in the group 3-5 MG of women with AML when compared to group 0-2 MG of female AML patients (29.4% *vs* 5.0%, respectively, OR=7.9, 95%CI: 0.8-76.3, p=0.08).
Summary/Conclusions: We conclude that polymorphism of the FMM genes could have an important role in mechanism of epigenetic disturbances at AML and MDS, associated with aberrant methylation of CpG islands of tumor-supressor genes.

PB1642

LOCALIZATION OF FLT3-ITD IS ASSOCIATED WITH RESPONSE TO INDUCTION CHEMOTHERAPY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: FLT3-ITDs (internal tandem duplications) represent the most frequent molecular aberrations in acute myeloid leukemia (AML) that are both virtually patient-specific and associated with a higher probability of relapse. Several studies addressed the question whether the diversity of FLT3-ITD affects the clinical outcome of AML patients. We present patient-specific sequence analysis of FLT3-ITD-positive AML patients and its correlation with clinical data.
Aims: FLT3-ITD sequence analysis focused on ITD localization and the presence of distinct amino acid motives. We sought to establish a relationship between the localization of the internal tandem duplication in FLT3 and response to therapy.
Methods: 43 patients (26 female, median age 57 years, 84% with *de novo* AML) diagnosed and treated in a single center were retrospectively analysed.All patients received intensive induction chemotherapy according to one of the following protocols of the Ostdeutsche Studiengruppe für Hämatologie und Onkologie (OSHO): AML96 or AML2002 protocol containing idarubicine for patients up to 60 years old and AML97 or AML2004 protocol containing mitoxantrone for elderly patients.
Results: FLT3-ITD localization more downstream can be correlated with impaired leukemia-free survival (LFS) while the level of significance is not reached in our small cohort of AML patients. In contrast, all investigated amino acid motifs (*e.g.* YVDFREY) had no impact on the clinical outcome. Importantly, the probability to achieve a complete remission (CR) after AML induction chemotherapy is affected by the localization of the internal tandem duplication. In detail, CR rate is significantly higher in those patients who present with FLT3-ITD localized towards the N-terminus (86.4% *vs* 57.1%, p=0.045, Chi squared test).
Summary/Conclusions: We hypothesize that FLT3-ITDs that are located closer to the C-terminus of FLT3 are associated with an unfavorable prognosis due to a lower CR rate following induction chemotherapy of AML.

PB1643

MITOCHONDRIAL SPECIFIC ROS HYPEROXIDATION VIA PEROXIREDOXIN III HAS IMPORTANT ROLES ON ARSENIC TRIOXIDE INDUCED APOPTOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA

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Background: Despite that the Arsenic trioxide (ATO) is an effective therapeutic drug for acute promyelocytic leukemia (APL), some APL cells are resistant to ATO treatment. Previous studies reported that the apoptotic processes by ATO treatment was due to the accumulation of ROS in the cells and its balance of redox enzymes. However, not only the mechanisms of apoptosis via reactive oxygen species (ROS) and peroxiredoxin (PRX) but its resistance during ATO treatment remain elusive.
Aims: Aims of current study are to elucidate that the upregulation of ROS production and the changes of redox enzyme may be major players of anti-leukemia effect in APL-derived NB4 cells during ATO treatment and to find ways to potentiate of the anti-leukemic effects by maniplating ROS and its redox enzymes.
Methods: NB4, one of the human acute promyelocytic leukemia cell lines, was treated with 2 μM arsenic trioxide to induce apoptosis for 16-48 hours in RPMI-1640 medium supplemented with 10% FBS in CO2 humidified atmosphere at 37°C. Apoptosis was measured by staining with 7-amino-actinomycin D (7-AAD) with flow cytometry. 2, 7-dichlrodihydro-fluorescein-diacetate (H2DCF-DA) and MitoSOX Red were used to detect cellular and mitochondrial levels of ROS. SO2 form for PRX I, PRX II, and PRX III were detected by western blot assay. Steady state level of sulfiredoxin (SRX) and caspase 3, 9 were also studied by western blot analysis. To evaluate of the effect of SRX depletion, NB4 cells were transfected with small interfering RNA (siRNA).
Results: Intracellular ROS of NB4 cells was increased significantly after 16 hour of ATO treatment but decreased after 24 hour of ATO treatment. Mitochondrial ROS of NB4 cells was increased significantly after 39 hour of ATO treatment. Apoptosis of NB4 cell after ATO treatment was increased as time elapsed (24% on 16hr, 26% on 24hr, 48% on 39hr, and 60% on 48hr). Increased cysteine sulfinic acid (Cys–SO2H) of PRX III, oxidized form, was observed as a hyperoxidation reaction in NB4 cells after ATO treatment in concordance with mitochondrial ROS increment of NB4 cells. Increased expressions of cleaved caspase-9 and cleaved caspase-3 were also observed during NB4 cell apoptosis by ATO treatment. Meanwhile, SRX expression was increased in NB4 cells after ATO treatment which was rather unexpected observation. Down regulation of SRX by siRNA promoted ROS generation and apoptosis in ATO-treated NB4 cells.
Summary/Conclusions: These findings suggest that ATO-induced anti-leukemic activity can be down regulated by an enhancing oxidized PRX III reduction after ATO-induced SRX activation and that down regulation of SRX can enhance the apoptosis in ATO-treated NB4 cells. This result might provide the insights for finding novel ways in the development of strategies, which may potentiate ATO-induced apoptosis in APL cells.

PB1644

HIGH EXPRESSION OF TIM-3 ON BLAST CELLS IS ASSOCIATED WITH GOOD PROGNOSTIC FACTORS IN *DE NOVO* NON-M3 AML

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Background: T-cell immunoglobulin and mucin domain-containing molecule3 (Tim-3) represents a novel mechanism of T-cell dysfunction and exhaustion. Tim-3 has also been identified in various solid tumors. However, the role of Tim-3 expression on blast cells in acute leukemia is not well understood.
Aims: In this study, we aimed to explore the role of Tim-3 in patients with *de novo* acute leukemia and the correlation between Tim-3 and clinicopathological prognosis.
Methods: The study cohort consisted of 121 patients including 76 cases with *de novo* non-M3 AML and 45 cases with ALL. These patients' bone marrow samples were collected and then bone Marrow mononuclear cells (BMCs) were isolated for flow cytometry to detect Tim-3 expression on blast cells. E_LINK3">clinicopathological prognosis.
Results: According to FAB type, 76 AML patients were diagnosed as M0(n=2), M1(n=16), M2(n=20),M4(n=20),M5(n=16) and M6(n=2), respectively. And ALL group were comprised of 38 cases with B-cell ALL and 7 cases with T-cell ALL. The results came out that Tim-3 expression on blasts in *de novo* AML patients significantly increased compared with that of ALL patients (P=0.00). Moreover, the frequency of Tim-3 high expression was higher in M4 patient than that in other AML patients according to FAB type (P=0.00). Tim-3 high expression was also closely associated with inv(16) (P=0.01) and C/EBPA mutation (P=0.03). The mutations of the following six genes, *i.e.*, FLT3-ITD, NPM1, C-KIT, IDH1/IDH2, DNMT3A, had little to do with the expression of Tim-3. Additionally, it is more likely to find higher level of Tim-3 in low-risk group than in intermediate- and high-risk groups (P=0.04). The expression of Tim-3 was positively correlated with CD13 (r=0.33,P=0.02), CD34 (r=0.51, P=0.00), CD7 (r=0.40, P=0.00) in AML patients. As for the ALL patients, Tim-3 expression significantly increased in the T-ALL group than in the B-ALL group (P=0.00). The expression of Tim-3 in ALL patients was positively correlated with CD34(r=0.32, P=0.00), CD7(r=0.56, P=0.01) and negatively with CD10(r=-0.35,P=0.02), CD19(r=-0.24,P=0.00), CD20(r=-0.31,P=0.01). Tim-3 expression was not significantly associated with potential prognostic factors, including age or cytogenetic risk in ALL patients. AML patients with high Tim-3 expression achieved significantly high CR rate (P=0.01) and then their Tim-3 expression significantly decreased after CR (P=0.00), but such trend did not occur in ALL patients.

Summary/Conclusions: These findings suggest that high Tim-3 expression on blast cells is associated with good prognosis and detection of Tim-3 expression may be helpful to predict clinicopathological prognosis in non-M3 AML patients.

PB1645

MEIS1 IS ESSENTIAL FOR THE MAINTENANCE OF HUMAN ACUTE MYELOID LEUKEMIA BLASTS INDEPENDENT OF MLL REARRANGEMENTS

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Background: Acute myeloid leukemia (AML) is one of the common hematopoietic malignancies with high mortality. Although the outcome of patients with AML has improved by optimized chemotherapy regimens and bone marrow transplantation, leukemia relapse remains one of the most challenging problems during clinical treatment. Sustained existence of AML blasts is a fundamental determinant for the leukemia development and resistance to therapy. Recent evidences suggest that Meis1 is tightly associated with the self-renewal capacity of normal hematopoietic stem cells. Meis1 was also found to be essential for the development of mixed lineage leukemia (*MLL*)-rearranged leukemia. Whether Meis1 functions independently of *MLL* abnormality in the context of leukemia is of major interest.
Aims: To elucidate whether Meis1 functions independently of *MLL* abnormality in the maintenance of leukemia blasts.
Methods: We performed siRNA-mediated gene silencing experiments in the cultured human AML cells without *MLL* rearrangements. Next, we detected the expression levels of Meis1 in the bone marrows from 95 patients with newly diagnosed AML excluding promyelocytic leukemia and 30 healthy donors. All patients did not express a set of recognized AML-associated fusion genes. The association between the Meis1 levels and the response to chemotherapy was further explored.
Results: Deficiency of Meis1 expression impaired the maintenance and survival of cultured human AML blasts, which is independent of *MLL* abnormality. In the patients with newly diagnosed AML and without *MLL* rearrangements, high levels of Meis1 expression were found in 64 of 95 (67.4%) AML patients whereas 31 of 95 (32.6%) patients showed the dramatically low levels, compared with the median level of Meis1 expression in healthy donors. We further demonstrated that high levels of Meis1 expression were associated with the poor response to conventional chemotherapy, compared with the group with low Meis1 levels (p=0.028).
Summary/Conclusions: We identified a distinct expression pattern of Meis1 levels in patients with newly diagnosed AML and highlighted a role of Meis1 in regulating maintenance and survival of human AML cells without *MLL* abnormality. These results implicate Meis1 functions as an important regulator and could be an independent prognostic factor during the progression of human AML.

PB1646

ACTIVATION OF AMP-ACTIVATED PROTEIN KINASE AMELIORATES CYTARABINE-INDUCED OVEREXPRESSION OF NUCLEOPHOSMIN AND DRUG-INDUCED-CHEMORESISTANCE IN HL-60 AML CELLS

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Background: Nucleophosmin (NPM or B23) is a ribosomal protein located mainly in nucleolus, and multifunctional enzyme in cancer cell growth and protein synthesis. Particularly, it has been suggested that NPM plays a role in the positive regulation of cell proliferation, thus observed the over-expression levels in pathological higher grades of tumor and actively proliferating cells than normal cells. On the other hand, AMP-activated protein kinase (AMPK) is a critical energy sensor to regulate homeostasis and plays a potential role for anti-cell proliferating activity.
Aims: We investigated the effects of AMPK activation on the cell death (apoptosis) and NPM expression in AML (acute myeloid leukemia) cells (HL-60) treated with low or high dose of an antileukemic drug cytarabine to understand the mechanisms responsible for AML cells chemoresistance.
Methods: Cell viability was assessed using MTS assay. Apoptosis and cell cycle progression were evaluated by flow cytometry assay. Protein and mRNA expressions were detected with real time-PCR and Western blot assay.
Results: Interestingly, we found that the level of NPM expression was increased significantly in cytarabine-treated cells without dose-dependency of cell death, indicating the drug-induced cell resistance. In the same point, cytarabine also inhibited the phosphor-activity (Thr172) and expression level of AMPK, which has mTOR-p70S6K pathway-repressor activity. As expected, single cytarabine treatment increased the ratio of p-mTOR/mTOR and p-p70S6K/p70S6K. Co-treatment of AMPK activator (phenformin or AICAR) in cytarabine-treated HL-60 AML cells inhibited significantly the induction of NPM expression level with the decrease of phosphor-activities of mTOR and its substrate p70S6K, resulted in the accelerated cell apoptosis.
Summary/Conclusions: Our results suggest that AMPK activation might be used to sensitize AML cells to cytarabine with the control of NPM expression levels, thus the lower therapeutic dose and the less adverse effects.(Correspondence to HG Yi and CS Park; Medical Research Center no. 2014009392)

PB1647

SERUM LEVELS OF CYTOKINES AND SOLUBLE ADHESION MOLECULES IN ACTIVE ACUTE MYELOID LEUKEMIA AND COMPLETE REMISSION: EVIDENCE OF ENDOTHELIAL CELL ACTIVATION

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Background: Acue myeloid leukemia cell are highly resistant to therapy. The presumed molecular basis of this resistance is the effect Tumor Necrosis Factor-α (TNF-α) and other cytokines on endothelial adhesion molecule expression.
Aims: The aim of this study was to test the hypothesis that cytokines and soluble adhesion molecules are involved in endothelial cell activation in acute myeloid leukemia (AML).
Methods: A total of 84 AML patients were studied. Two subgroups comprising 84 samples collected in active disease and 45 samples collected at AML complete remission (CR) were evaluated. Samples obtained after allogeneic stem cell transplantation were not included. We evaluated serum levels of the following 29 analytes: interleukins (IL-1α,IL-1β,IL-2,IL-3,IL-4,IL-5,IL-6,IL-7,IL-8,IL-10,IL-12,IL-13,IL-15), Epidermal Growth Factor (EGF), GM-CSF, IFN-γ, Macrophage Inflammatory Protein-1α (MIP-1α), Monocyte Chemotactic Protein-1 (MCP-1), TNF-α, Vascular Endothelial Growth Factor (VEGF), E-selectin (E-SEL), P-selectin (P-SEL), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Matrix Metalloproteinase-9, soluble IL-2 receptor-α (sIL-2Rα) and soluble receptors for IL-6 (sIL-6R) and TNF-α type I and II (TNFR-1,2). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox). Probability values (P)<0.05 were considered significant.
Results: In active disease, the levels of VCAM-1 correlated with ICAM-1 (P<0.0001), E-SEL (P=0.0011), leukocyte count (P=0.0006), TNF-α (P<0.0001), TNFR-2 (P<0.0001), TNFR-1 (P=0.0047), LDH (P<0.0001), IL-2Rα (P=0.0224) and IL-6R (P=0.0240). The ICAM-1 levels correlated with E-SEL (P=0.0285), TNFR-1 (P=0.0007), LDH (P=0.0344) and IL-6 (P=0.0646). E-SEL correlated with P-SEL (P<0.0001), leukocyte count (P<0.0001), LDH (P<0.0001), TNFR-1 (P=0.0152), TNFR-2 (P=0.0202). CRP levels correlated with IL-6 (P<0.0001), ICAM-1 (P=0.0122) and negatively with albumin levels (P=0.0175). The platelet count correlated with IL-7 (P<0.0001), EGF (P<0.0001) and VEGF. There was no correlation of age or haemaglobin levels with any evaluated analyte. In CR the levels of IL-7 (P<0.0001), EGF (P<0.0001) and VEGF (P<0.0001) were higher, which was related to normalized platelet count. The levels of IL-6 were lower in CR with no significant correlation with CRP levels. The levels of VCAM-1, ICAM-1, E-SEL and P-SEL were decreased compared to active disease, which was not significant after Bonferroni correction of P. The P-SEL correlated with platelet count (P<0.0001) and IL-8 (P<0.0001), which was not observed in active disease. VCAM-1 correlated with ICAM-1 (P=0.0027), but not with E-SEL or P-SEL. The E-SEL did not correlate with P-SEL.
Summary/Conclusions: Our findings are in agreement with the results of studies showing that cytokine and chemokine expression are largely independent of variables, such as age, gender and haemoglobin levels. In active disease the adhesion molecule levels are influenced as a whole and correlate with leukocyte count, LDH and levels of TNF-α, TNFR-1 and TNFR-2. Understanding the relationship of evaluated analytes in active AML and CR is more important than simple comparison of their levels. Endothelial cells can release both E-SEL and P-SEL. This happens in active disease, where the correlation of E-SEL and leukocyte count is significant. In CR there was no significant correlation of E-SEL with P-SEL or leukocyte count. Platelets release only P-SEL and are the major source of P-SEL in CR. We interprete these data as direct evidence of endothelial cell activation in active AML.
The work was supported by a long-term organisation development plan 1011 (FMHS).

PB1648

ARE TP53BETA AND TP53GAMMA EXPRESSION LEVELS CORRELATED TO NPM1/FLT3 MUTATIONAL STATUS?

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Background: *TP53* encodes a tumor suppressor protein which consists of transactivation, DNA-binding and oligomerization domains. Due to alternative splicing it may exist in 13 different isoforms. Alternative splicing of intron 9 leads to production of 2 different proteins, p53β and p53γ, without oligomerization domain (stop codon is localized in exon 9b). These isoforms can be present in acute myeloid leukemia (AML) cells. p53β binds to *BAX* promoter

and can induce apoptosis independent from p53 wt. It also regulates p53 activity. In AML high expression of p53β and p53γ proteins may play role in response to treatment by enhancing cells sensitivity to chemotherapy. It has been showed that patients have longer survival after treatment. *NPM1* (nucleophosmin gene) mutations are frequent alterations in normal karyotype AML (NK AML). Until now 56 different mutations of exon 12 of *NPM1* have been described, mostly insertions. The NPM protein plays an important role in cell cycle and apoptosis control. It cooperates with several proteins, including p53 and ARF. While patients with *NPM1* mutations are stratified to favorable risk group, internal tandem duplications (ITD) in the fms-like tyrosine kinase-3 gene *(FLT3) (i.e. FLT3ITD)* are poor prognosis factors.
Aims: The aim of the study was to assess mutational status of *NPM1/FLT3* in association with *TP53*beta and *TP53*gamma expression levels.
Methods: 56 NK AML patients with *NPM1* and/or *FLT3*ITD mutations were included in the study. Analysis of *TP53*β and *TP53*γ expression levels was possible only in 36 patients. Relative expression results were analyzed with ΔΔCt method, with *ABL* as a control gene and K562 cell line as a calibrator.
Results: In all 36 cases, *TP53*β and *TP53*γ transcripts were detected. 17 patients were *NPM1+/FLT3-*, 14 were *NPM1+/FLT3+*and 5 were *NPM1-/FLT3+*. Assessed median expression level of *TP53*β was much higher (ΔΔCt 43,87) than *TP53*γ (ΔΔCt 10,52; p=0,000027). Furthermore, according to statistical analysis, expression level of *TP53*γ was significantly associated with *NPM1/FLT3* mutations (p=0,008). We also classified patients according to median expression value of *TP53* to two groups: with overexpression or with small expression. Median WBC count in patients with overexpression of both isoforms was higher (75,4 G/L) than in group where expression of both isoforms was below median value (30G/L). Expression level of *TP53*γ was also correlated to WBC (p=0,05) and patients' age (p=0,015).
Summary/Conclusions: Obtained results may indicate a clinical importance of analysis *TP53* isoforms expression together with clinical data of patients.

PB1649

IMPACT OF ADDITIONAL CYTOGENETIC ABERRATIONS ON OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH RUNX1-RUNX1T1 ACUTE MYELOID LEUKEMIA

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Background: *RUNX1-RUNX1T1*-positive acute myeloid leukemia (AML) is considered as a favorable group. Allo-HSCT has been generally used for relapsed patients (pts), although prognostic factors are still unclear and prognostic significance of additional cytogenetic aberrations (ACA) in transplanted pts is contradictory.

Table 1.

Number of patients	25 (100%)
Patient sex, n (%)	
Male	15 (60%)
Female	10 (40%)
Age at HSCT, median, (range) years	**20.2 (2-58)**
Age group	
≤18 yo	12 (48%)
≥18 yo	13 (52%)
Cytogenetics, n (%)	
t(8;21) without additional cytogenetic aberration	12 (48%)
t(8;21) with additional cytogenetic aberration	13 (52%)
Complex karyotype -	16 (64%)
Complex karyotype+	9 (36%)
Time from diagnosis to HSCT, n (%)	
<360 days	14 (56%)
>360 days	11 (44%)
Clinical stage at HSCT, n (%)	
CR	13 (52%)
Active disease	12 (48%)
HSC source, n (%)	
Bone marrow	13 (52%)
Peripheral blood	11 (44%)
Both	1 (4%)
Conditioning regimen, n (%)	
MA	12 (48%)
Non-MA	13 (52%)
Donor type, n (%)	
HLA-id sibling	7 (28%)
Matched unrelated	12 (48%)
Haploidentical	6 (24%)

Aims: To evaluate impact of additional cytogenetic aberrations on outcome of allogeneic hematopoietic stem cell transplantation in patients with *RUNX1-RUNX1T1* acute myeloid leukemia
Methods: In this study, outcomes in 25 *RUNX1-RUNX1T1*-positive AML patients (pts), who were transplanted in a single institution between 2008 and 2015, were analyzed. All patients and transplant characteristics are listed in Table 1.
Results: The median follow-up was 566 (8 – 2127) days. Overall survival (OS) was 33% (95% CI 14-53) and relapse free survival (RFS) was 26% (95% CI 9-45) at 4 years estimated with Kaplan-Meier method. In univariate analysis, prognostic factors associated with increased OS and RFS were age (>18 *vs* <18 years; p=0.03, p=0.0006, respectively), donor type (match related *vs* match unrelated *vs* haploidentical; p=0.0003, p=0.02, respectively), disease status at transplant (complete remission *vs* active disease; p=0.0002, p=0.005, respectively), the interval from diagnosis to transplant (<360 *vs* >360 days; p=0.008, p=0.9, respectively), ACA (ACA- *vs* ACA+; p=0.02, p=0.009, respectively) (Figure 1), complex karyotype (CK- *vs* CK+; p=0.004, p=0.0003, respectively). In multivariate analysis, the ACA (HR-13.5; P=0.04), the donor type (HR-6.86; P=0.01), the interval from diagnosis to HSCT (HR-6.80; P=0.02) remained statistically significant for OS. Moreover, age (HR-0.11; P=0.004) and the donor type (HR-4.16; P=0.04) were independent predictors of RFS.

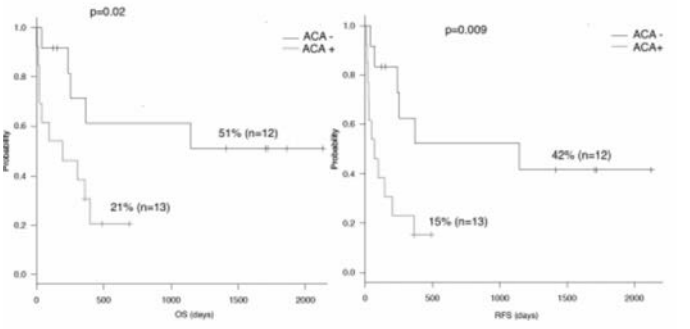

Figure 1.

Summary/Conclusions: The study demonstrates that the ACAs are independent prognostic factor for *RUNX1-RUNX1T1*-AML in transplanted pts. Since chemotherapy and cytotoxic conditioning regimen may be responsible for production of new ACAs, these regimens should be change by substitution of more toxic alkylating agents to less toxic.

PB1650

ABERRANT METHYLATION OF PROMOTER REGIONS OF SOX7, P15INK4B AND WNT PATHWAY ANTAGONIST GENES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Aberrant methylation of CpG islands of tumor-supressor genes may be involved in the development of acute myeloid leukemia (AML) and be associated with the natural history of the disease.
Aims: To reveal the features of aberrant methylation of promoter regions of *SOX7, p15^{INK4b}* and Wnt pathway antagonist genes in AML patients.
Methods: The data of 57 AML patients with median age of 51.0 years were analyzed. Next AML variants were diagnosed: 2 patients with M0, 7 M1, 23 M2, 4 M4, 8 M5, 3 M6 and 10 with myelodysplasia. Cytogenetic data were available in 56 patients. Normal karyotype was identified in 31 (54.4%) patients. Other 25 patients had different chromosomal aberrations including complex karyotype in 8 patients. Methylation-specific PCR was used to study the methylation status of promoter regions of *SOX7, p15^{INK4b}* and Wnt pathway antagonist genes.
Results: The frequency of aberrant methylation was next: *SFRP1* 68.4%, *p15^{INK4b}* 63.2%, *SOX7* 47.4%, *SFRP4* 42.1%, *SFRP5* 35.1%. Absence of aberrant methylation was found in 5 (8.8%) patients. The most frequent finding was methylation of 2 and 3 genes simultaneously: 29.8% and 21.1% accordingly. Half of the patients with aberrant methylation status of all 5 studied genes had AML with dysplasia. The most part of patients with complex karyotype (62.5%) had aberrant methylation of 3-5 genes. The difference in the number of patients with complex or normal karyotype who had aberrant methylation of all 5 studied genes was significant: 50.0% *vs* 9.7% OR=9.3 95% CI 1.5-58.0; *p=0.022*. The patients with normal karyotype and without *FLT3* and *NPM1* mutations were separated according to the number of genes with aberrant

methylation: 0-2 *vs* 3-5. There was no significant difference in overall and relapse free survival between these groups.
Summary/Conclusions: Aberrant methylation of *SOX7, p15^{INK4b}* and Wnt pathway antagonist genes is recurrent biologic phenomenon in AML patients. More often this finding occurs in AML patients with myelodysplasia and complex karyotype.

PB1651

INCIDENCE AND MAIN CHARACTERISTICS OF T(12;22)(P13;Q12)/MN1-ETV6-POSITIVE ADULT BULGARIAN ACUTE MYELOID LEUKEMIA PATIENTS: A SINGLE INSTITUTION EXPERIENCE
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Background: Background: Translocation t(12;22)(p13;q12) is a recurrent, but rare genetic abnormality in acute myeloid leukemia (AML). To our knowledge, only 14 cases of t(12;22)-positive AML have been published so far. This translocation results in the *MN1-ETV6* fusion gene formation, however, in only 5 of the reported t(12;22)-positive AMLs, the presence of *MN1-ETV6* fusion gene was confirmed molecularly [1;2;3]. Due to the rareness of the disease data about the clinical and laboratory features of t(12;22)-positive AML patients are still limited.
Aims: To determine the incidence and the main characteristics of t(12;22)-positive AML.
Methods: Bone marrow aspirates of 645 adult AML patient were tested in the Laboratory of Cytogenetics and Molecular Biology of the National Specialized Hospital for Active Treatment of Hematological Diseases – Sofia during a 10-years period. Successful karyotypes were obtained in 555 patients (86%). In all cases with t(12;22), the presence of *MN1-ETV6* rearrangement was additionally tested by fluorescence *in situ* hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR). The AML diagnosis and subclassification were performed according to the World Health Organization Classification criteria (2008).
Results: Overall, t(12;22) was detected by conventional cytogenetics in three patients (0.54%). In all of them the translocation was identified as a sole chromosome abnormality and the *MN1-ETV6* fusion was confirmed by FISH and RT-PCR. None of the patients was positive for *BCR-ABL1*, *CBFb-MYH11, AML1-ETO, FLT3-ITD, JAK2 V617F*, and *NPM1* mutations. Two of the patients were male and one was female, aged 60, 65 and 71 years, respectively. White blood cell counts varied from 4.6x10^9/L to 77.5x10^9/L, platelet counts from 14x10^9/L to 104x10^9/L, and hemoglobin from 81 g/L to 125 g/L. Morphology was myelomonocytic in all patients, associated with granulocytic dysplasia in one of the cases and presented as a hypoplastic leukemia in another. Bone marrow blasts accounted for 58%>88%. Flow cytometry revealed CD34 positive expression and aberrant myelomonocytic phenotypes in all three patients, with CD56 positive and CD4 positive immune labeling in 2 patients each. Aberrant expression of other lymphoid-associated antigens (CD7/CD19) was also detected in one case. No splenomegaly was found in all of the patients, while mild liver enlargement was observed in one of them. Two of patients received conventional cytarabine/antracycline-based induction therapy. Unfortunately, early death occurred in both cases. The third patient refused chemotherapy and was lost of follow up.
Summary/Conclusions: Our study confirmed the low incidence of t(12;22)/*MN1-ETV6*-positive AML. Though heterogeneous in terms of clinical and laboratory presentation, the reported patients were characterized by absence of additional chromosomal aberrations, aberrant myelomonocytic phenotype and a high induction-induced death rate.
Acknowledgements: This study was partially supported by the National Science Fund, Ministry of Education, Youth and Science.

References
1. Buijs A, *et al.* Oncogene. 1995;10(8):1511-9.
2. Vieira L, *et al.*, Br J Haematol. 2000;110(1):238-9.
3. Nofrini V, *et al.*, Leuk Res. 2011;35(7):e123-6.

Acute myeloid leukemia - Clinical

PB1652

INCREASED RISK OF TUBERCULOSIS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA IN AN ENDEMIC AREA: A NATIONWIDE POPULATION-BASED STUDY
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Background: To date, few studies have investigated the association between tuberculosis (TB) and acute myeloid leukemia (AML).
Aims: We therefore aim to assess the impact and the risk factors of TB in patients with AML for a better risk stratification strategy.
Methods: We conducted a nationwide population-based study using data retrieved from Taiwan's National Health Insurance Research Database to determine the incidence of TB and to analyze the risk factors for TB in patients with newly diagnosed AML. From 2000 to 2011, we identified 6,771 AML patients and the same number of subjects without AML matched for sex, age, and comorbidities. Cox proportional hazards models were applied for further analysis. AML-related therapies were assessed as time-dependent covariates to avoid immortal time bias.
Results: Compared with the matched cohort, AML patients exhibited a higher risk for TB (adjusted hazard ratio [HR] 4.31, 95% confidence interval [CI] 3.27–5.67, p <0.001), with an increased risk for both pulmonary (adjusted HR 4.22, 95% CI 3.13–5.69, p <0·001) and extrapulmonary (adjusted HR 4.86, 95% CI 2.40–9.84, p <0.001) TB. Adjusted HRs of TB occurrence for follow-up periods of <1, 1–2, 2–5, and ≥5 years were 9.61 (95% CI 5.69–16.25), 5.40 (95% CI 2.88–10.13), 1.89 (95% CI 0.94–3.79), and 1.81 (95% CI 0.71–4.63), respectively. Multivariate analysis showed that being male (adjusted HR 1.47, 95% CI 1.06–2.04, p=0.020) and having liver cirrhosis (adjusted HR 2.08, 95% CI 1.17–3.70, p=0.012) were identified as independent risk factors for developing TB in AML patients. However, treatment with idarubicin, daunorubicin, high-dose cytarabine, or hematopoietic stem cell transplantation did not increase the risk of TB development. AML patients who developed TB had a higher mortality rate than those who did not (adjusted HR 1.63, 95% CI 1.33–1.99, p <0.001) (Figure 1).

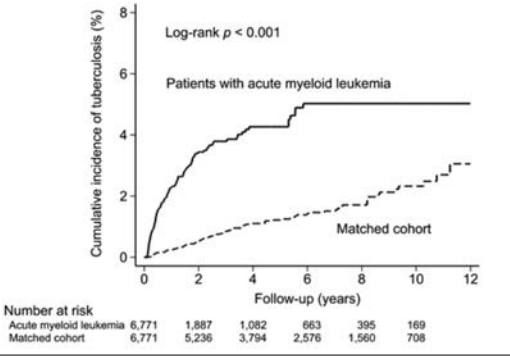

Figure 1.

Summary/Conclusions: Patients with AML had a higher risk of TB, especially within two years of diagnosis. Being male and having liver cirrhosis were independent risk factors for TB in AML patients. Physicians should be aware of the TB situation among their respective populations, particularly those in endemic areas.

PB1653

DECREASING EARLY DEATHS IN ACUTE PROMYELOCYTIC LEUKEMIA (APL) IN AN ACADEMIC CENTER IN THE US BY USING A SIMPLIFIED TREATMENT ALGORITHM AND DEDICATED APL EXPERTS
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Background: APL is a highly curable malignancy with reported survival above 90% in many large co-operative group studies. Treatment of low and intermediate risk patients showed an expected survival of 99% with retinoic acid (ATRA)/Arsenic (ATO) combination *versus* 91% with ATRA/chemotherapy at 2

years. These spectacular results are not evident in the general population. US SEER data showed that 1 and 5 year relative survival in APL patients is 71% and 65%. Studies from Swedish Cancer Registry and Brazil showed that early deaths (ED) can be approximately 30%. This is in contrast to observation in clinical trials where the early mortality is approximately 5%. Common causes of death are hemorrhagic complications (HC), differentiation syndrome (DS), infection and multi-organ failure (MOF). It is now agreed that the most common cause of treatment failure in APL is ED; and decreasing ED will improve population wide survival in this most curable leukemia. We report our institutional experience that showed a decrease in ED by using a streamlined set of guidelines and expert advice as needed.

Aims: To identify the impact of streamlined guidelines

Methods: We reviewed charts of patients with newly diagnosed APL and treated at our institution between January 2007 and August 2013 (earlier group). We compared these outcomes with those diagnosed from September 2013 to January 2016 (newer group) when we put in place a set of streamlined guidelines to improve ED. In addition to the guidelines, three faculty members with an interest in APL provided input as needed in the management of all newly diagnosed patients.

Results: From 01/2007 to 01/2016, 108 patients were treated; 75 patients managed prior to and 33 after establishment of guidelines. One Jehovah's Witness patient was excluded from the newer group for refusing transfusions and subsequently died. Median age was similar at 45 years in both groups (range 19-86 and 21-81 respectively). Patients above 60 years were approximately 30% (23/75 and 10/32) and platelet count (29 and 30) were similar in both groups. In the earlier group, median white cell count (WBC) (4.2 and 2.2) and proportion of high risk (WBC >10,000/ml) patients (36% *vs* 21%) were higher. In the earlier group, induction regimen consisted of ATRA in combination with anthracycline (50, 66%), arsenic (12, 16%), other chemotherapy (8, 10%) or single agent ATRA in (5, 6%) patients. In contrast in the newer group, ATRA with ATO was the preferred induction regimen in 24 (75%) and anthracycline based treatment was used in 6 (18%) patients. 2 patients received ATRA alone. Overall the ED rate was higher in the earlier group with 19 patients (25%) dying in the first month. The causes of death were DIC (9), DS (7) and infection/MOF (3). 5 patients presented with large intracranial bleeds and were extremely ill. Excluding the patients that were referred late, the mortality was 18%; similar to some single institution studies but more than what is seen in clinical trials. After the institution of our algorithm, mortality was reduced to 9.4% (3/32 ED). Cause of ED was DS (2) and DIC (1). The incidence of severe DS (18% *vs* 6%) and sepsis (28% *vs* 16%) were lower in the newer group. The improved outcomes (25% *vs* 9.3%) is probably multifactorial due to lower number of high risk patients, more frequent arsenic based induction, use of streamlined guidelines and advice by dedicated APL experts.

Summary/Conclusions: Our experience shows that a streamlined treatment algorithm along with input from experts will result in better outcomes in this most curable hematological malignancy even at academic centers. We believe our experience warrants large scale implementation of our model and is presently approved as an ECOG/ACRIN trial.

PB1654

CLINICAL SIGNIFICANCE OF IMMUNOPHENOTYPIC CHANGES AT THE FIRST RELAPSE IN ADULT ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Immunophenotypic changes (IPC) between diagnosis and the first relapse are found in up to 90% of relapsed acute myeloid leukemia (AML) cases, and are considered as a result of major clone shift and/or clonal evolution of AML cells, just like additional cytogenetic abnormality (ACA) which we reported that was the strongest negative prognostic factor in relapsed AML patients.

Aims: Because the clinical significance of IPC has not been elucidated in adult AML patients, we conducted a large-scale retrospective study to address this unsolved issue.

Methods: Of the 375 adult patients diagnosed with non-APL AML between 1990 and 2010, 108 relapsed patients whose immunophenotypic data both at diagnosis and at the first relapse were available were included in this study. All these patients underwent intensive chemotherapy. AML cells were sorted with usual CD45/SSC gating, and CD7, CD19, CD13, CD33, CD34, HLA-DR, and CD56 were analyzed. IPC was diagnosed when more than 50% increase or decrease of at least one antigen-positive from diagnosis to the first relapse was observed. Overall survival (OS) was defined as the interval from the date of the first relapse to the date of death. Fisher's exact test was used to compare binary variables. OS was estimated with the Kaplan-Meier method and compared using the log-rank test. Multiple logistic regression analysis and the Cox proportional hazard model were used for multivariate analysis of predisposing factors and prognostic factors, respectively. Values of $p < 0.05$ were considered to indicate statistical significance.

Results: Of the 108 patients included in this study, 65 patients were male, and 43 were female. The median age was 56 years (range, 18–78 years). According to the definition described above, 46 patients (43%) experienced IPC at the first relapse. With univariate analysis, t(8;21) was extracted as a statistically significant predisposing factor for IPC at the first relapse (69%). Multivariate analysis revealed t(8;21) and M4/5 as independent predisposing factors. Excluding one patient that directly proceeded to allo-SCT without re-induction chemotherapy, the 107 patients with IPC showed a similar second remission rate when comparing with those without IPC (42.1% *vs* 44.0%, respectively; p=0.848). Additionally, among all 108 patients, there was no significant difference in the 2-year OS rates after the first relapse between patients with and without IPC (34.4% *vs* 29.3%, respectively; p=0.212). Multivariate analysis extracted ACA, high-risk karyotype, fewer than 12 months duration of the first remission, and no allogeneic stem cell transplantation (allo-SCT) after the first relapse, but not IPC. Of the 46 patients with IPC (median age: 55, range; 18–78 years), 18 underwent allo-SCT after the first relapse. The 2-year OS rates after the first relapse were significantly different between patients undergoing allo-SCT after the first relapse and those treated only with chemotherapy (62.7% *vs* 17.9%; p <0.001). With multivariate analysis, undergoing allo-SCT after the first relapse was identified as an independent prognostic factor, in addition to ACA and duration of the first remission.

Summary/Conclusions: These findings suggested that IPC had no prognostic impact in relapsed AML patients, unlike acquisition of ACA. As t(8;21) was extracted as an independent predisposing factor for IPC as well as ACA, susceptibility for clonal evolution might be high in t(8;21) AML patients. Clarification of the biological background of IPC may contribute to the development of novel therapeutic strategies and improved treatment outcomes in adult AML patients.

PB1655

HIGH FREQUENCY OF OCCULT CENTRAL NERVOUS SYSTEM INVOLVEMENT IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM AT DIAGNOSIS: ROLE FOR INTRATHECAL PROPHYLAXIS?

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare aggressive myeloid neoplasm which shows a high rate of central nervous system (CNS) recurrence (≈30%) and a short overall survival (OS), usually <1 year. Despite this, screening for CNS involvement is not routinely performed at diagnosis and intrathecal (IT) prophylaxis is not regularly administered in BPDCN patients.

Aims: To analyse the incidence of CNS involvement in BPDCN patients at diagnosis and to evaluate the potential benefit of intrathecal prophylaxis administration.

Methods: Forty-one stabilized cerebrospinal fluid (CSF) samples from 13 consecutive BPDCN patients were evaluated for the presence of CNS involvement by next generation flow cytometry immunophenotypic studies. Cases were evaluated at diagnosis (n=10) or at relapse (n=3) and subsequently, after IT therapy. The 10 patients studied at diagnosis received high-risk acute lymphoblastic leukaemia (ALL)-type treatment, including one dose of triple intrathecal therapy (TIT), as CNS prophylaxis at each treatment phase. For CSF-positive cases, additional IT treatment was given until two consecutive CSF-negative samples were obtained. In order to validate the impact of CNS involvement and CNS-directed therapy on patient outcome, an independent validation cohort of 23 BPDCN was retrospectively analysed. Informed consent was obtained from all patients.

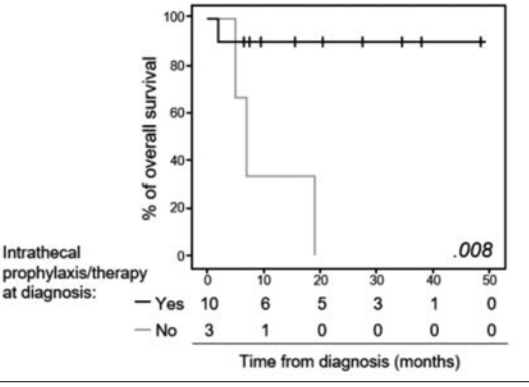

Figure 1. Prognostic impact on OS of intrathecal prophylaxis/therapy administration at diagnosis in BPDCN patients.

Results: Despite none of the patients presented with neurological symptoms at disease staging, occult CNS involvement was detected in 6/10 cases evaluated at diagnosis and 3/3 studied at relapse/progression. Detection of tumour cells in CSF at diagnosis was associated with a ≥20% bone marrow infiltration by tumour cells. BPDCN patients evaluated at diagnosis received IT treatment -either CNS prophylaxis (n=4) or active therapy (n=6)- and all but one remain alive (median follow-up of 20 months). In contrast, all three patients assessed at relapse/progression died (Figure 1). Follow-up CSF samples obtained after TIT showed absence of tumour cells in 6/6 CSF^{+}cases studied at diagnosis, either after one -5/6 cases- or 4 doses of therapy (6/6 cases). The potential benefit of IT treatment administered early at diagnosis on OS and CNS recurrence-free survival (RFS) of BPDCN was further confirmed in a retrospective cohort of another 23 BPDCN patients. Univariate analysis of prognostic factors performed in the whole patient cohort (n=36) showed a favourable impact on CNS-RFS and/or OS for children, patients receiving ALL-type therapy, allogeneic hematopoietic stem cell transplant and IT prophylaxis/treatment at diagnosis, the later variable emerging as the only independent (favourable) prognostic factor for CNS-RFS (p=.02, hazard ratio [HR]=11.2, 95% confidence interval [CI]: 1.4 – 88.8) and OS (p=.001, HR=7.6, 95% CI: 2.2 – 25.9).
Summary/Conclusions: Our findings show that BPDCN patients studied at diagnosis frequently display occult CNS involvement; moreover, these data also indicate that treatment of occult CNS disease might lead to an improved outcome for BPDCN. These results suggest that the CNS could be a persistent blast-cell sanctuary in BPDCN patients with leukemic presentation, due to the limited power of cytostatic drugs to cross the blood-brain barrier into the CSF and brain parenchyma. This reservoir of leukemic cells may also contribute to the high rate of bone marrow/systemic disease recurrence observed in these patients.

PB1656

APPLICABILITY OF THE PATIENT'S "FITNESS" CRITERIA IN ELDERLY AML IN CLINICAL PRACTICE

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Background: The prognosis for acute myeloid leukemia (AML) patients older than 65 years of age is poor. Besides age, comorbidities, a proper assessment of patient's fitness represents a important step in the therapeutic decision-making process in elderly AML patients. Recent study proposed a set of objective criteria to define pts fit or unfit to conventional intensive chemotherapy (i-CT), non-intensive chemotherapy (ni-CT) or best supportive care (BSC)(Ferrara *et al.,* Leukemia, 2013).
Aims: The aim of this study was to investigate: a) applicability of "fitness" criteria; b) the concordance between "fitness" categorization of pts and the type of treatment they actually received; c) the outcome of pts according to "fitness", to the prognostic stratification of European Leukemianet (ELN) and to the treatment received.
Methods: This single-center study involved 201 adult patients with nonpromyelocytic *de novo* AML aged ≥65 years (median age 70 years, range 65-83 years) diagnosed between January 2009 and December 2015. Pts were categorized according to "fitness" criteria, retrospectively: as fit to i-CT (FIT), unfit to i-CT (UNFIT), or unfit even to ni-CT (FRAIL). According to ELN recommendation, pts were at low-risk (6 pts: 3.2%), intermediate-I (91 pts: 48.4%), intermediate-II (39 pts: 20.7%), or high risk (52 pts: 27.7%). The patients were treated with i-CT 89 (44.5%), 66 pts (33%) ni-CT, such as low-dose cytarabine and 45 pts (22.5%) BSC, including cytoreductive therapies (hydroxyurea) and/or transfusion.
Results: Among 201 pts, 98 (49%) were FIT, 79 (39.5%) were UNFIT, and 23 (11.5%) were FRAIL. Their median age was 67, 73 and 72 years. Median overall survival (OS) of FIT, UNFIT and FRAIL pts was 4, 3 and 1 months, (FIT *vs* others: p<0.0001, UNFIT *vs* FRAIL: p=0.012). Overall concordance between "fitness criteria" and the treatment actually received by pts were 77% in FIT, 84% in UNFIT and 96% in FRAIL pts. Median OS of pts receiving i-CT, ni-CT or BSC was 6, 3 and 3 months in FIT (p=0.001); 2,3,4 months in UNFIT (p<0.047). Median OS in FRAIL pts receiving ni-CT or BSC was 1 *vs* 2 months, (p<0.107). CR rate was 50% in LR, 40% in Int-I, 25.6% in Int-II, 25.5% in HR, (p=0.039). OS rate was 5 in LR/Int-I *vs* 3 months in Int-II/HR pts (p<0.0001). Comprising ELN prognostic stratification with fitness, the use of i-CT obtained a significantly better median OS of 12 months in FIT pts at ELN LR/Int-I compared to 4 months in pts at Int-II/HR (p=0,008).
Summary/Conclusions: This study has shown that a fitness was significantly related to patient's outcome. Applying the fitness criteria could be useful in clinical practice for therapeutic decision-making in elderly AML patients.

PB1657

CHARACTERIZATION AND PROGNOSTIC IMPACT OF FLT3-ITD MUTATIONS IN INTERMEDIATE RISK ACUTE MYELOID LEUKEMIA

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Background: The adverse impact of internal tandem duplications (ITDs) of the *FMS-like tyrosine kinase 3 gene (FLT3)* in acute myeloid leukemia (AML) has been related to specific ITD characteristics such as the *FLT3*-ITD/wt allelic ratio (AR), length of mutation and insertion site.
Aims: To evaluate *FLT3*-ITD mutations, as well as *NPM1* mutations, and the prognostic impact of the *FLT3*-ITD AR, size, number and the insertion site of ITDS in AML patients with intermediate-risk karyotype.
Methods: This single-centre study included 148 AML patients with intermediate-risk karyotype according to the European LeukemiaNet criteria. Screening of *FLT3*-ITD and *NPM1* mutations was performed in diagnostic bone marrow samples by PCR with labeled primers and GeneScan-based fragment-length analysis. In *FLT3*-ITD patients, size and number of ITDs were collected and *FLT3*-ITD AR was calculated. The ITD insertion site was determined by direct sequencing of PCR products in a subgroup of patients. For survival analysis, median (Md) value was used to dichotomize continuous variables.
Results: *FLT3*-ITD mutations were found in 29% (43/148) of patients. The majority of patients (36/43) had a single ITD, four patients – two ITDs and one patient - three ITDs. Median ITD size was 42 bp (range, 18 to 108 bp). The *FLT3*-ITD AR varied from 0.02 to 13.48, with a median of 0.57. DNA sequence analysis of 26 single *FLT3*-ITDs patients revealed that the insertion site was localized in juxtamembrane domain (JMD, amino acids 572-609) in 77% patients and in tyrosine kinase I domain (TKD1, amino acids 610-615) in 23% of patients. A trend for worst overall survival (OS) was noted for patients with insertion site in TKD1 (*P*=.09). *FLT3*-ITD was significantly longer in cases with an insertion site in TKD1 compared with ITD insertion in JMD (69 *vs* 36 bp, *P*=.008). Concerning pretreatment patients characteristics, white blood counts (*P*<.001), serum lactate dehydrogenase (*P*=.002), and bone marrow blasts (*P*=.03) differed significantly with increasing AR. Regarding clinical outcome, only patients candidates to intensive chemotherapy were included (N=128). *FLT3*-ITD patients with high mutant level (AR>0.57) had significantly worst OS, median of 7.6 months, than low mutant level and wt patients (25.6 *vs* 23.2 months, *P*=.003). A trend for worst Disease Free Survival (DFS) was noted for patients with AR>0.57 (Md=5.7 months) compared with low ratio (Md=15.6 months) and wt patients (Md=23 months), *P*=.05. When the analysis was restricted to *NPM1*mut AML patients (60/146), *FLT3*-ITD patients with high AR had significantly worst OS (Md=7.6 months) than low mutant level (Md=34.7 months); OS for wt patients was not reached (*P*<.001). *FLT3*-ITD patients with high AR had a DFS significantly lower (5.8 months) than low AR (15.6 months) and wt type patients (37.9 months), *P*=.01. No significant difference was observed between low ratio and wt patients for DFS (*P*=.09), whereas for OS was (*P*=.02). No significant difference was observed in DFS and OS regarding insertion site, size and number of ITDs.
Summary/Conclusions: We confirmed that *FLT3*-ITD allelic ratio was the only ITD characteristic with significant prognostic marker for OS and DFS within our cohort of *FLT3*-ITD intermediate-risk AML patients. Nevertheless we were able to identify a trend for worst OS in patients with ITD insertion site in TKD1. Moreover, we were able to show that, among patients with concomitant *NPM1* mutation, DFS was not significantly different between ITD low burden and wt patients.

PB1658

POST-TRANSPLANTATION MONITORING OF ACUTE MYELOID LEUKEMIA BY SERIAL MEASUREMENT OF WT1 GENE EXPRESSION LEVELS

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Background: The major problem in the treatment of acute myeloid leukemia (AML) with allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the relapse of the underlying disease, poorly responsive to chemotherapy. For its early detection, molecular monitoring methods were introduced, of which serial measurement of Wilms Tumor-1 (WT1) gene expression levels is considered to be the most efficient (Candoni *et al.*, 2011; Zhao *et al.*, 2011; Mamaev *et al.*, 2015).
Aims: We aimed to estimate the molecular monitoring potential for early detection of relapse and the quality of its treatment in AML patients after allo-HSCT.
Methods: A serial measurement of WT1 gene expression levels by quantitative real-time polymerase chain reaction (PCR) was conducted in 65 AML patients in the post-transplant period. The threshold for identification of WT1 gene overexpression was above $250x10^4$ copies of the ABL gene. The clinical and laboratory characteristics of AML patients after allo-HSCT are presented in Table 1.
Results: The maximum initial level of WT1 gene expression ranged from 259 to 56884 copies/10^4 copies of ABL gene (9869 copies on average). Its expression hardly depended on the cytological type of AML or the cytogenetic type of the cells. According to the obtained data on WT gene expression, two groups of patients were formed. The first group consisted of 28 patients with stable normal WT1 gene expression during the post-transplantation period; the second group consisted of 37 patients with impaired WT1 gene expression. In the period after transplantation WT1 gene expression levels were consistent with or

surpassing those of specific markers of minimal residual disease in parallel analysis. Our data showed that in one-third of the patients WT1 overexpression preceded the increase in blast cells in the bone marrow, thus predicting a relapse. For post-transplantation relapse therapy hypomethylating agents in combination with or without donor lymphocyte infusion were applied in 31 patients. The therapy results were not encouraging. In 22 patients mortality was due to the relapse of the underlying disease. Complete remission was only achieved in 6 patients with AML. Three of them later died from GVHD, acute heart failure and sepsis on D+101, D+541 and D+350 after allo-HSCT respectively.

Table 1.

Number of patients	65
AML	
AML *de novo*	51 (0-4, 1-6, 2-12, 3-1, 4-20, 5-6, 6-1, 7-1)
Secondary AML	14
Patient sex, n	
Male	36
Female	29
Age at HSCT (mediana)	26 (4-56)
Cytogenetics, n	
Favorable	0
Intermediate	50
Infavorable	15
Molecular markers, n	
Yes	16
No	49
HSC source, n	
Bone marrow	34
Peripheral blood	31
Conditioning regimen, n	
MA	44
Non-MA	21
Donor type, n	
HLA-id sibling	12
Matched unrelated	41
Haploidentical	12
Number of transplanted CD34+cells (mediana)	$4,78*10^6$ (2,2-11,9)

Summary/Conclusions: Molecular monitoring of acute myeloid leukemia after allo-HSCT using serial measurement of WT1 gene expression levels allows to reveal cytological relapses at an earlier stage and may soon be used to assess the treatment of prognostically adverse post-transplant relapses of AML.

PB1659

EFFICACY AND SAFETY OF ADJUSTED CAG IN COMBINATION WITH DECITABINE IN NEWLY DIAGNOSED OR REFRACTORY/RELAPSED ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Priming regimen(*e.g.*, CAG) combined with Decitabine is increasingly being used to treat acute myeloid leukemia, especially in elderly patients. However, the particular mode of administration and dosage of Decitabine has not been unified.
Aims: This pilot study was designed to evaluate the efficacy and safety of adjusted CAG (aCAG) regimen combined with Decitabine treatment for elderly patients with newly diagnosed or refractory/relapsed acute myeloid leukemia (AML).
Methods: All patients (age≥60 years, PS≤2) in this study were treated with aCAG-D regimen: Cytarabine (10mg/m^2/12h, days 1-8), Aclarubicin (5-7mg/m^2/day, days 1-8), Granulocyte colony-stimulating factor (300μg/day, days 0-8 or stopped when WBC count >20×10^9/L) and Decitabine(25mg/m^2/day, days 5-8) for two cycles.
Results: The median age of the twelve patients (seven men and five women) able to be evaluated, which contained nine *de novo* patients and three refractory/relapsed patients, was 67 years (range of 60-75 years). A total of nine patients (75.0%) achieved complete remission (CR) after the aCAG-D treatment, including eight of whom achieved CR after only one treatment cycle. In nine newly diagnosed patients eight cases achieved CR (88.9%) and another got partial remission (PR). Meanwhile, three other refractory/relapsed patients achieved CR/ partial remission (PR)/no response (NR) respectively. The overall response rate (ORR) and CR after two treatment cycles were 91.6% and 75.0%, respectively. In patients with CR, the median time was 24.2 days (range of 16-43 days) for granulocyte recovery and 17.9 days (range of 11-37 days) for platelet recovery. After a median follow-up of 11 months (range of 4.5-22 months), one patient died due to secondary infections; one patient showed a relapse; five patients died due to progression of the disease; five patients maintained CR. The median overall survival (OS) of 13 months(range of 4.5-22 months) for those who achieved CR was significantly longer than that of patients who did not achieved CR(5 months). The main adverse events were grade IV bone marrow suppression(12/12 patients) and grade I-IV secondary infection(11/12 patients). There was no treatment-related mortality during remission induction (Figure 1).

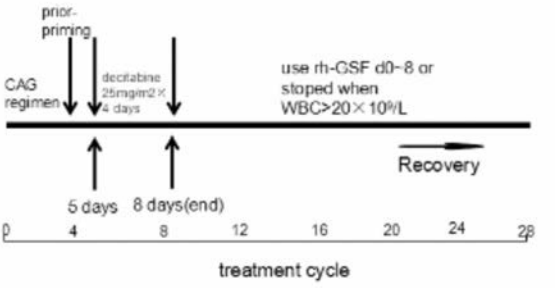

Figure 1.

Summary/Conclusions: The regimen combined aCAG and Decitabine, which designed to use CAG scheme for priming firstly, then add Decitabine to target tumor cells in the S-phase of the cell cycle, demonstrates good efficacy for elderly AML patients, especially in *de novo* patients. Meanwhile, this regimen didn't give rise to serious myelo-suppression and secondary infection. In general, aCAG-D regimen was well tolerated by the elderly patients with *de novo* or relapsed/refractory AML and showed promising clinical efficacy.

PB1660

HOME CARE ADMINISTRATION OF SUBCUTANEOUS AZACITIDINE (AZA) AND DOMICILIARY MANAGEMENT OF FRAIL PATIENTS AFFECTED BY ACUTE MYELOID LEUKEMIA/MYELODYSPLASTIC SYNDROME: A FEASIBILITY STUDY

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Background: Home delivery of parenteral antineoplastic agents may allow an appropriate treatment for frail or disable patients (pts) with acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS), whose management would be otherwise hampered by clinical barriers or conditioned by hospitalization requirements.
Aims: To describe the feasibility of a home care (HC) program that provides a managing package capable of supporting pts with their parenteral AZA therapy at home.
Methods: A retrospective analysis of the clinical features, use of resources, follow-up and HC satisfaction rating of frail homebound pts affected by AML or intermediate-high risk MDS with a clinical frailty scale score >4, who received at home subcutaneous AZA (75 mg/sqm/7 days).
Results: Overall, 106 pts with AML/MDS received AZA therapy; in 13 out of 31 pts (4%) with AML and in 7 out of 75 pts (9%) with MDS, the drug was administered at home. Reasons for the HC solution were: i) disability in 15 pts (11 AML/4 MDS), ii) early discharge from the ward in 5 (2 AML/3 MDS). The pts' median age was 76 yrs (range 59-83), Karnofsky PS 50% (range 40-60%), Activity Dayly Living score 4 (range 1-6), Charlson co-morbidity index 2 (range 1-3). The mean No. of AZA courses in AML and MDS patients was 3 (1-6) and 2 (1-4), respectively. At home, 409 units of packed red cells were transfused, with a mean No. of 20 units transfused/per patient (range 0-94). During AZA therapy, 13 pts had infections with a mean No. of infections/patient of 1.9 (range 0-7). In 37 out of 38 cases (97%), infections were managed at home with i.v. antibiotic administration. Overall, hospitalization occurred in 8 out of 20 pts (40%). AZA therapy at home was started as a first course in 6 pts, whereas it was continued in 14 pts following courses already delivered at hospital, with a mean No. of 2.8 courses (range 1-4) and 2.1 (range 1-6), administered respectively. In 83% of cases, pts rated HC as more than good. Eighteen pts discontinued HC AZA: 15 due to disease progression and 3 because of an infection-related hospitalization. The median length of HC after AZA interruption was 111 days (1-343). Two pts are still on treatment with AZA, 18 pts have died: 11 at home or at a hospice, 7 while hospitalized. The pts median survival time from the start of AZA was 143.2 days (range 5-284) for AML pts and 229.7 days (range 49-411) for MDS pts.
Summary/Conclusions: Home administration of AZA proved feasible through the availability of a domiciliary service that guaranteed a high number of transfusions, blood sampling and i.v. antimicrobial therapy, combined with an approach of palliative care. Pts with poor prognosis AML/MDS had the opportunity of undergoing a specific epigenetic treatment at home, while living a con-

siderable time interval outside the hospital with a high satisfaction level for HC. Inappropriate hospitalizations were reduced, with an overall median time at home of nearly 5 months for AML and 7.6 months for MDS pts. Death during the hospice care occurred in 61% of cases.

PB1661

ELANE MUTATION C233TER PREDISPOSES PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA TO ACUTE MYELOID LEUKEMIA

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Background: Mutations in *ELANE* are the most frequent cause for cyclic and congenital neutropenia. In a genotype-phenotype study, we previously reported that mutations G214R which replaces glycine (nonpolar, neutral) with the much larger arginine (polar, strongly basic) amino acid, and C151Y which disrupts the second disulfide bond of the protein are associated with severe outcomes. (Makaryan *et al.,* Curr Opin Hematol 2015;22:3-11) Termination mutations were associated with leukemic transformation, but the number of these patients was relatively small.
Aims: To investigate the natural history and long-term consequences of mutations in *ELANE*.
Methods: The Severe Chronic Neutropenia International Registry (SCNIR) has enrolled 3 patients with the termination mutation C223ter. All three have developed acute myeloid leukemia.
Results: Patient #1 is a 38 year old female residing in Michigan USA. She was diagnosed with SCN in 1989 at age 11. She started G-CSF in 1989, 11.5 mcg/kg/day. Because of a poor response, G-CSF was gradually increased to 24 mcg/kg/day. After 15 years, she was switched to peg G-CSF, 3 mg every 10 days for the next 11 years. In February 2015 at age 37, she had blasts in the blood and marrow revealing 65.8% blasts. Ten months ago, she underwent HSCT, GvHD has resolved, platelets are holding at 59K without transfusions and she receives darbepoetin alfa to maintain hemoglobin. Patient #2 is a 25 year old female residing in Western Australia. She was diagnosed with SCN in 1991 at age 4.5 months. She was started at age 5 months on G-CSF at 5 mcg/kg/day and the dose increased to 20 mcg/kg/day because of a poor response. After 12 years, she was switched to peg G-CSF 6 mg q 14 days after 12 years because of her poor neutrophil response. She received Neulasta 6 mg every 14 days for 11 years. She became pregnant in May 2015 having had 3 previous pregnancies (2 terminations and one live birth). In the second month of her fourth pregnancy, she appeared to be losing her response to peg G-CSF. About two months later, she was found to have hypertrophied gums with infiltration of immature myeloid cells, consistent with the abnormal cells also found in her bone marrow. She was maintained on G-CSF, prednisone, and blood products because without the growth factor she was severely ill. With this approach, she delivered a healthy male infant at 30 weeks via C-section. She had a HSCT five weeks after delivery and is now 2 months post-transplant, in serious condition with the donor marrow beginning to respond. Patient # 3 was a 6 year old female who resided in Chile. She was diagnosed with SCN in 1997 at 10 months and started G-CSF at 15 mcg/kg/day. There was a poor response requiring an increase of G-CSF to 37.5 mcg/kg/day. After 3 years on G-CSF, she developed AML presenting with increased blasts, anemia and thrombocytopenia. She received chemotherapy; HSCT was not available. She died from complications of AML in 2002. Mutations in G214R, C152Y and C223ter have no obvious common features in terms of effects on the active site, glycosylation sites, or disulfide bonds of the enzyme. These mutations and others associated with evolution to AML are often relatively resistant to treatment with G-CSF, but the biological basis for resistance to G-CSF as well as the risk of evolution to AML is not known.
Summary/Conclusions: Understanding the biological basis for the diversity of genotype-phenotype relationships for mutations in *ELANE*, particularly how some mutations predispose to a high risk of AML, is critical for improving the diagnosis and treatment of this form of hereditary neutropenia.

PB1662

ACUTE MYELOID LEUKEMIA IN THE ELDERLY TREATED AGGRESSIVELY IN A NON-TRANSPLANT CENTER: A SINGLE CENTER OF EXPERIENCE

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Background: Most cases of Acute Myeloid Leukemia (AML) occur in older patients in whom, considering biological variation associated with chemoresistance and comorbidities, defines a challenging population. While overall survival (OS) in patients with AML decreases with age, most studies performed in elderly patients showed Complete Remission (CR) rates between 50 and 60% if they are treated aggressively.
Aims: This study aims to characterize an elderly population with a diagnosis of AML (considering age subgroups, cytogenetic risk groups, type of intended treatment and use of consolidation) and to analyze the impact of different treatment approaches on complete response (CR) and their overall survival (OS).
Methods: We analysed 157 patients aged over 60 years with the diagnosis of AML (excluding acute promyelocytic leukemia, according the 2008 World Health Organization classification) treated agressively. Retrospective analysis of clinical and laboratory data, between 1st January 2005 and 31st December 2015.
Results: Of all patients, 55% were male. The median age at diagnosis was 67 years and 68% of patients were over 65. In this population 49% (N=77) of AML were secondary to another haematological disorder (40%, N=63), or as consequence of prior chemotherapy for another malignancy (9%, N=14). According to the French-British-American classification, the most frequent subtypes were M4 (23%), followed by the M2 and M1 subtypes (22% each) and M5 (19%). We obtained conventional karyotype cytogenetic results in 64% of our patients, of whom 4% were of low risk, 45% of intermediate risk and 15% of high risk. We choose an aggressive treatment strategy in these 157 patients, pre-selected according to age, performance-status and comorbidities, attaining a CR rate of 52%. The main induction regimens were MIC/MICE (N=113), ICE/3+7 (N=30), ARA-C (N=12). OS was respectively 43%, 49% and 8% at 10 months, with significant difference between MIC/MICE and ICE/3+7 when compared with ARA-C regimen ($p<0.05$). During hematological recovery, 62% presented febrile neutropenia, 10% presented mucositis (grade ≥3) and 8% presented palmar-plantar rash with cytarabine. The median days to hematological recovery was 28. The use of consolidation therapy after CR in this age group didn't improve overall survival (62 *vs* 53% at 12 months, p=N.S.). OS was 15% at 12 months and 10% at 24 months with a statistically significant difference in survival between the 60-65, and over 65 age groups at 12 months (20% *vs* 12%; $p<0.05$). Progression of the haematological malignancy was the primary cause of death in 43% of patients and infection in 22%; 6% died from bleeding complications.
Summary/Conclusions: Although we concluded that a significant proportion of patients treated aggressively obtain CR, in our series the use of consolidation didn't improve OS. However aggressive treatment strategies may induce several complications so treatment decisions should be individualized to each patient, according to risk factors, such as age and cytogenetics, performance status and co-morbidities. The recommendation is to whenever possible enroll the patient in a clinical trial.

PB1663

ACUTE PROMYELOCYTIC LEUKEMIA: A CONTEMPORARY SINGLE-INSTITUTION EXPERIENCE

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Background: Acute promyelocytic leukemia (APL) is a rare hematologic malignancy, with an incidence of 600-800 cases per year in the United States. At our institution, fewer than 10 patients with a new diagnosis of acute promyelocytic leukemia are treated each year.
Aims: We aim to describe our experience with patients treated for acute promyelocytic leukemia at our institution over the last 15 years.
Methods: After Mayo Clinic institutional review board approval, we searched our institutional electronic database for those 18 years and older with a new diagnosis of acute promyelocytic leukemia between January 1, 2000 and December 31, 2015. We meticulously gathered all patient information through electronic chart review.
Results: Out of a total of 89 patients reviewed, 59 patients had complete data available. The median age at diagnosis was 61 years (range: 18-80 years). 34 patients were male, and 25 were female. 9 patients (15.3%) presented with high-risk disease, 27 (45.8%) had intermediate-risk disease, and 23 (39.0%) had low-risk disease. 5 patients (8.5%) had received chemotherapy for prior malignancies. 50 patients (84.7%) received ATRA with anthracycline-based chemotherapy, and 9 received ATRA and arsenic induction. 58 of 59 (98.3%) patients achieved complete remission after induction chemotherapy. One patient died during induction due to diffuse alveolar hemorrhage. After a median follow-up of 51 months (range: 1-180 months), 53 of 59 (89.8%) patients remain alive. 2 patients died of causes related to APL, 1 of a non-APL-related cause, and 3 died of unknown causes. Among 9 patients treated with ATRA and arsenic induction, all achieved complete remission and were alive at the time of last follow-up. 28 patients (47.5%) presented with disseminated intravascular coagulation (DIC) at diagnosis. Out of 28 patients with DIC, 9 (32.1%) experienced hemorrhage, and 19 (67.9%) experienced thrombotic events. 23 of 59 patients (40.0%) developed differentiation syndrome; 3 of 9 patients (33.3%) treated with arsenic and 20 of 50 patients (40.0%) treated with chemotherapy. 6 of the 9 patients (66.7%) treated with ATRA-arsenic had grade 3 or 4 neutropenia for 15 days or more during induction therapy. 46 of 50 patients (92.0%) treated with ATRA-chemotherapy had grade 3 or 4 neutropenia during induction. Infection was documented during induction therapy in 5 of 9 patients (55.6%) treated with arsenic and 26 of 50 patients (52.0%) receiving chemotherapy. 4 of the 6 patients (66.7%) who developed hepatic toxicity were receiving arsenic. Overall,

7 of 59 patients (11.9%) have relapsed, and 10 of 59 patients (16.9%) developed a secondary malignancy in our cohort.
Summary/Conclusions: Herein, we summarize our single-institution experience with acute promyelocytic leukemia treated over the last fifteen years. A comparison of the adverse events and efficacy of ATRA and chemotherapy *versus* ATRA and arsenic needs further exploration.

PB1664

SUCCESSFUL BL-8040 TREATMENT FOR RELAPSED AML PATIENTS SINGLE CENTER EXPERIENCE

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Background: BL-8040 is a high-affinity antagonist of CXCR4, a chemokine receptor that is directly involved in tumor progression, angiogenesis, metastasis, and cell survival. BL-8040 binds to CXCR4 on leukemic cells, inhibiting its function, thus releasing them from the protective microenvironment of the bone marrow (BM) resulting in amplification of their sensitivity to chemotherapy. BL-8040 also has a direct anti-tumor effect by selectively inducing apoptosis of malignant cells.
Aims: To assess the effectiveness of BL-8040 in combination with cytarabine (Ara-C) in three patients with relapsed AML who were treated under compassionate protocol.
Methods: Treatment of relapsed AML patients with SC BL-8040 1.25mg/kg on days 1-7 together with Ara-C 1.5 gr/m^2 from day 3-7.
Results: Case 1: A 75 year old male with relapsed AML, 7 years after initial diagnosis. At the time of diagnosis he had normal karyotype and treated successfully with 7+3 followed by consolidation with Ara-C. At time of relapse he presented with dysplastic changes and additional chromosome 13 karyotype. At relapse the patient was treated with Ara-C and BL-8040. On day 35 his BM was in complete remission (CR) with less than 5% blasts. Maintenance therapy with azacitidine began three months later. Nine months post induction the patient is still in CR. Case 2: A 23 year old male with a history of Hodgkin lymphoma at age 17. At the age of 20 he was diagnosed with AML with the t(8;21) chromosomal abnormality. He was treated with 7+3 followed by consolidation with Ara-C. A year later he relapsed and received an allogeneic transplant (allo-HSCT). Two years post-transplant the patient relapsed with intra and extramedullary disease represented by a large chest wall mass. He was treated with BL-8040 and Ara-C as a salvage treatment. There was some resolution of the extramedullary mass on the first 2 days of monotherapy treatment with BL-8040 (before the onset of Ara-C). The patient experienced severe injection site pain and generalized muscle pain which required narcotics for analgesia. The patient entered CR on day 34 and underwent a second allo-HSCT. Almost a year and half post BL-8040 salvage treatment the patient is still in CR. Case 3: A 66 years old female with relapsed AML, 14 months after first diagnosis. At the time of diagnosis she presented with a complex karyotype. She received induction treatment followed by allo-HSCT but relapsed 3 months later. She was then treated with Ara-C and donor lymphocyte infusion (DLI) reaching CR; 7 months later she relapsed again. A second salvage treatment with BL-8040 and Ara-C was provided. During treatment she suffered from severe muscle pain (chest and legs). The patient didn't reach response showing 52% myeloblasts on day 24 BM examination. Two days later the patient died. The direct cause of her death is likely to be disease related.
Summary/Conclusions: Three patients were treated with BL-8040 and Ara-C as salvage therapy for relapsed AML. The safety profile was similar and the adverse events were well managed. These were characterized by injection site reactions and severe muscle pain. BL-8040 in combination with Ara-C can be administered safely to relapsed, heavily pretreated, AML patients. BL-8040 in combination with Ara-C has been shown to be an effective regimen for bone marrow as well as extramedullary AML. BL-8040 in combination with Ara-C should be considered as a bridging therapy for allo-HSCT.

PB1665

IMPACT OF GRANULOCYTE COLONY STIMULATING FACTOR FOR OUTCOMES OF NON-M3 AML PATIENTS TREATED WITH ANTHRACYCLINE-BASED INDUCTION (7+3 REGIMEN) CHEMOTHERAPIES

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Background: Currently most guidelines recommend primary granulocyte colony-stimulating factor (G-CSF) prophylaxis in patients with solid cancer who have an approximately 20% or higher risk for febrile neutropenia. However, these recommendations are not as clear for patients with acute myelogenous leukemia (AML).
Aims: To identify the role of G-CSF in induction treatment in patients with newly diagnosed AML, we analyzed the efficacies of administration based on clinical situations such as the development of neutropenia or fever, and investigated the impact of G-CSF exposure on the anti-leukemic efficacies of induction chemotherapy.
Methods: A total of 285 patients enrolled in the Korea University AML registry from September 2001 to March 2015 were analyzed and classified based on G-CSF administration: (1) no G-CSF exposure during induction (no G-CSF group), (2) administration initiated immediately after the development of neutropenia (absolute neutrophil counts, <1000/μL) but before the development of febrile neutropenia (preemptive group), and (3) administration initiated after the development of febrile neutropenia (therapeutic group).
Results: G-CSF administration resulted in faster ANC recovery compared to that in the no G-CSF group (p <0.001), but did not significantly affect the duration of neutropenia or chemotherapy-induced febrile neutropenia (CIFN) in both the preemptive and therapeutic group. In treatment-related mortality (TRM) multivariate analysis, the therapeutic group had higher TRM than the preemptive group (OR 5.921, 95% confidence interval 1.316–26.634, p=0.020), with no significant difference between the preemptive and no G-CSF groups (OR 2.454, 95% CI 0.482-12.495, p=0.280). Only quinolone prophylaxis was shown to be effective in reducing the incidence of CIFN (p=0.001). There were no significant differences in remission rate, cumulative incidence of relapse, overall survival, and relapse-free survival among the groups (Table 1).

Table 1. Univariate and multivariate analysis for risk factors associated with treatment-related mortality.

Factors	Treatment-related mortality			
	Univariate	Multivariate		
	P value	OR	95% CI	P value
Group(Ref. Pre-emptive G-CSF)	**0.040**			**0.022**
Pre-emptive G-CSF vs. No G-CSF	0.076	2.454	0.482 – 12.495	0.280
Pre-emptive G-CSF vs. Therapeutic G-CSF	**0.014**	5.921	1.316 – 26.634	**0.020**
Age< 60 (Ref.) vs. Age≥ 60	**<0.001**	2.366	0.979 – 5.716	0.056
Sex	0.139			
ECOG 0-1(Ref.) vs. ECOG≥ 2	**0.001**	3.392	1.239 – 9.288	**0.017**
CCI 0-1(Ref.) vs. CCI ≥ 2	**0.001**	2.813	1.093 – 7.237	**0.032**
Cytogenetic risk	0.558			
Type of anthracyclin	0.481			
Prophylactic quinolone	0.836			

Abbreviations: G-CSF, Granulocyte-stimulating factor; CCI, Charlson comorbidity index; HR, Hazard ratio; CI, Confidence interval; NA, Not applicable

Summary/Conclusions: G-CSF administration during induction chemotherapy in non-M3 AML patients can accelerate neutrophil recovery without affecting treatment outcomes. It is best administered at least before the development of febrile neutropenia in order to prevent TRM. Quinolone prophylaxis might be effective in reducing CIFN.

PB1666

ADULT BIPHENOTYPIC ACUTE LEUKEMIA: THE EGYPTIAN NATIONAL CANCER INSTITUTE EXPERIENCE

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Background: Biphenotypic acute leukemia is a rare form of leukemia. Knowledge concerning the clinical and biological presentation, as well as the outcome of treatment, in adult is limited
Aims: Our objective was to analyze the biological features and outcome of patients diagnosed with BAL in our institute.
Methods: This is a retrospective analysis of the clinical, biological, and immunophenotypic features of 30 biphenotypic acute leukemias (BALs), fulfilling modified EGIL's score, and treated in the medical oncology department at the National Cancer Institute (NCI-Cairo) between 2005& 2010. Myeloid and T-lineage features were demonstrated by cytoplasmic myeloperoxidase and CD3; B-lineage features were demonstrated by CD19, CD22 and CD10.

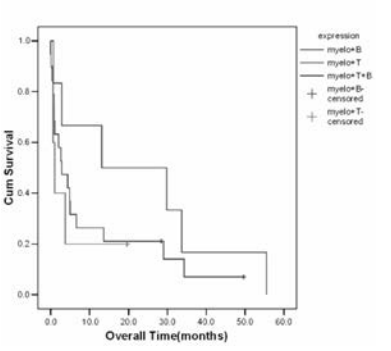

Figure 1.

Results: There were 18 men and 12 women; all were adult with a male to female ratio 3:2. The median age of the patients at diagnosis was 40 years (range, 19-62).The median white blood cell (WBC) count, hemoglobin concentration and platelet count were 31x10^3/dl (range, 2-275), 7g/dl (range, 4.1-11) and 53x10^3/dl (range, 14-539), respectively. Morphological assessment showed myeloid features in 14, and undifferentiated in sixteen patients. According to the EGIL classification, there were 20 cases of myeloid+B-lymphoid leukemia (66.7%), 5 cases of myeloid+T-lymphoid (16.7%), and 5 cases of trilineage myeloid+B+T-lymphoid leukemia (16.7%). The most common phenotypic feature was the expression of CD45 antigen which was positive in 27 (93.1%) patients. Cytogenetic results were available for only 4 patients. Eight patients received ALL-tailored therapy, 14 received AML-tailored therapy while 8 were either unfit for chemotherapy or died before induction treatment. Patients that received ALL-tailored chemotherapy had a better CR achievement rate (87.5%) over the patients that received AML-tailored chemotherapy (35.7%). The 6, 12 & 24 months overall survival (OS) were 33.3%, 30.0% & 26.6% respectively. Although patients with trilineage phenotype had better OS at 6, 12 & 24 months this was not of statistical significance (Figure 1).
Summary/Conclusions: Biphenotypic acute leukemia is a poor-risk disease. Despite the progress in the treatment of acute leukemia there are no uniform criteria about whether to treat BAL patients as ALL or AML. Further prospective collaborative studies are needed to investigate proper treatment protocols for this entity.

PB1667

SALVAGE REGIMENT WITH FLAT FOR REFRACTORY AND RELAPSED AML: EXPERIENCE A SINGLE CENTRE

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Background: Outcomes in patients with acute myeloid leukemia (AML) who are primary refractory or early relapsed are dismal, and there is not one standard therapy for all patients. Allogeneic hematopoietic stem cells transplantation (HSCT) is the treatment with the highest probability of cure when is possible to reduce the leukemia burden with salvage chemotherapy regimen prior to transplantation.
Aims: In this study, we report our experience of salvage chemotherapy regimen with fludarabine 30 mg/m^2, cytarabine 2 g/m^2 and topotecan 1.5 mg/m^2 on days 1 to 4 (FLAT), for refractory or relapsed AML treated in our institution.
Methods: Analytical, observational and retrospective study. We included all patients treated with FLAT from 2008 to 2016 in our center. We studied disease status prior to salvage therapy (refractoriness to one or two previous regimens *Vs* early or late relapse), cytogenetic risk profile (favorable, intermediate or adverse), response (complete response [CR], partial response [PR] or refractory disease [RD]) and survival (overall survival [OS]).
Results: Twenty-four patients were treated with FLAT in the last eight years in our center. Median age at time of treatment was 55 years old (range 39-69). AML was the underlying condition in all individuals. Cytogenetic risk profile at diagnosis (ELN) was favorable in 2 patients, intermediate in 10 and adverse in 9 of them. It was not determinate in 3 patients. Ten patients received FLAT salvage course for primary refractory AML, 1 for secondary refractory AML, 11 for relapsed AML after chemotherapy and 2 for relapsed AML after stem cell transplant (allo-SCT). Median OS was 16 months (range 1-86), with median follow up of 41 months. OS in primary refractory AML was 20 months (1-84) and 14 months (3-86) in relapsed AML. RC rate after FLAT was 45.8% (11 patients), higher in refractory disease (7 out of 11). Treatment related mortality was 16%. After reaching CR or PR, 7 patients underwent allogeneic transplantation. In this group, OS was 23 months (2-84). Four patients did not undergo transplantation despite reaching CR because of infection complications or early relapse while unrelated donor search was activated. Four patients underwent a sequential approach with a third salvage chemotherapy and allo-SCT despite refractoriness to FLAT; two of them could reach CR with this approach.
Summary/Conclusions: FLAT is an efficient salvage regimen for refractory and relapse AML. An acceptable CR rate allowed patients to continue with allogeneic-SCT and a longer overall survival. This combination has an acceptable safety profile, even for the patients who were treated after transplant.

PB1668

KINETICS OF WT1 OVER-EXPRESSION IN ACUTE MYELOID LEUKEMIA PATIENTS WHO RECEIVED STANDARD CHEMOTHERAPY OR UNDERWENT ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Wilms' tumor 1 (WT1) gene expression is well-known pan leukemia marker which overexpressed in more than 90% of newly diagnosed acute myeloid leukemia patients. However, the clinical utility of WT1 monitoring has been somewhat controversial.
Aims: The aim of this study is to compare WT1 transcript level kinetic changes in patients with AML either received standard chemotherapy (Arm A) or underwent allogeneic stem cell transplantation (Arm B).
Methods: Peripheral blood samples collected from 26 patients diagnosed with AML at initial diagnosis. Further samples collected from 9 patient who received standard chemotherapy (Arm A) at post-induction and post-intensification time points. Further samples collected from 8 patients who underwent allogeneic stem cell transplantation (Arm B) before conditioning, at day 30 and at day 100. The remaining 9 patients were not included in the analysis due to either early death or a negative WT1 at diagnosis. Quantitative RT-PCR detection of WT1 gene transcript level using Ipsogen WT1 profile Quant was performed on peripheral blood samples in both arms at the different time points mentioned before.
Results: We observed significant difference in the median values of WT1 transcript level at the 3 time points for patients with Arm A (P value 0.011) while for allogeneic arm B the median values were nearly the same at the 3 time points (P value 0.687). We found that WT1 level post-induction correlates with morphologic response (P value: 0.04). The median values for WT1 level were more for relapsed rather than non-relapsed cases in both arms at the 3 time points, but this difference was statistically insignificant except at D100 in arm B (P-value 0.046) and about to be significant at post-intensification (P value was 0.064) in arm A (Figure 1).

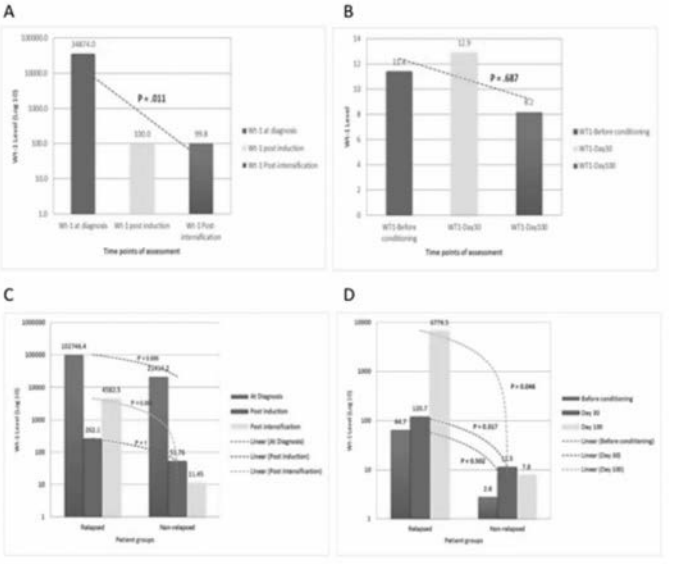

Figure 1. WT1 kinetics. A: WT1 kinetics for arm A. B: WT1 kinetics for arm B. C: WT1 transcript level between relapsed and non-relapsed case in arm A. D: WT1 transcript level between relapsed and non-relapsed case in arm B.

Summary/Conclusions: We identified a positive correlation between WT1 transcript level and morphological response. Therefore, elevation or rising WT1 transcript levels after intensifications or beyond day 100 post-transplant may be a marker of impending relapse that if validated in larger studies, may warrant either close observation or pre-emptive intervention. Although our results are poorly reaching the level of significance, probably due to the small sample size, WT1 transcript level showed dynamic changes with treatment and may be a marker for relapse. We recommend further studies with larger number of patients in different centers to confirm these results.

PB1669

STANDARD INDUCTION CHEMOTHERAPY IN CHILDREN WITH MLL-AF9 ACUTE MYELOID LEUKEMIA AND SEVERE DISSEMINATED INTRAVASCULAR COAGULATION IS ASSOCIATED WITH HIGH MORTALITY

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Background: MLL-AF9 AML is a rare subgroup of unfavorable prognostic leukemia secondary to t(9;11)(p22;q23) with particular clinical aspects, mainly severe coagulopathy.
Aims: To analyze the clinical, hematological and coagulation parameters at diagnosis and correlation with the outcome after chemotherapy initiation in children with MLL-AF9 leukemia treated according to a BFM 2004 AML protocol in a single center.

Methods: Six consecutive patients with MLL-AF9 AML were identified by fluorescence *in situ* hybridization and Nested PCR in our center in the last 10 years. The diagnostic was completed by bone marrow morphology and immunophenotyping, standard kariotype and coagulation tests (AP, aPTT, INR, D-Dimers, PDF, Fbg, PC, PS, AT III). Patients were treated according to the BFM 2004 AML protocol. All patients received fresh frozen plasma. Informed consent has been obtained from parents.
Results: Between 2006-2015 we treated 74 patients with AML, 6 (8,1%) being diagnosed with MLL-AF9 AML, fusion type 6A in all cases. The kariotype has been performed in all patients, but we obtained interpretable result only in 2 patients, all of them without abnormal cytogenetics. The patient characteristics are shown in Table 1. All patients had laboratory signs of variable degrees of DIC at diagnosis documented by prolonged coagulation times, presence of fibrin degradation products (FDP) and elevated D-Dimer levels. Three patients developed during the first 2 days after chemotherapy initiation a respiratory distress syndrome and seizures associated with cerebral hemorrhage with fatal outcome in the next 2-5 days. The initial hemostasis/coagulation analysis in these patients showed platelet counts $30x10^9$/L, $70x10^9$/L, respectively $165x10^9$/L, but with high D-Dimer levels 1670, 2430, respectively >60.000 µg/L (upper detection limit for our laboratory). The other 3 patients completed the induction chemotherapy without special complications. The BM aspirate and MLL-AF9 monitoring confirmed the complete remission (CR). The hemostasis/coagulation analysis in these patients showed similar platelet counts (60 $x10^9$/L, 54 $x10^9$/L, 30 $x10^9$/L), but associated with lower levels of D-Dimers (1070, 960, 1020µg/L).

Table 1.

Patient characteristics	No. 6 cases
Sex ratio M/F	5/1
Age median	2y 6 mo
FAB type	M1:1/M5:3/M7:2
Hg median	7.6 g/dl
PLT median	$62x10^9$/L
WBC median	$19.13x10^9$/L
Blasts median	$13.88x10^9$/L
Hepatomegaly	4/6
Splenomegaly	3/6

Summary/Conclusions: MLL-AF9 is a poor prognostic AML subtype, associated with a higher mortality at induction treatment. Half of the patients in our cohort had a severe DIC associated at onset, with high D-Dimers levels (more than twice upper normal limit) that worsened after chemotherapy initiation leading to respiratory failure and cerebral hemorrhage in absence of severe thrombocytopenia. For our cohort the presence of significant coagulopathy at onset is associated with poor prognosis, suggesting a clinical evolution of this syndrome that is similar with AML M3 outcome in the absence of all-trans retinoic acid associated chemotherapy.

PB1670

CD34 POSITIVE CELL COUNT DETECTED BY MULTI-COLOR FLOW CYTOMETRY (MFC) AFTER FIRST INDUCTION THERAPY IN ACUTE MYELOID LEUKEMIA (AML) EFFECTS ON SURVIVAL AND RELAPSE

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Background: Minimal residual disease (MRD) is defined as detection of low levels of leukemic cells and had been shown to correlate with an increased risk of relapse and shortened survival in Acute Lymphoblastic Leukemia. However there is a controversy in definition of MRD in AML.
Aims: Our aim is to evaluate the effects of CD34 positive cell count of >1.0% after first induction therapy on survival and relapse in AML in our cohort.
Methods: CD34 positive cell count of >1.0% was measured in newly diagnosed 148 AML (excluding acute promyelocytic leukemia) patients by multi-color flow-cytometry (MFC) based detection after first induction regimen in between 2007 and 2015. Chi-square test and kaplan-meier curves were used for statistical analysis. P<0.05 was considered statistically significant.
Results: The median age was 47 (range, 21-82). 78 (52%) of patients were male. AML risk stratification at diagnosis were as follows; low risk (21%), standart risk (38%), high risk (22%). For the induction regimen, 11 geriatric patients received azacytidine and 137 patients got standart 3+7 (ARA-C/daunorubicin). 23.5% of patients (35/148) were not in remission and received reinduction therapy. CD34 positive cell count>1.0% was detected in 49.7% patients (74/148) after induction. High dose ARA-C consolidation treatments (mean 2 cycles, range 1-6) were administered and 87 patients (58%) underwent allogeneic-stem cell transplantation due to their risk status. The relapse rate of patients with CD34 positive cell count >1.0% was significantly higher than CD34 positive cell count lower than 1.0% (58% *vs* 32%, P=0.01). RFS rate at 12 months was higher in CD34 positive cell count lower than 1.0% (72% *vs* 41%, P=0.1). 2 year OS significantly improved in patients with in CD34 positive cell count lower than 1.0% (83% *vs* 66%, P=0.049) (Figure 1).

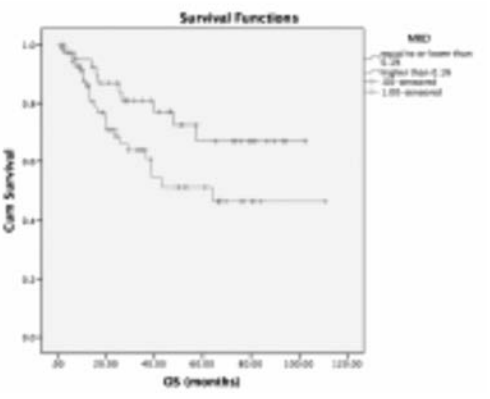

Figure 1.

Summary/Conclusions: CD34 positive cell count >1.0% detected by MFC after first induction is related to higher risk of relapse and decreaced OS. Despite techniques for detection of MRD in AML evolve rapidly, MFC remains a useful tool.

PB1671

ACUTE MYELOID LEUKEMIA WITH MYELODISPLASIA-RELATED CHANGES: A REVIEW OF 58 CASES AT A SINGLE CENTRE

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Background: The WHO-defined category (2008) of acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) includes cases with 20% or more blasts in the peripheral blood or BM and (1) dysplastic morphology in 50% or more of the cells in two or more myeloid lineages and/or (2) a preceding MDS or MDS/MPN phase and/or (3) specific myelodysplasia-related genetic abnormalities. In consecuense, this is a heterogeneous disorder defined by morphologic, genetic, or clinical features.
Aims: Our objective was to analyze the main characteristics of patients with a diagnosis of AML-MRC who were treated and followed in our institution.
Methods: We have retrospectively studied all the cases of AML-MRC that were seen at our hospital in the last fiveteen years. Main recorded data included age, sex, a previous diagnosis of myelodysplastic síndrome (MDS), peripheral blood cell counts, serum lactate-dehydrogenase level, bone marrow aspirate features, cytogenetics, immunophenotype by flow cytometry, therapeutic approach, response to treatment, overall survival and mortality. Statistical analysis was performed using the SPSS 17.0 version.
Results: From 2001 to 2015, 58 patients were diagnosed with AML-MRC at our hospital. Mean age at diagnosis was 72 years (median 75, range 23-90) and 60,3% were men. A previous diagnosis of MDS was noted in 43 patients (74%), with the following distribution: refractory cytopenia with multilineage dysplasia, 22 cases (38%); RAEB-1 or 2, 17 (29%); refractory anemia, 3 (5%); and refractory anemia with ring sideroblasts, 1 (1,7%). According to WHO criteria, cytogenetic abnormalities sufficient to diagnose AML-MRC were found in 7/31 patients with a conclusive caryotypic result (22,6%). A diagnosis of AML-MRC based only in morphological findings was performed in 9 patients (15,5%).
With respect to the findings on blood at diagnosis, leukopenia was present in 53% and leukocytosis in 28%; anemia in 93% (macrocytic 30%) and thrombopenia in 84%. Pancytopenia was found in 47% of cases and a high level of serum lactate-dehydrogenase in 42%. The more frequent phenotypic markers of blast cells, analysed by flow cytometry, were HLA-DR (80% of cases), CD34 (74%), CD33 (72%), CD13 (68%), CD117 (68%), CD38 (62%) and MPO (48%). Some aberrant expressions were found, being CD7 positivity the most frequent (34% of cases); positivities for CD56 (10%) and CD4 (3%) were also observed. The "3+7" induction regimen was applied in 24% of patients, while 5-azacytidine was the drug of choice in 21%. Paliative or supportive treatment was used in the majority of cases (55%). Nine of 14 patients (64%) obtained complete remission after "3+7" scheme (four still alive), and only one (8%) among those treated with 5-azacytidine. At the moment of closing this study, 52 patients had died (89,7%), with a mean overall survival of 14 months (range 0-60), and 6 were still alive after a mean follow-up of 53 months (range 16-168). Mean age of survivors at diagnosis was 57.5 years, compared with 74 years of that who have died. Among the recorded data, those associated with a better outcome were younger age (p=0.028), positivity for CD56 (p=0.005) and intensive treatment (p=0.026).

Summary/Conclusions: In our experience, AML-MRC occurs mainly in elderly patients and is associated with a poor prognosis. The age at diagnosis (and therefore, the probability of receiving intensive chemotherapy) seems to be a strong determinant of prognosis; also, CD56-positive cases exhibited a lower mortality rate than those negative. Further studies are needed to confirm these findings and new therapeutic strategies should be investigated to improve outcome in this group of patients.

PB1672

THE ROLE OF DAILY ECG-MONITORING IN THE MYOCARDIAL INJURY DIAGNOSIS ON THE BACKGROUND OF ANTHRACYCLINE LOW CUMULATIVE DOSES IN PATIENTS WITH ACUTE LEUKEMIA IN COMBINATION WITH ISCHEMIC HEART DISEASE

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Background: Anthracycline-induced cardiotoxicity is a complicated problem of treating patients with acute leukemia (AL), which can lead to the acute cardiac events development. Ischemic heart disease (IHD) is one of the cardiotoxicity risk factors, which requires monitoring of changes in myocardial bioelectric activity on the background of anthracycline low cumulative doses (CDs) in these patients.
Aims: To assess the nature of electrocardiogram (ECG) changes on the background of anthracycline low CDs in patients with AL taking into account concomitant ischemic heart disease.
Methods: The study involved 93 patients with newly diagnosed AL (acute lymphoblastic leukemia – 21 pts, acute myeloid leukemia – 72 pts), mean age 16-72 years, 48 (51.8%) men, 45 (48.2%) women, ECOG I-II. Their polychemotherapy (PCT) programs included anthracyclines. According to the presence of concomitant IHD patients were divided into two groups: I (n=57) – AL patients without concomitant IHD; II (n=36) – AL patients with concomitant IHD. The standard 12-lead ECG and daily ECG-monitoring were performed for patients of both groups in achieving the CD from 100 to 200 mg/m^2 for doxorubicin, which amounted 179.5±24.11 mg/m^2 and 172.1±23.15 mg/m^2 in patients of groups I and II respectively.
Results: The sinus tachycardia, repolarization processes violations and QRS complex voltage reduction were registered in 16 (28%) pts of group I according to the standard 12-lead ECG. In 29 (80.5%) pts of group II on the sinus tachycardia background the following changes were revealed: right bundle branch block – in 2 (5.6%) pts, left anterior fascicular block – in 2 (5.6%) pts, first-degree atrioventricular block – in 2 (5.6%) pts, supraventricular extrasystoles – in 4 (11.1%) pts, lower voltage and repolarization processes reduction – in 8 (22.2%) pts. The ST segment depression was registered in 13 (36.1%) pts, the Q-T interval prolongation – in 6 (16.6%) pts, T wave changes – in 6 (16.7%) pts of group II. According to the daily ECG-monitoring in 28 (49%) pts of group I with minimal physical activity on the tachycardia background the episodes of solitary supraventricular extrasystoles were detected. In all 36 (100%) patients of group II the periods of tachycardia were recorded, that were accompanied by the increasing number of single supraventricular extrasystoles, episodes of paired and group supraventricular extrasystoles – in 24 (66.6%) pts, single episodes of ventricular extrasystoles – in 19 (52%) pts and increased number of clinically significant ST segment depression periods – in 29 (80.5%) and Q-T prolongation – in 14 (38.8%) patients.
Summary/Conclusions: Low CDs up to 100-200 mg/m^2 for doxorubicin in AL patients without ischemic heart disease are accompanied by the cardiotoxic effects development in the form of arrhythmias: sinus tachycardia, supraventricular extrasystoles. In case of concomitant ischemic heart disease presence the complex of myocardial bioelectric activity disorders develops, such as arrhythmias, conduction abnormalities and silent myocardial ischemia. With the purpose of early anthracycline-induced cardiotoxicity diagnosis in patients with AL receiving PCT it is necessary to conduct daily ECG-monitoring, which has greater sensitivity compared with standard 12-lead ECG.

PB1673

MOLECULAR DIAGNOSTIC ON BONE MARROW BIOPSY SPECIMEN: AN INNOVATIVE CLINICAL TOOL WITH MAJOR IMPACT ON CLINICAL DECISIONS.

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Background: Genetics is currently central to diagnosis, prognosis and measure of response to therapy (minimal residual disease, MRD) for patients with acute leukemia, myeloid or lymphoid. These tests are usually conducted on bone marrow aspirate by various techniques (karyotype, fluorescence *in situ* hybridization, reverse transcription polymerase chain reaction, or DNA sequencing). In case of a dry tap these most valuable information are often lost.
Aims: Since January 2014, we implemented a method for DNA and RNA extraction from bone marrow biopsy specimen in case of a dry tap at diagnosis or during follow up. Here we present the method, results and relevance of the analysis conducted so far.
Methods: Bone marrow biopsy is immediately sunken in either RNAlater (QIAGEN, as for other products listed below) for transcript expression analysis such as acute myeloid leukemia (AML) recurrent genetic anomaly or MRD determination. In case DNA-based analysis is warranted, such as analysis of BCR rearrangements and NOTCH mutations, the biopsy specimen is put into phosphate buffer saline (PBS). Biopsy material is then manually crushed and solved in Qiazol for RNA and PBS for DNA. Manual extraction according to the phenol/chloroform is performed prior to RNA purification, quantification and reverse transcription according to manufacturer's instruction. DNA purification and quantification is performed similarly. Analyses are performed according to established standards for bone marrow aspirates.
Results: We retrospectively identified 32 analysis from 20 patients with either acute lymphoid leukemia (ALL, n=6), acute myeloid leukemia (AML, n=12) or high risk myelodysplastic syndromes (MDS, n=2). Dry tap occurred either at diagnosis (n=11) or during follow up (n=21), regardless of diagnosis (lymphoid or myeloid neoplasia), blast count (median 5%, range: 1-95%) or marrow fibrosis (median 2, range 0-4). In seven samples, this technique allowed to identify a mutation in NMP1, with impact on diagnosis and prognosis, which is not amendable to other techniques (karyotype or FISH). Seventeen more samples allowed to quantify minimal residual disease (levels of WT1 or BCR-ABL transcripts), which is either not possible or less refined by alternative cytogenetic methods. Seven patients had more than one sample, which allowed correlation with and refinement of response assessment by histology. On 11 occasions (37.5%) the result of molecular analysis had a major impact on therapy (mostly distinction of Philadelphia positive *vs* negative ALL n=5, targeted therapy for FLT3-ITD transcript n=1, identification of relapse/refractory disease n=2, decision between autologous *vs* allogeneic transplantation for consolidation n=2).
Summary/Conclusions: The method presented here is a readily accessible variant of the commonly used molecular diagnostic techniques. Its use can confirm, expand and usually refine the analysis available on the bone marrow biopsy specimen, and in one third of the cases, have a significant impact on therapy options.

PB1674

ACUTE MYELOID LEUKEMIA IN OLDER PATIENTS: COMPARISON OF THE EFFECTIVENESS OF THERAPY AND SURVIVAL ANALYSIS. A SINGLE CENTER EXPERIENCE

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Background: Acute myeloid leukemia (AML) is disease of older adults with median age over 65 years. The effect of age and severe comorbidities are associated with high incidence of early death, low rate of complete remission (CR) and poor survival. Treatment options for elderly patients include: intensive chemotherapy, less-intensive regimens, best supportive care as well as enrollment in clinical trials. Therapeutic decisions in older AML patients are highly challenging and need to be individualized since the outcome in this group remains still unsatisfactory.
Aims: The aim of the study was to compare results of different treatment strategies in elderly patients with AML and to analyze overall survival in examined patients.
Methods: Patients. We describe a single center experience of 101 patients (41 females and 60 males) with diagnosis of AML, hospitalized in the Department and Clinic of Hematology, Blood Neoplasms, and Bone Marrow Transplantation, Wroclaw Medical University between 2007 and 2014. Median age was 70,6 years (range, 60-90 years). In 85 patients AML was diagnosed *de novo*, whereas 16 patients had secondary AML. Adverse, intermediate and favorable karyotype was found in 14, 82 and 5 patients, respectively. All patients were enrolled into therapeutic procedures according to the age, karyotype, performance status and comorbidity index. Fifty-five patients were treated with intensive chemotherapy regimen based on daunorubicin and cytarabine (DA, "3+5-7"), 22 patients obtained azacitidine (AZA), 14 patients were treated with low-dose cytarabine (LDAC), and only 10 patients received best supportive care (BSC). We estimated percent of CR, early mortality rate, leukemia free survival (LFS) and overall survival (OS).
Results: CR was achieved in 24 patients (44%) who received DA, and in 10 patients (28%) with less-intensive therapy. LFS was comparable in both these groups (5 *vs* 4 months), but superior in patients who received DA. Eight-week early mortality rate was up to 15% after intensive induction therapy and 10% after AZA and LDAC regimens. Nevertheless, median OS in patients treated with DA was marginally superior (5 month) than in patients who received low intensive treatment (4 month) and significantly longer than in patients with BSC (1 month).
Summary/Conclusions: The DA regimen remains still a best standard therapy in older AML patients with good performance status and low comorbidity index. Low-intensive treatment strategies (azacitidine, low-dose cytarabine) seem to be good therapeutic options for older patients with severe comorbidities.

PB1675

ARSENIC TRIOXIDE AS THE FIRST LINE THERAPY FOR PATIENTS WITH SECONDARY MITOXANTRONE-RELATED ACUTE PROMYELOCYTIC LEUKEMIA ON THE BACKGROUND OF MULTIPLE SCLEROSIS

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Background: Mitoxantrone (MT) is a topoisomerase II inhibitor which impairs DNA repair mechanisms. It is well known to induce acute leukemia with absence of myelodysplastic phase. MT therapy is often associated with translocation t(15;17), which defines the development of acute promyelocytic leukemia (APL). MT remains an effective drug for treatment of remitting progressive phase of multiple sclerosis (MS). The APL risk in patients with MS depends on MT dose (≤60mg/m^2, ≥60mg/m^2). Long MS anamnesis, severity of somatic status and evolvement of therapy-related acute leukemia (TRAL) in such patients requires new approaches to treatment.
Aims: To describe the optimal treatment protocols for MS patients with APL, taking into account the patients somatic status.
Methods: From January 2014 to February 2016 in the Department of Hematological Oncology and BMT 15 pts with APL were treated, and 2 of them (39 and 58 y.o.) were with long anamnesis (over 15 years) of MS (remitting progressive phase, cerebral-spinal form). First patient (pt) due to fast progression of MS got MT therapy several times a year, in total≥60mg/m^2, second pt received MT with 60mg in total. Both pts developed APL after 2 years of MT injections. Severity of somatic status was determined by hemorrhagic syndrome (skin and mucosa), coagulation disturbances, granulocytopenia and lower paraparesis with pelvic organs dysfunction due to MS. Pts had intermediate risk APL, cytogenetic analysis showed translocation t(15;17), molecular – bcr1 transcript in both cases.
Results: For the first patient we started all trans-retinoic acid (ATRA) therapy with arsenic trioxide (ATO). Good tolerance to treatment was stated. After 1 month of treatment APL molecular remission was established. The neutropenia period lasted 6 days; catheter-associated sepsis, vaginal and urinary infections, QTc lengthening to 0,48ms were diagnosed. The course of treatment was completed according to ATRA and ATO protocol (E. Estey, 2006). APL remission retains for 16 months. Neurologic symptoms of MS stay stable since the start of APL therapy. The second pt was started with chemotherapy protocol (AIDA), on day 14 he developed septic shock, severe pneumocystis pneumonia with acute respiratory failure. In 2 days he was transferred to the intensive care unit for prolonged invasive mechanical ventilation. Due to long lasting cytopenia (30 days), severe infectious complications, the polyneuropathy deteriorated and tetraplegia developed after status stabilization. ATO therapy with further combination ATRA+ATO was started. Morphological complete remission (CR) and cytogenetic CR were achieved after chemotherapy, molecular CR – after 28 days of ATO. 5 courses of ATO and ATRA+ATO treatment completed. No severe complications were noted and the duration of CR is 8 months. Upper limbs function has recovered, lower limbs – only partially.
Summary/Conclusions: Our experience shows high toxicity of chemotherapy protocol in APL patients with MS. Such protocols cause severe infectious complications due to long period of immunosuppression and may worsen the neurologic status. ATO and ATRA combination as the first line therapy of therapy-related APL in such pts allows to achieve molecular remission with lower toxic effects and without deterioration of MS course.

PB1676

THE ACUTE CLINICAL AND LABORATORY CONSEQUENCES OF ORAL MUCOSITIS DEVELOPED AFTER REMISSION INDUCTION CHEMOTHERAPY IN ACUTE LEUKEMIA PATIENTS

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Background: Chemotherapeutic (CT) agents frequently cause oral mucositis (OM) as a side effect during the treatment of acute leukemia. Patients with OM are generally more predisposed to intraoral infections. OM also affects the nutrition, swallowing, and quality of life.
Aims: To analyze the consequences of post chemotherapy developed OM in acute leukemia patients.
Methods: Seventy-two adult patients with newly-diagnosed Acute Myeloblastic leukemia (n=59), Acute Lymphoblastic Leukemia (n=13) were included. CTs were 7+3 ARA-C+idarubicin (n=43), Azacitidine (n=13), idarubicin+ATRA (n=2), low dose ARA-C (n=1), Hyper-CVAD (n=8), CALGB (n=5). Patients divided into two groups as post-chemotherapy OM or not. Evaluation was performed by same physician daily according to WHO (World Health Organization) OM classification. Stage 1: Oral soreness, erythema; stage 2: Oral erythema, ulcers, solid diet tolerated; stage 3: Oral ulcers, liquid diet only; stage 4: Oral alimentation impossible.
Results: OM occurred in 27 (37%) of 72 patients. Appearance time of the OM after the initiation day of the CT was 6.3±3.4 days and OM were continued for 9.7±5.8 days. There were no significant differences in sociodemographic data, type of leukemia, CT protocol, and smoking status between the two groups. Mostly OM were stage 2 (40.7%, n=11). Others were stage 1 (14%, n=4), stage 3 (29.6%, n=8), stage 4 (14.8%, n=4). Microorganisms in the oral mucositis swab cultures were detected in 12 out of 27 patients with OM. They were Pseudomonas aeruginosa (n=4 patient, 14.8%), Streptococcus viridans in (n=2; 7.4%), Enteroccus faecium in (n=2), Candia albicans (n=1; 3.7%), Acinetobacter baumannii (n=1), Morganella morganii (n=1), Enterobacter cloacae (n=1). Microorganisms in blood cultures of patients with OM were detected in 7 (25.9%) out of 27 patients. This ratio was low as 11% (5 out of 45) in patients without OM. There wasn't any relationship between microorganism in swab culture and blood culture as same in patients. Microorganism in blood cultures of patients with OM were: Staphylococcus hominis (n=2 patients, 7.4%), ESBL Negative E.coli (n=2; 7.4%), Acinetobacter baumannii (n=1; 3.7%), Staphylococcus Epidermidis (n=1), Coagulase negative staphylococcus (n=1). Microorganisms in blood cultures of patients without OM were such as: Staphylococcus Epidermidis (n=2; 4.4%), ESBL Negative E.coli (n=1; 2.2%), Staphylococcus capitis (n=1), Staphylococcus hominis (n=1). The mean duration of neutropenia in OM group was 32 days compared to 22 days in the without OM group (p=0.02). The pre-treatment level of serum albumin was lower, and the levels of CRP and LDH during mucositis were higher in the patients with OM (p<0.001, p<0.001and p=0.002, respectively). Mortality was higher in the group with OM, 9 (6 patients with stage 3 or 4 OM; 3 patients with stage 1) in 27 *vs* 6 in 45 patients (p=0.04). Six of 12 patients who developed severe mucositis (stages 3 and 4) died. The fever appeared on day 7.1±0.87 of the CT in the patients with OM, whereas fever was observed on day 4.1±0.67 in the patients without OM (p=0.002).
Summary/Conclusions: The development of OM may have a role about prognostic value in the patients receiving CT because of the extended duration of neutropenia, higher levels of LDH and CRP, and a higher rate of mortality in patients with OM. Furthermore, we would like to highlight that the patients with low levels of albumin prior to chemotherapy should be more closely followed for OM.

PB1677

ACUTE MYELOID LEUKEMIA IN ELDERLY PATIENTS. ANALYSIS OF 102 CASES

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Background: Acute myeloid leukemia (AML) is more frequent in elderly patients and the prognosis is very poor. There is no established standard treatment, since the survival of patients treated with intensive chemotherapy is 15% at 3 years. Most patients are not candidates for intensive treatment due to age, comorbidity or other biological characteristics. The management of old patients with AML remains controversial, specially in those cases affecting very old patients (aged ≥70).
Aims: It is presented the experience of our centre (single centre experience) with a group of patients from the period 1995-2014.
Methods: We conducted a retrospective analysis of 102 consecutive patients 65years of age or older, diagnosed for AML between January 1995 and December 2014, in Department of Hematology, County Hospital, Timisoara, Romania. Without selection (33.1%) had evolved from prior myelodysplastic syndrome. Patients were divided into 3 groups according to the treatment: no treatment (supportive treatment), low intensity treatment (low doses Ara-C: 10 mg/m^2/12h s. c. days 1-21) and high intensity treatment (Idarubicin 10 mg/m^2 days 1 and 3; Ara-C 100 mg/m^2/12h days 1-3; Etoposide 100 mg/m^2 days 1-3).
Results: Total number of subjects was 57 men and 45 women. Median patient age was 73 years (range, 65-87 years); mean Karnofsky index was 70; 72 patients received treatment and 30 of them did not; M3 subtype was excluded, overall survival was 6,2 months, significant differences were observed in the mean overall survival between the treated and no-treated groups (12,5 *vs* 2,5 months respectively; p=0,015). The patients treated with supportive therapy had a median length of hospital stay of 16 days, while the patients treated with chemotherapy had a median length of hospital stay of 75 days. In the low intensity group (45 patients) an overall response of 30% was observed; in the high intensity one (27 patients), overall response was 50% no statistical differences were observed between both groups considering all subgroups of response (p=0,14).
Summary/Conclusions: Overall survival in the treated group is higher than in the non-treated one, differences reached statistical significance. Comparing both arms of treatment no statistical differences were observed in the quality of response, though a higher proportion of complete responses were observed in the high intensity group. An adequate selection of patients for chemotherapy treatment leads to a low mortality rate. Further research is needed to establish optimal management and improve outcomes of elderly patients with AML.

Aggressive Non-Hodgkin lymphoma - Clinical

PB1678

HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION RESULTS IN EXCELLENT LONG TERM SURVIVAL IN PRIMARY MEDIASTINAL B CELL LYMPHOMA

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Background: Primary mediastinal B cell lymphoma (PMBL) is a rare subtype of diffuse large B cell lymphoma and has some clinical and pathologic features of classical nodular sclerosing Hodgkin lymphoma. Patients are predominantly young women and often present with a locally invasive anterior mediastinal mass. The optimal treatment of PMBL is unknown and differs in clinical practice. Standard immunochemotherapy alone is inadequate and has resulted in routine consolidation with mediastinal radiation. There is only a paucity of prospective data and best results were achieved with rituximab based regimens combined with radiotherapy or dose intensified therapy (DA-EPOCH-R) without radiation. Here we present a single center 10 year retrospective survey indicating that high dose therapy with autologous stem cell support (HDT/ASCT) results in excellent long term outcome.
Aims: Aim of this study is to investigate the infrequent population of PMBL patients, as a single center survey, regarding treatment strategies and long-term outcome.
Methods: All patients with PMBL who presented at Freiburg University Medical Center since 2005 were evaluated according to their baseline characteristics, treatment modality and treatment outcome. From 2005 on patients were treated with upfront HDT/ASCT after R-CHOP induction and R-VCPE (rituximab, epirubicin, etoposide, cisplatin, cyclophosphamide) intensification. Conditioning regimen was BEAM (group A). From 2011 onwards patients were treated with an early intensified protocol: Dense R-MTX/CHOP-14 (6 cycles CHOP-14, dose dense rituximab on days 0, 1, 4, 8, 15, 22, 29, 47, 61 and 75. High-dose MTX was administered on days 30 and 76 right before standard CHOP). Patients who did not achieve a complete metabolic response were intensified as described above (group B). Radiotherapy consolidation was not routinely performed in both treatment groups.
Results: 25 patients were identified. Median age was 32 y (range 19-57y), with predominantly females (64%). Disease stage was mostly III/IV (76%). All patients presented with bulky disease and elevated LDH. 36% had an ECOG-Score of ≥2. 72% of patients presented with more than one extranodal site involvement. 88% presented with high-intermediate or high risk aaIPI. Nine patients were intended to receive upfront HDT/ASCT (group A). Of these patients one was primary refractory to R-CHOP-21 and died early in the course of disease. Three patients received consolidating radiotherapy. 2-year PFS and OS were 88.9%, respectively. 14 patients were treated with upfront dense R-MTX/CHOP-14 (group B). Six patients achieved only partial remission and received HDT/ASCT as consolidation. Five patients received additional mediastinal radiotherapy. 2-year PFS and OS were 85.7% and 100%. In addition to both groups, two patients were treated with six cycles of R-CHOP-21+2xR (group C). With a median follow-up of 35.9 months estimated 3-year progression-free and overall survival for all patients were 83.2% and 83.6%, respectively. Despite a complete metabolic response after R-MTX/CHOP-14 induction two relapses were observed in this group. One relapse was observed after six cycles R-CHOP-21 and mediastinal radiation. Patients who underwent upfront HDT/ASCT or received HDT/ASCT consolidation in addition to dense R-MTX/CHOP-14 induction had an excellent long-term outcome with a 3-year PFS and OS of 100%, respectively. According HDT/ASCT or no-HDT/ASCT survival curves do significantly differ (Figure 1). There was no transplant related mortality. No second malignancies were observed yet.

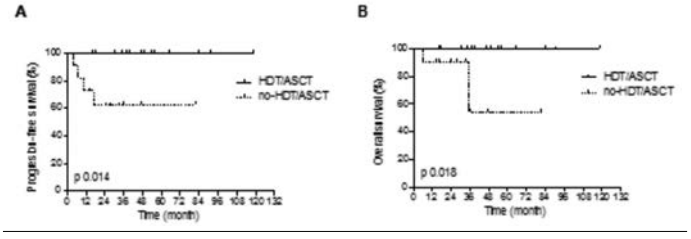

Figure 1. Kaplan-Meier curve for A) progression-free survival and B) overall survival stratified by high dose treatment (HDT/ASCT) or no high dose treatment (no-HDT/ASCT) for all PMBL patients.

Summary/Conclusions: Our results support that dose intensification is necessary in PMBL patients. Regarding the role of HDT/ASCT our data suggest that an approach using upfront HDT/ASCT (group A) or as intensification for patients failing complete metabolic response after induction (group B) is safe and results in excellent long term survival.

PB1679

THE REVISED INTERNATIONAL PROGNOSTIC INDEX IS MORE USEFUL IN COMBINATION WITH SERUM INTERLEUKIN-2 RECEPTOR LEVEL FOR DETERMINING THE PROGNOSIS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: The current standard of care for diffuse large B-cell lymphoma (DLBCL) is rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). The International Prognostic Index (IPI) was developed to predict the outcome prior to the availability of rituximab. The addition of rituximab to CHOP dramatically improved clinical outcomes in patients with DLBCL. Therefore, some major modifications to the IPI have been reevaluated in the rituximab era. The revised IPI (R-IPI), a clinical tool used to predict patient outcome, has been implemented and validated; however, it has not been established for general practical use. Serum soluble interleukin-2 receptor (IL-2R) level has been identified as a marker of poor outcome in patients with DLBCL.
Aims: In this study, we analyzed to determine whether incorporation of serum IL-2R level into the factors of R-IPI allow for wider differentiation of outcomes.
Methods: The clinical records of 227 consecutive patients in our center with DLBCL diagnosed between 2003 and 2013 who received rituximab were retrospectively analyzed. Data for IPI factors (age >60 years, stage III/IV disease, elevated serum lactate dehydrogenase level, Eastern Cooperative Oncology Group performance status ≥2, and >1 extranodal site of disease) and serum IL-2R level upon diagnosis were collected retrospectively. The normal upper limit of serum IL-2R level was set at 519 U/mL using an enzyme immunoassay system. The R-IPI segregated patients into 3 outcome groups. A combination of R-IPI and serum IL-2R level identified 6 groups. The survival period was calculated from treatment initiation and estimated using Kaplan-Meier method. Death from any cause or relapse was defined as an event. Survival was compared between risk groups using the log-rank test. This study was conducted in accordance with the Declaration of Helsinki and its amendments. The protocol was approved by the Ethics Committee of the Japanese Red Cross Kyoto Daiichi Hospital.
Results: A total of 227 patients with DLBCL were enrolled, and 54 patients had normal serum IL-2R levels, on the basis of which, 12 were categorized as a very good prognostic group, 39 a good prognostic group, and 3 a poor prognostic group according to the R-IPI; similarly 173 patients had elevated serum IL-2R levels, on the basis of which, 4 were categorized as a very good prognostic group, 81 a good prognostic group, and 88 a poor prognostic group. With a median observation period of 39 months (range, 1 - 205), the 3-year overall survival (OS) was 72.9% for all patients. and 100%, 94.4%, and 100% for 3 prognostic groups of patients with normal serum IL-2R levels, according to the R-IPI. Similarly, the 3-year OS for patients with elevated serum IL-2R level was 100%, 81.7%, and 68.7%, respectively (p=.002). Moreover, 12 patients with a very good prognostic group per the R-IPI showed favorable prognoses regardless of serum IL-2R level (100% 3-year OS). However, serum IL-2R level significantly differed between with good and poor prognostic groups. According to the serum IL-2R level, 3-year OS was 94.4%, and 81.7%, respectively, for patients with the good prognostic group (p=.02) and 100% and 60.5%, respectively, for those with the poor prognostic group (p=.02).
Summary/Conclusions: A new scoring system is needed for better risk stratification and establishing the appropriate therapeutic strategy in the rituximab era. R-IPI is a useful prognostic marker, particularly in patients with elevated serum IL-2R levels. The combination of serum IL-2R level with R-IPI might provide a more precise risk stratification and serve as a useful prognostic predictor in DLBCL patients to identify candidates for experimental therapy other than R-CHOP.

PB1680

SALVAGE TREATMENT WITH R-IEV (RITUXIMAB, IFOSFAMIDE, EPIRUBICIN, ETOPOSIDE) AND AUTOLOGOUS STEM CELL TRANSPLANT FOR RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

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Background: The better salvage treatment before Autologous Stem Cell Transplant (ASCT) for relapsed/refractory Diffuse Large B-Cell Lymphoma (DLBCL) hasn't been still definitively established. The phase III CORAL study, comparing R-ICE (Rituximab, Ifosfamide, Carboplatin, Etoposide) *versus* R-DHAP (Rituximab, Dexametasone, Cisplatin, Cytarabine) in relapsed DLBCL patients didn't demonstrate a significant difference in terms of response rate and Progression-Free Survival (PFS) between the two groups. Therefore, the ESMO (European Society of Medical Oncology) guidelines recommend the use of R-ICE or R-DHAP or R-GDP (Rituximab, Gemcitabine, Dexamethasone, Cisplatin) as

salvage regimen for relapsed/refractory DLBCL, considering the similar efficacy of all these treatment schemes.
Aims: The aim of the study was to evaluate the efficacy and safety of salvage treatment with R-IEV (Riruximab, Ifosfamide, Epirubicin, Etoposide and Dexamethasone) followed by ASCT in relapsed/refractory DLBCL patients.
Methods: From July 2007 to December 2015, 42 consecutive adult patients with relapsed (n=17) or refractory (n=25) DLBCL patients underwent a salvage treatment with three courses of R-IEV (Rituximab 375 mg/m^2 on day 0, Ifosfamide 2500 mg/m^2, Etoposide 150 mg/m^2, Dexamethasone 20 mg, on days 1-3, Epirubicin 100 mg/m^2 on day 1) every 21 days in two different Hematology Institutions of Rome (Regina Elena National Cancer Institute, n=20; San Giovanni Addolorata Hospital, n=22). For all patients we planned a Peripheral Blood hematopoietic Stem Cell (PBSC) harvest after the second or the third course of chemotherapy, if the bone marrow wasn't or was involved at salvage treatment start, respectively, using Filgrastim or Lenograstim 5 mcg/Kg. All patients underwent a febrile neutropenia prophylaxis with Filgrastim (n=14), Lenograstim (n=18) or Pegfilgrastim (n=10) in all courses of treatment. All patients underwent a baseline evaluation of Performance Status (PS) and comorbidities, and left ventricular ejection assessment.
Results: The median age of patients at the start of salvage treatment was of 48 years (range: 23-62). At diagnosis, 38 patients (90%) were in advanced (III-IV) Ann-Arbor stage and 18 (43%) presented intermediate-high IPI score. Moreover, 35 patients (83%) had a 0-1 ECOG PS and 15 (36%) had not any relevant comorbidities. As first line treatment, 29 patients (69%) were treated with R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine and Prednisone), whereas the remaining 13 with other chemotherapeutic regimens. At the time of salvage treatment start, 25 patients (59%) were defined as refractory, 7 (17%) were relapsed within 12 months from the end of previous treatment line and 10 (24%) were relapsed after more than 12 months. After the end of R-IEV, a Complete Remission (CR) was obtained in 30 patients (71%), 9 patients were in Partial Response (PR, 21%), whereas the remaining 3 patients (8%) were defined as refractory to salvage treatment. PBSC collection was successfully performed in 40 patients, whereas in the remaining two wasn't carry out for early progression (n=1) or harvest failure (n=1). Actually, 37 patients underwent ASCT after BEAM (Carmustine, Etoposide, Cytarabine, Melphalan) conditioning chemotherapy. Out of 40 patients in which a PBSC collection was successfully performed, three patients didn't undergo ASCT for progression of disease (n=1), clinical choice (n=1) and patient refusal (n=1). A grade 3-4 neutropenia and a febrile neutropenia occurred in 78% and 31% of patients, respectively. However, we didn't observe any cases of toxic death or cardiac relevant complication. After a median follow-up of 36 months (range: 3-116), the 2-year-PFS and OS (2y-PFS and 2y-OS) from the start of salvage treatment were of 73.8% and 83.3%, respectively.
Summary/Conclusions: From our experience, R-IEV salvage regimen with consequent ASCT was highly effective in relapsed/refractory DLBCL patients, permitting to obtain an impressive 2y-PFS, with a good safety profile. Further prospective randomized studies comparing R-IEV with other standard salvage regimens should be useful to establish the better therapeutic approach for these patients.

PB1681

A COMPARISON STUDY BETWEEN ALLOGENEIC AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANT FOR HIGH-RISK PERIPHERAL T-CELL LYMPHOMAS (PTCL)

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Background: Peripheral T-cell lymphomas (PTCLs) are heterogeneous malignancies sharing common elements of chemotherapy resistance and poor outcome with standard treatments. Attempts to improve outcomes have included autologous or allogeneic hematopoietic cell transplantation (autoHCT or alloHCT). both modalities lead to durable remissions in recurrent disease settings and might be important in consolidating first remission. However, key questions remain, including relative efficacy of autologous *versus* allogeneic approaches, and HCT timing (first-line consolidation v relapse).
Aims: Herein, we analyzed outcomes of 60 cases recieved autoHCT or alloHCT in our center.
Methods: From July 2007 to July 2014, Outcomes of 60 patients undergoing autologous HCT (autoHCT) or allogeneic HCT (alloHCT) were analyzed retrospectively. Primary outcomes were nonrelapse mortality (NRM), relapse/progression, progression-free survival (PFS), and overall survival (OS).
Results: All 60 patients were at high risk group (carried with IPI≥3), with a median age of 31 (13-55) years old. Of the 60 cases, 22 were PTCL-not otherwise specified (PTCL-NOS), 22 with ALK negative anaplastic large cell lymphoma (ALK-negative ALCL) and 16 with angioimmunoblastic T-cell lymphoma (AITL). Before receiving transplantation, 40/60 patients were in complete remission (CR) and 20/60 patients were not remission (NR). Twenty-one (21/60) received allo-HSCT and thirty-nine patients (39/60) received auto-HSCT. In the 20 NR patients before transplant, 11 patients received allo-HCT and 9 patients received auto-HCT. After a median follow-up of 39 (range 1-96) months, the K-M analysis showed that the 5-year PFS for auto-HSCT and allo-HSCT were 61% and 60% (*P*=0.724). 1-year NRM was higher (22.5% *vs* 7.8%) for alloHCT. The 5-year OS for auto-HCT and allo-HCT were 62% and 61% (*P*=0.724). There were no statistically significant differences between the auto-HSCT and allo-HSCT. However, autoHCT recipients were more likely in complete remission (CR; 76.9% *vs* 47.6%; P <0.01) and with chemotherapy-sensitive disease.

Table 1. Baseline characteristics of the patients.

Parameters	Auto-HCT	Allo-HCT
Number	39	21
Histological subtypes		
PTCL-NOS	12	4
ALK-negative ALCL	13	4
AITL	7	9
NK/T	7	4
Conditioning regimen		
BEAM	39	0
BUCY	0	11
TBI+BUCY	0	10
Donor source	N	
Matched unrelated donor		8
Matched sibling donor		4
Haploid donor		8
Cord blood		1
Disease status before HCT		
CR	30	10
PR	2	0
NR	7	11
Disease status after HCT		
CR	35	19
PR	0	0
NR	4	0
Uncertain	0	2

Summary/Conclusions: Both autoHCT and alloHCT approaches can benefit patients with PTCL. We did not find a difference in PFS and OS between auto-HCT and alloHCT, although NRM increased significantly in alloHCT. However, our results suggest that outcomes of alloHCT are better for refractory and relapsed patients with PTCL. nd overall survival (OS).

PB1682

PROGNOSTIC SIGNIFICANCE OF SARCOPENIA IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH RITUXIMAB PLUS CHOP (R-CHOP)

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Background: Sarcopenia, defined as the loss of skeletal muscle mass and strength, is one of the criteria for cancer cachexia. A computed tomography (CT) scan has been accepted as one of the most preferred tools toassess sarcopenia. Measuring the extent of sarcopenia by CT in patients with malignancy has severaladvantages, including accurate quantification of muscle mass, precise differentiation between fat andmuscle, and wide availability as a routine diagnostic tool in cancer. Sarcopenia evaluated by CT isknown to be related to an increased risk of chemotherapy toxicity, poorer functional status, andreduced survival in malignancy.
Aims: In this study, we investigated the prognostic impact of sarcopenia in all age groups of patients with DLBCL using an extended cohort. Additionally, a new prognostic model, including sarcopenia and other significant prognostic factors, was constructed using a nomogram.
Methods: In total, 187 consecutive DLBCL patients treated with induction R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) immunochemotherapy were reviewed.Sarcopenia was defined as the lowest sex-specific quartile of the skeletal muscle index, calculated bydividing the pectoralis muscle area by the height. Clinical outcomes were compared between thesarcopenic and non-sarcopenic groups. A nomogram was constructed from the Cox regression model for overall survival (OS).
Results: Treatment-related mortality (21.7% *vs* 5.0%, p=0.002) and early discontinuation of treatment(32.6% *vs* 14.9%, p=0.008) were more common in the sarcopenic group than in the non-sarcopenic group. The 5-year progression-free survival (PFS) rates were 35.3% in the sarcopenic group and 65.8% in the non-sarcopenic group (p <0.001). The 5-year OS rates were 37.3% in the sarcopenic group and 68.1% in the non-sarcopenic group (p <0.001). Sarcopenia and the five variables of the International Prognostic Index (IPI) were independent prognostic factors in a multivariate analysis for PFS and OS, and were used to construct the nomogram. The calibration plot showed good agreement between the nomogram predictions and actual observations. The c index of the nomogram (0.80) was higher than those of other prognostic indices (IPI, 0.77, p=0.009; revised-IPI, 0.74, p <0.001; National Comprehensive Cancer Network-IPI, 0.77, p=0.062).

Summary/Conclusions: This study demonstrates the role of sarcopenia as a poor prognostic marker in DLBCL patients. We suggest that dose adjustment and intensive supportive care should be considered in DLBCL patients with sarcopenia receiving induction R-CHOP immunochemotherapy. Further prospective studiesare warranted to confirm the prognostic role of sarcopenia and to externally validate the nomogramincluding sarcopenia in DLBCL.

PB1683

GOOD RESULTS WITH R-CHOP-14 THERAPY IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMAS. THE CHEMOKINE TARC IS AN EXCELLENT MARKER OF TUMOR ACTIVITY AND IN TREATMENT EFFECTIVITY IN MEDIASTINAL LYMPHOMAS.

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Background: Primary mediastinal large B-cell lymphoma (PMBCL) is a rare disease that belongs to the group of aggressive, diffuse large B-cell lymphomas and is associated with a characteristic clinical feature. Data from evidence-based treatments are not available. Retrospective studies suggest that the so called third generation treatments are more effective than the previous standard treatment with 21-day cycles R-CHOP. Nowadays, dose-dense DA-EPOCH-R treatment is well tolerated by young patients and appears to be even more effective.
Aims: Our research shows that dose-dense R-CHOP-14 treatment is as effective as the more aggressive DA-EPOCH-R. There is no earlier data for the role of TARC (thymus activation-regulated chemokine) in PMBCL as a disease activity marker. The change of TARC level during the treatment was monitored.
Methods: Between July 2005 and June 2015, 56 newly diagnosed, untreated PMBCL patients were subject to treatment with R-CHOP-14. The average age of the 33 (59%) female and 23 (41%) male patients was 32 years (range: 21-53 years). Using the R-IPI, 9 patients were the very good, 46 patients were the good and one patient was in the worse prognostic group. The combination of CHOP-14 and rituximab administered in 14-day cycles was used as treatment; the treatment regimen also included preventive administration of filgastrim. The average number of cycles of the chemotherapeutic treatments was 6.6 (range: 4-8). After the final R-CHOP-14 treatment high energy photon radiation was used to the residual mediastinal abnormalities in 46 cases as consolidation treatment. Before diagnosis could be performed two patients received life-saving irradiation. Since May 2013 we have investigated the role of TARC to follow the disease activity in 13 cases.
Results: The planed treatment was completed in 55 (98.2%) patients and in 54 (96.4%) patients we achieved complete remission verified by PET/CT. One patient, who did not get complete remission, was cured with high-dose chemotherapy and autologous stem cell transplantation. One patient died after the fourth cycle R-CHOP-14, due restrictive pulmonary disease and ARDS. The average duration of the follow-up period was 57 months (range: 10-126 months), during which time only one relapse was shown. The relapse free survival is 98.1%. One patient did not wish to receive further oncological treatment, she was only one who died due lymphoma. The lymphoma specific mortality is 98.2%. Two young female patients (with hereditary mental retardation) died in lung tuberculosis 9 and 13 month after the end of the treatment. The overall survival rate is 92.9%; the event free survival is 91.1%. Before the treatment all but one out of 13 cases extremely high TARC level was found. The TARC levels were dropped quickly in the normal range due the treatment effect.
Summary/Conclusions: In the treatment of PMBCL the R-CHOP-14 was found highly effective, short-term and well tolerable. Further investigation and research is needed to find out if radiation therapy is necessary. The TARC proved to be a very good marker in PMBCL which signs the activity of disease and the effectivity of treatment well and early.

PB1684

PROGNOSTIC VALUE OF PRETREATMENT ADVANCED LUNG CANCER INFLAMMATION INDEX (ALI) IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH R-CHOP CHEMOTHERAPY

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Background: It has become evident that cancer-related inflammation plays an important role in the development and progression of various types of cancer and adversely influences response to anti-cancer therapy. Advanced lung cancer inflammation index (ALI), a novel inflammation-based prognostic system, has been demonstrated to be a prognostic factor of survival in some solid cancers. However, the prognostic usefulness of ALI has not been investigated in lymphoma.
Aims: The authors investigated the ability of ALI to predict response to chemotherapy and survival in patients with diffuse large B-cell lymphoma (DLBCL).
Methods: The records of 212 patients with newly diagnosed DLBCL treated by R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) chemotherapy were reviewed. ALI values were calculated by dividing the product of body mass index and blood albumin level by peripheral blood neutrophil/lymphocyte ratio. Patients were allocated to a low pretreatment ALI group (n=82, 38.7%) or a high pretreatment ALI group (n=130, 61.3%) using on optimal ALI discriminatory cutoff value for survival of 15.5 as determined by receiver operating curve analysis.
Results: The proportion of older patients (aged >60 years) was significantly higher in the low ALI group than in the high ALI group (63.4% *vs* 36.2%, p<0.001). More patients had advanced disease (Ann Arbor stage III/IV) in low ALI group at initial diagnosis (72% *vs* 54.6%, p=0.014). Poor Eastern Cooperative Oncology Group performance status (ECOG PS, p=0.001), presence of B symptoms (p=0.002), and high-risk of International Prognostic Index (IPI, p=0.029) were observed more frequently in the low ALI group. Complete remission rates in the low and high ALI groups after completing R-CHOP chemotherapy (after 6 to 8 cycles) were 54.9% (45/82) and 75.4% (98/130), respectively (p=0.008). During a median follow-up of 54 months (range, 6-92 months), patients in the low ALI group had shorter 5-year PFS (58.1% *vs* 77.3%, p=0.006) and OS (64.2% *vs* 80.2%, p=0.008) than patients in the high ALI group. Multivariate analysis showed advanced disease (hazard ratio [HR] 2.425, 95% confidence interval [CI] 1.514-3.128, p=0.013), bulky disease (diameter ≥10 cm, HR 1.568, 95% CI 0.678-4.287, p=0.043) and low pretreatment ALI (HR 1.927, 95% CI 0.893-3.598, p=0.021) were significantly associated with poorer PFS. Regarding OS, age >60 (HR 2.298, 95% CI 1.628-6.118, p=0.015), ECOG PS ≥2 (HR 1.814, 95% CI 0.874-6.521, p=0.024), high-risk IPI (HR 2.872, 95% CI 1.658-5.723, p<0.001), advanced disease (HR 2.431, 95% CI 1.422-6.249, p=0.002) and low pretreatment ALI (HR 2.645, 95% CI 1.542-5.974, p=0.001) were all found to be independently associated with shorter OS. Interestingly, a low ALI before R-CHOP chemotherapy reverted to a high ALI during treatment in 58 patients (27.4%) and median OS of these patients (not reached) was better than that of patients whose ALI remained low (n=24, 25 months; p<0.001). Conversely, patients with a persistent low ALI during treatment showed worse survival.
Summary/Conclusions: This study shows a significant association exists between pretreatment ALI and response to R-CHOP chemotherapy and prognosis in DLBCL. Changes in ALI during the course of chemotherapy might also provide additional information about survival, particularly for patients with a low pretreatment ALI. Our findings suggest ALI is an inexpensive, potential prognostic marker in patients with DLBCL that can be readily used in routine practice.

PB1685

FACTORS RELATED TO NON-HODGKIN'S LYMPHOMA LONG-TIME TO TREATMENT INITIATION AND IMPACT ON SURVIVAL IN A POPULATION BASED STUDY IN FRANCE: IS THERE A ROLE OF SOCIOECONOMIC STATUS?

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Background: Diffuse large B cell lymphoma (DLBCL) is one of the most frequent non-Hodgkin's lymphoma histological subtype for which care management is well codified and remaining still an aggressive disease potentially curable with a combination of anthracycline-based chemotherapy. In this context, time to treatment initiation (TTI) could be having an impact on the patient's survival.
Aims: The aim of this study was to identify the socioeconomic and medical factors predicting a long TTI and determine the impact of a long delay on overall (OS) and relative (RS) survival (excess mortality due to lymphoma) for DLBCL patients.
Methods: We performed a population-based "high-resolution" study including all patients diagnosed with DLBCL in three registry-areas in France (Gironde, Côte d'Or and Basse-Normandie) between 2002-2008. Medical and socioeconomic factors (GP's density, rural area, distance to the nearest reference centre, index deprivation) were collected. TTI was the delay in days between diagnosis and treatment initiation. A long TTI was considered for 75thpercentile and very long TTI for the 90th percentile. Three multivariate analysis logistic regressions were performed for the first aim using median, 75th and 90th percentiles TTI as dependant variables. For each patient, the follow-up period continued from the date of diagnosis to the death or until June, 30, 2013. For survival outcome we fitted a multivariate cox model for OS and an Esteve model for RS (3 and 5 years). Net survival (NS) using Pohar Perme unbiaised method was also calculated for 1, 3 and 5 years.
Results: We included 1084 DLBCL patients (median age 71(9-99) years) and sex ratio H/F 1.12.The median TTI was 27 days, interquartile (15-42); The long

TTI was 42 days and a very long TTI was considered >63 days. Each dependant variable has been analysed in details. Among these variables in multivariate logistic regression, adjusted on age and sex, we first confirmed that elevated serum lactate dehydrogenase and disseminated stage of disease were independently associated with a TTI>63 days (OR: 0.37 IC 95%: 0.22-0.63 and OR: 0.59 IC 95%: 0.37-0.93, respectively). The registry areas and outpatient diagnosis were both independently associated with a long TTI (OR: 1.89 IC 95%: 1.38-2.60 for patients of north of France; OR: 1.55 IC 95%: 1.01-2.39 for outpatients). In contrast, the socioeconomic status (European Deprivation Index) was not associated with the delay of treatment initiation. Median of follow up was 5 years. For the respective long TTI groups, 5 years OS estimates were 55% and 60.5%; 5 years NS estimates were 62.3% and 67.7%. We found no association between a TTI>63 days and overall survival or relative survival.

Summary/Conclusions: This study shows that medical factors such as LDH and stage of disease influence a longer delay of treatment. To be diagnosed in the same place of care management that treated seems to be also a factor of earlier treatment. Socioeconomic status is not associated with TTI whatever the delay. Finally, our results emphasize disparities according to France area. This retrospective cohort suggest that time to treatment initiation don't may influence outcomes.

PB1686

Abstract withdrawn.

PB1687

R-CHOP-21 PLUS LOW DOSE ETOPOSIDE *VERSUS* DA-EPOCH-R IN PATIENTS WITH AIDS-RELATED GCB DIFFUSE LARGE CELL B CELL LYMPHOMA; SINGLE CENTRE EXPERIENCE

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Background: Treatment of AIDS-related diffuse large cell B cell lymphoma (DLBCL) with low CD4 count (<100 mm3) remains a great challenge for hematologists. Balancing between competing needs of lymphoma treatment and HIV management is crucial for achieving long term survival. Previous studies have shown strong advantage of DA-EPOCH-R *versus* R-CHOP in AIDS-related lymphomas. However hematological toxicity profile of this regimen is quite considerable especially in patients with AIDS with decreased bone marrow reserves. Combination of standard R-CHOP with low dose etoposide (R-CHOEP) could be effective alternative option in AIDS-related GCB-type DLBCL management.
Aims: The aim of our study was to evaluate if R-CHOEP benefit of R-DA-EPOCH in patients with AIDS-related GCB DLBCL considering hematological toxicity.
Methods: Thirty one patients (pts) with AIDS-related GCB DLBCL have been enrolled in study. All pts had low CD4 count with mean amount 78 cells/mm3 and did not receive HAART before the enrolment. They have been divided into two arms. First arm (n=14) received standard R-CHOP with 50 mg/m2 etoposide infusion on days 1-3. Second arm received DA-EPOCH-R as it has been described in previous studies. FDG-PET scans have been done before and after the treatment.
Results: Complete response (CR) has been achieved in 93% in DA-EPOCH-R arm and in 85% in R-CHOEP arm. Hematological toxicities have been observed in both arms in all cycles of treatment. However in DA-EPOCH-R arm neutropenia mean period was 16 days when in R-CHOEP arm 11 days; thrombocytopenia period was 11 and 3 days respectively. Septic complications in DA-EPOCH-R arm have been observed in 53% when in R-CHOEP arm in 31%.
Summary/Conclusions: Patients treated with R-CHOEP had shorter periods of neutropenia and thrombocytopenia in comparison with DA-EPOCH-R group, where periods of neutropenia and thrombocytopenia were longer and resulted in higher rate of infectious complications. Considering comparable CR rate in both groups R-CHOP plus low dose etoposide could be effective option with lower hematological toxicity profile in AIDS-related DLBCL with CD4 count less than 100 cells/mm3.

PB1688

THE PROGNOSTIC IMPACT OF SERUM ALBUMIN IN DOUBLE HIT/DOUBLE EXPRESSING AGGRESSIVE B CELL LYMPHOMAS: A PILOT STUDY EVALUATING SAAB SCORING SYSTEM

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Background: Double hit lymphomas (DHL) [rearrangements of MYC and BCL2/BCL6, and double expressing lymphomas (DEL) [expression of MYC and BCL6/BCL2 by immunohistochemistry] represent a subset of diffuse large B-cell lymphoma (DLBCL) with poor overall survival (OS). Several prognostic scores have been tested to clinically stratify these patients with the double hit score (DHS) being the most recently developed model (Petrich 2014).
Aims: We explored the use of serum albumin (SA) as prognostic factor in DHL as previously shown in DLBCL (Dalia 2013).
Methods: We included 100 patients from Moffitt Cancer Center database from January 2008 to December 2015 with DHL and performed a retrospective chart review. Recorded clinical data included SA, hemoglobin, age, International Prognostic Index (IPI), DHS, and lymphopenia. Pairwise Cox Comparison was used to develop a weighted scoring system. Kaplan-Meier method with Cox proportional hazard model was used to determine OS.
Results: The median age was 63 (18-87), stage III/IV in 73.1%, bulky disease (>10cm) in 47.4%, B symptoms in 41.67%, CNS involvement in 14.1%, extranodal (EN) involvement in 34.7% of the patients. Patients with DHL and DEL were 60.2% and 39.8%, respectively. The median OS for the cohort was 15 months. Multivariate analysis identified Age >60 (1 point), SA <3.7 (4 points), bulky disease (2 points) and Stage III/IV (1 point) as prognostically significant. LDH and EN dropped from the model. SAAB scoring consisted of 3 groups: Very good (0-2), Good (3-5), Poor (6-8). The 3-years OS was compared among scoring systems (Table 1).

Table 1.

Score (N=100)	3 Year-OS	P-value
SAAB		0.005
Very good (SAAB=0-2)	45%	
Good (SAAB 3-5)	30%	
Poor (SAAB=6-8)	NA*	
R-IPI		0.437
Very Good (R-IPI=0)	100%	
Good (R-IPI=1-2)	30%	
Poor (R-IPI=3-5)	32%	
Double Hit Score (DHS)		0.281
Low (DHS=0)	26%	
Intermediate (DHS=1)	29%	
High (DHS=2 or more)	27%	

**Highest survival was 18 months.*

Summary/Conclusions: Compared to the R-IPI and DHS, the new SAAB score better discriminated prognostic groups in DHL/DEL. Confirmation in a larger and multicenter study is needed to validate these findings. SA might represent a surrogate of comorbid status and worse biology in patients with DHL/DEL.

PB1689

CLINICAL CHARACTERISTICS AND OUTCOME OF PATIENTS WITH PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA TREATED WITH INTENSE SHORT-PULSE CHEMOTHERAPY, R-CHOP OR R-DA-EPOCH A RETROSPECTIVE OBSERVATIONAL STUDY

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Background: Primary mediastinal large B-cell lymphoma (PMBL) is a locally highly aggressive tumour and accounts for approximately 2.4% of adult non-Hodgkin's lymphoma (NHL). Overall survival rates (OS) between 46 – 79% have been reported but optimal treatment regimens remain to be defined.
Aims: Retrospective, longitudinal assessment of clinical risk factors and outcome of three different treatment regimens for patients with primary mediastinal large B-cell lymphoma.
Methods: We performed a longterm longitudinal retrospective study to analyze the clinical characteristics and the treatment outcome of 63 adult patients with primary mediastinal large B-cell lymphoma in two large hematology departments in Berlin. The study was approved by the Charité Ethic Committee. Between 1996 and 2016 patients were registered at the time of treatment start and data on demographics, clinical features and laboratory parameters were recorded during treatment and follow up. The prognostic impact of IPI-value, pleural or pericardial effusion, LDH, gender and presence of bulky disease was analyzed with univariate analysis with respect to overall survival (OS) and event-free survival (EFS). Survival analyses were performed with the Kaplan-Meyer method and the log-rank test for comparison using SPSSÒ.
Results: Between 1996 and 2016 37 female patients with a median age of 31 years and 26 male patients with a median age of 36 years were registered. Initial presentation in Ann Arbor stage I: 22%, stage II: 41%, stage III: 9% and

stage IV: 28%. B-symptoms: 42%. Bulky disease with a tumor >10cm was present in 36.2% of all patients. Extranodal manifestations were detected in 30% and either pleural or pericardial effusions was present in 56% of all patients. 16 pts. (26%) were treated according to an intensive short-pulse B-NHL type chemotherapy protocol without Rituximab (B-NHL) and 22 patients (36%) with Rituximab (R-B-NHL), respectively. R-CHOP and R-DA-EPOCH was administered in 12 pts. (20%) and 6 pts. (10%), respectively. 4 pts. received either CHOP without Rituximab or R-ACVBP. Almost all patients received adjuvant involved field radiotherapy after completion of chemotherapy, usually with a total dose of 36 Gy. The probability of event-free survival (pEFS) and OS at 5 years for all patients was 73.5±6.7% and 81.3±6.0%, respectively. For 16 pts. treated with a short-pulse B-NHL type protocol without Rituximab 5-year EFS and OS was 64.3±12.8% and 78.7±11.0%, respectively. With the addition of Rituximab the 5-year EFS and OS (22 pts.) increased with this therapy to 79.4±10.7% and 91.7±8.0%, respectively. Treatment with R-CHOP for 12 pts. led to a 5year EFS and OS of 77.1±14.4% and 75.0±15.3%, respectively. 5-year survival analysis for the 6 pts. treated with R-DA-EPOCH was not calculated due to short follow up (Figure1). Except for IPI-value there was no significant prognostic impact of pleural or pericardial effusion, LDH, gender or presence of bulky disease.

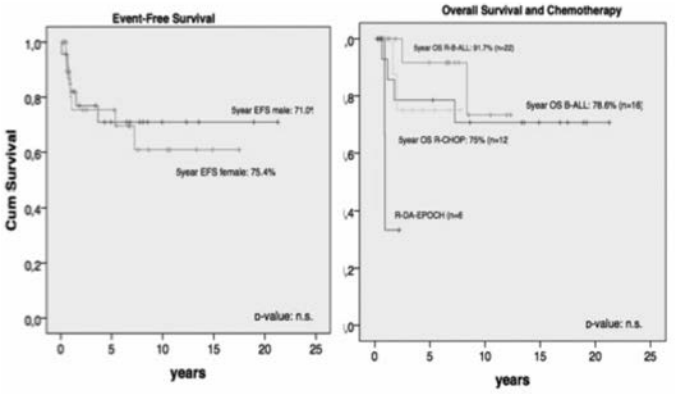

Figure 1.

Summary/Conclusions: The addition of Rituximab to the intensive short-pulse B-NHL type chemotherapy protocol led to an improved 5 year EFS and OS. Treatment with R-CHOP seems to be slightly inferior with respect to clinical outcome. We did not find a higher predilection of young female patients and there was no significant prognostic impact of bulky disease, pleural or pericardial effusion, elevated LDH or gender. The best treatment regimen for this rare entity of B-cell lymphoma remains be determined in a randomized multicenter trial.

PB1690

POSITRON EMISSION TOMOGRAPHY COMPUTED TOMOGRAPHY FEATURES OF TYPE II ENTEROPATHY ASSOCIATED T CELL LYMPHOMA

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Background: Type II enteropathy associated T cell lymphoma (EATL) is a rare malignancy in the West and the only form of EATL in the East. 18F-fluorodeoxyglucose (FDG) positron emission tomography computed tomography (PET/CT) findings have not been described for type II EATL.
Aims: Type II EATL is increasingly recognized as a separate entity from type I EATL, we report herein the PET/CT findings of in a large cohort of patients, so that more can be known for this new disease entity.
Methods: Consecutive patients with type II EATL diagnosed between January 2008 and July 2015 were studied. The diagnosis was based on typical histopathological and immunophenotypical features. Standard clinical and biochemical evaluations for staging and response assessment were undertaken. Treatment protocols evolved over this period and were heterogeneous.
Results: Six men and four women with type II EATL investigated by PET/CT at diagnosis and relapse were retrospectively analysed. On presentation, the primary involved sites were the small bowel (N=8), stomach (N=1) and large bowel (N=1). The uninvolved small bowel did not show undue FDG-avidity to suggest enteropathy. Distant lymph nodes and organs were involved in four cases (40%). The primary lesions were hypermetabolic except in one case, where the colonic lesion was eumetabolic. At relapse, the stomach and large bowel might be involved even if the primary tumours arose from the small bowel, and multiple extra-intestinal metastases occurred. Interestingly, the lung and the brain were frequently involved (40% and 20% respectively).
Summary/Conclusions: These findings showed that in contrast to classical EATL, where the small bowel is the exclusive primary site (owing to its origin from coeliac disease associated enteropathy) and distant metastases even during relapse are exceptional, type II EATL could on presentation and during relapse involve any part of the gut, and metastasize to multiple extra-intestinal sites. These observations support the proposition of classifying this lymphoma as a distinct clinicopathological entity (monomorphic epitheliotropic intestinal T-cell lymphoma) to differentiate it from classical EATL.

PB1691

THE INFLUENCE OF GENETIC POLYMORPHISMS BCL-2 AND C-MYC IN PATIENTS WITH DLBCL TREATED WITH RCHOP TO ACHIEVE COMPLETE REMISSION

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Background: Non -Hodgkin's diffuse large B- cell lymphoma (DLBCL) represents a heterogeneous group of aggressive lymphomas, which are characterized by the presence of large, transformed B cells. DLBCL with both translocations C-MYC and BCL-2 provides *double-hit* lymphoma and is characterized by poor response to standard therapy with an aggressive clinical course.
Aims: What is the influence of BCL-2 and C-MYC in DLBCL treated with RCHOP to achive complete remission.
Methods: We analyzed the population of 62 patients primary DLBCL, treated with immunochemotherapy R-CHOP/21 day. Testing BCL-2 and C-MYC was conducted with classical FISH analysis. Complete remission (CR) was defined as the absence of disease after the therapy. We tested the sensitivity and specificity, and statistical analysis was performed in Windows SSPS (22.00), a statistical significance defined as p <0,05.
Results: The study group consisted of 32 men and 30 women, mean age was 59.76+/- 14,38. The patients were in CS III and IV in 81.9%. After applying the first therapeutic line RCHOP, 30 patients or 48.7% achieved a complete remission. The frequency of FISH/BCL2+in the group with complete remission was 40% and in the group without a complete remission of 46.2%. Achieving complete remission was not statistically significantly different between subjects FISH/ BCL-2 positive and negative (χ 2 test, p=0,585). Frequency FISH/C-MYC in the group with complete remission was 36.7%, while in the group without CR it was 43.8%. Achieving complete remission was not significantly different between patients FISH/ C-MYC positive and negative (χ 2 test, p=0,570). The frequency of both positive FISH/ BCL-2 and C-MYC, with the presence of at least one positive polymorphism and negative both, it is not statistically significantly different in comparison to achieving a complete remission (χ 2 test, p=0,600). Respondents with BCL-2 and C-MYC negative were slightly over-represented in the group with the complete remission of 43,3%, compared to the patients without complete remission of 31,3%. A bit higher frequency BCL-2 positive patients 31,3% was observed in group without achieving the complete remission, but in patients with complete remission of 20%. The sensitivity observed in the presence of both polymorphisms as well as the sensitivity of FISH positive BCL-2 or C-MYC findings was the same, about 45%. Specificity was the same in the presence BCL-2 or C-MYC gene polymorphism at about 60%, while it was the lowest in the presence both genetic polymorphism
Summary/Conclusions: In our study, the sensitivity observed in the presence of both or single mutation was the same, about 45%. Specificity in all observed parameters was relatively small, which means that the absence of the observed risk factors does not mean the emergence of the complete remission. The existence C-MYC and BCL-2 is not a good predictor of achieving complete remission in the era of rituximab.

PB1692

ANAPLASTIC LARGE CELL LYMPHOMA IN ADULTS: RESULTS AND NEW APPROACH OF TREATMENT

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Background: Anaplastic Large Cell Lymphoma (ALCL) represents supgroupe of peripheral T-cell lymphoma and constitutes 10-12% of this groupe. According to WHO classification 2008 ALCL is subdivided by two types depending on presence of *ALK* gene rearrangement: ALK-positive (ALCL ALK+) and ALK-negative (ALCL ALK-). ALCL ALK+has a favorable prognosis with CHOP in patients without adverse prognostic factors and event free survival (EFS) reaches 70-75%. ALCL ALK- has worse treatment results: when using CHOP overall survival (OS) and EFS doesn't reach more than 50%. Autologous stem cell transplantation (auto-SCT) in first complete remission takes an advantage.
Aims: to estimate an efficacy of intensive chemotherapy in treatment of ALCL ALK+and ALCL ALK-
Methods: The study included 64 patients with ALCL (38 ALCL ALK+26 ALCL ALK-)
Results: 36 patients with ALCL ALK+were treated according NHL BFM-90: OS and relapse-free survival were 90% and 83%, respectively, median survival was 67 months. In 16 patients with ALCL ALK- was used NHL-BFM-90 protocol – common response (CO) was achieved in 8 from 16 patients (50%), however 6 patients had disease progression and 3 had a relapse. Thus, OS and EFS were 37,5% and 31% respectively. 8 patients were treated by TL-REZ-09 protocol.

Complete/partial remission were acieved in 4 patients, 3 had disease progression. The highest efficacy was observed in 5 patients underwent auto-SCT in first complete remission: 4 from 5 patients alive in remission and one in relapse.
Summary/Conclusions: Patients with ALCL ALK+have favorable prognosis and high treatment results on NHL BFM-90 protocol. Selection of induction chemotherapy regimen before auto-SCT in patients with ALCL ALK- continue to be unsolved question. In patients who didn't achieve a common response like second line therapy could be DexaBEAM protocol with subsequent auto-SCT.

PB1693

DOSE ADJUSTED REPOCH IN UNTREATED *DE NOVO* DIFFUSE LARGE B CELL LYMPHOMA - A SINGLE CENTRE SINGAPORE EXPERIENCE

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Background: The current standard of care for untreated Diffuse Large B Cell Lymphoma (DLBCL) remains as Rituximab plus CHOP chemotherapy (RCHOP). Some studies suggested that certain subtypes of DLBCL respond better to dose adjusted REPOCH. However, data comparing the efficacy of RCHOP with REPOCH is scarce.
Aims: To compare the outcome of untreated *de novo* DLBCL of dose adjusted REPOCH chemotherapy with historical controls.
Methods: This is a retrospective analysis of all patients between age 18 – 70 with any stage of treatment naïve *de novo* DLBCL; who received RCHOP chemotherapy between 2009 to 2013, and REPOCH chemotherapy between 2013 to 2016. DLBCLs that were transformed from low grade lymphomas were excluded. Demography, histology, stage, immunohistochemistry biomarkers for cell of origin (GCB *versus* ABC using Hans algorithm), cytogenetics, details of chemotherapy, responses and duration of follow-up were included for analysis. 2 years overall survival (OS) and progression free survival (PFS) were calculated.
Results: 46 evaluable patients were followed up between 2009 and 2016 with 20 in the RCHOP cohort and 26 in the REPOCH cohort. The characteristics between the RCHOP and REPOCH groups were comparable: average age were 54 and 53.9 years respectively, 15 of 20 (75%) and 18 of 26 (69%) patients had stage 3 or 4 disease respectively. 50% and 20% of the RCHOP group; and 73% and 23% of the REPOCH group have the ABC and GCB subtypes of DLBCL respectively. The median follow-up period for the RCHOP and REPOCH groups were 40.5 (range 8 -70) and 16 (range 3 - 35) months. Kaplain Meier survival curves showed that the OS and PFS of REPOCH were greater than that of RCHOP, although they were not statistically significant (OS Log-rank test: z=0.71, p=0.48; PFS Log-rank test: z=1.52, p=0.13). The 2 years OS of the RCHOP and REPOCH groups were 89% and 100% respectively; and the 2 years PFS of the RCHOP and REPOCH groups were 75% and 95% respectively (Figure 1).

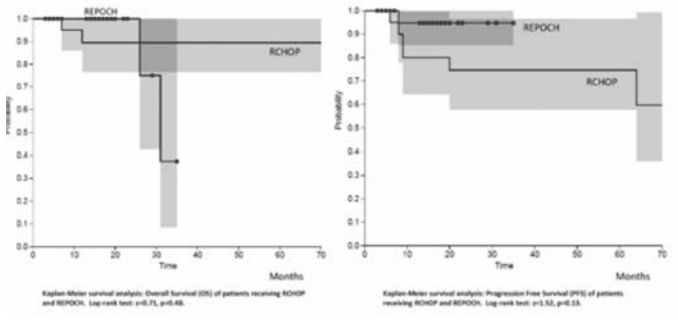

Figure 1.

Summary/Conclusions: In the literature, the GCB subtype is generally more than twice as common as the ABC subtype and confers a better overall outcome. In our institution, the ABC subtype was around 3 times more prevalent than the GCB subtype, suggesting the biology of the disease may differ with ethnicity and imply a more aggressive phenotype. Although our data suggests REPOCH to be superior to RCHOP in treatment naïve *de novo* DLBCL patients, a longer follow-up period may be required to observe a statistical difference in the OS and PFS. Further prospective randomized comparison between these two regimens in the Asian population is warranted.

PB1694

BASELINE RENAL FUNCTION AS A PROGNOSTIC INDICATOR IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH R-CHOP IMMUNOCHEMOTHERAPY

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Background: Recently the prognostic value of baseline renal impairment (RI) has been evaluated in patients with newly diagnosed cancers: baseline RI assessed by blood urea nitrogen showed significant relationship to overall survival (OS) of patients with advanced non-small cell lung cancer (Zhang *et al.,* Int J Cancer 2015;136:382) or to early mortality among patients with resectable pancreatic adenocarcinoma (Sohal, *et al.,* Cancer 2015;121:1779).
Aims: In this study, we evaluated the prognostic value of baseline RI in patients with diffuse large B-cell lymphoma (DLBCL) uniformly treated with conventional three-weekly rituximab plus cyclophosphamide, adriamycin, vincristine, and prednisolone (R-CHOP21) immunochemotherapy in single institution.
Methods: Patients with newly diagnosed *de novo* DLBCL treated with ≥1 cycle of R-CHOP21 were included. Glomerular filtration rate (GFR) was calculated using serum ceratinine level from pre-treatment serum chemistry data and other clinical information. RI was defined as a GFR of <60 mL/min/1.73 m^2according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.
Results: 185 patients treated from Mar. 2006 to Jan. 2015 were included and analyzed. For the median follow up period of 44.1 months (95% CI, 37.5 - 50.7), 53 patients (28.6%) underwent any event and 44 (23.8%) died. 3-year Event-free (EFS) and OS were 71.1% and 76.5%, respectively. Both standard and the NCCN-IPI showed good prognostication. However, NCCN-IPI more efficiently separated patients of low-intermediate *vs* high-intermediate risk compared to standard IPI, and the patients of high risk group according to NCCN-IPI showed poorer OS compared to those according to standard IPI. 19 patients had RI according to CKD-EPI formula: 5 patients had pre-existing CKD, 2 had post-renal acute kidney injury (AKI) caused by DLBCL, and another 2 had hypercalcemia and/or hyperuricemia, suggesting AKI due to the electrolyte imbalance due to lymphoma. We could not define the reason of RI of the other 10 patients: they were aged ≥70 years (range 71 – 89) except 2 patients with 49 and 69 years, respectively. Patients with RI showed inferior OS compared to those without RI (3-yr OS 26.7% *vs* 81.8%, p <0.001). There was no difference of OS between patients with a GFR of ≥90 *vs* ≥60 - 89 mL/min/1.73 m^2 (3-yr OS 82.4% *vs* 76.7%, p=0.436), suggesting that only RI defined by GFR below 60 mL/min/1.73 m^2 is a meaningful prognostic indicator of OS. In multivariate analysis, RI was an IPI-independent prognostic biomarker (Table 1). Among patients with high or high-intermediate risk according to standard IPI, patients with neither anemia of Hb <10 g/dL nor RI had superior OS *vs* patients who had one of them or *vs* those with both of them. These findings were maintained when the analysis was restricted to patients with high risk according to standard IPI. Use of NCCN-IPI instead of standard IPI showed same result.

Table 1. Univariate and multivariate analysis for event-free and overall survival.

Parameters	For overall survival	
	HR* (95% confidence interval)	*p*-value
Univariate analysis		
High-intermediate or high risk by standard IPI†	6.3 (3.3 – 12.0)	< 0.001
Age > 60 years	2.5 (1.4 – 4.7)	0.003
Elevated lactose dehydrogenase	3.6 (1.8 – 7.2)	< 0.001
Ann Arbor stage III or IV	3.6 (1.8 - 7.2)	< 0.001
ECOG‡ performance status ≥ 2	13.0 (6.6 – 25.0)	< 0.001
≥ 2 extranodal sites	4.4 (2.4 – 8.0)	< 0.001
High-intermediate or high risk by NCCN§-IPI	8.3 (4.0 – 17.3)	<0.001
Presence of B symptom	3.4 (1.9 – 6.1)	< 0.001
Bone marrow involvement	5.2 (2.8 – 9.6)	< 0.001
Hemoglobin < 10 g/dL	4.9 (2.7 – 8.9)	< 0.001
Bulky lesion	0.9 (0.5 – 1.9)	0.845
Non-germinal center B-cell type by Hans	1.4 (0.7 – 2.9)	0.376
Renal impairment	5.6 (2.9 – 11.0)	< 0.001
Multivariate analysis; IPI as separate 5 marker		
Elevated lactate dehydrogenase	-	-
ECOG performance status ≥ 2	9.6 (4.7 – 19.4)	< 0.001
Hemoglobin < 10 g/dL	2.5 (1.3 – 4.7)	0.006
Renal impairment	2.3 (1.1 – 4.7)	0.025
Multivariate analysis; with standard IPI as a single marker		
Hemoglobin < 10 g/dL	2.3 (1.1 – 4.5)	0.020
Renal impairment	4.1 (1.9 – 8.6)	< 0.001
High-intermediate or high risk by standard IPI	5.1 (2.5 - 10.1)	< 0.001
Multivariate analysis; with NCCN-IPI as a single marker		
Hemoglobin < 10 g/dL	2.8 (1.5 – 5.3)	0.002
Renal impairment	2.0 (1.0 – 4.2)	0.050
High-intermediate or high risk by NCCN-IPI	5.8 (2.7 – 12.7)	< 0.001

*HR, Hazard Ratio, †IPI, International Prognostic Index, ‡ECOG, Eastern Cooperative Oncology Group, §NCCN, National Comprehensive Cancer Network

Summary/Conclusions: Patients with baseline RI had inferior OS compared to those without RI. Because RI was an independent prognostic factor for OS in multivariate analysis whereas age >60 years (or ≥70 or even ≥75 years instead) was not, RI might have a role of the biological age-indicator of patients with DLBCL and therefore affect to OS. In combination with Hb <10 g/dL, RI enhanced prognostication in patients classified as higher risk group according to either standard or NCCN-IPI.

PB1695

INCIDENCE AND PROGNOSIS OF EPSTEIN-BARR VIRUS-POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA IN VERY ELDERLY PATIENTS (AGED OVER 80 YEARS)

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Background: Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma (DLBCL) of the elderly is a newly-described lymphoproliferative disorder (LPD) as a provisional entity in the 2008 WHO classification. The definition is a clonal B-cell proliferation associated with EBV, age at diagnosis over 50 years, and no secondary immune deficiencies. The prognosis is reportedly significantly inferior compared with EBV-negative DLBCL.
Aims: The aim of this study was to clarify the incidence of EBV infection and its related clinical features in very elderly patients with DLBCL.
Methods: We retrospectively analyzed 190 DLBCL patients diagnosed in our institute from January 2008 to December 2013. Diagnoses were immunohistochemically confirmed and the *in situ* hybridization detection technique for EBV-encoded small RNAs (EBER) was performed in all cases. EBV results were compared with clinical and immunochemical data according to age.
Results: The median age was 70.0 (22.7-97.1) years, and the median follow-up duration was 33.6 (0.2-96.5) months. The average EBER positivity was 4.6% in all patients. The mean EBV positivity of tumor samples in age groups were: 20-49 years (n=25), 0%; 50-59 years (n=32), 3.6%; 60-69 years, (n=38), 3.8%; 70-79 years (n=61), 8.9%; and over 80 years (n=34), 3.4% (1/34). The 5-year overall survival (OS) of all 190 patients was 58.2%, and that of EBV-positive patients (n=8) was 62.5%; the difference was not significant.
Summary/Conclusions: The reported incidence of EBV-positive DLBCL among patients of Asian and Latin American origin ranges from 9 to 15%, and the incidence of EBV-associated LPD increases with advancing age (Castillo JJ, *et al*. Oncologist 2011; 16: 87-96). Only 4.6% of the current DLBCL patients were EBER-positive; the reported peak (8.9%) was in the 70-79 years group, whereas the peak in that group in our cohort was only 3.4%. The immunophenotypic analysis results were similar in each age group, suggesting the patient population of this study was not deviated. In contrast to previous reports, the prognosis for EBV-positive and -negative DLBCL was similar. In conclusion, the characteristics of the EBV-positive DLBCL of the elderly identified in this study differ in several ways from that previously reported, and may be a new subtype of B-cell lymphoma; further studies to examine this possibility are warranted.

PB1696

OLDER PATIENTS WITH DLCLB-SHOULD ALL BE TREATED WITH INTENT TO CURE?

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Background: The incidence of diffuse large B cell lymphoma (DLCLB) increases in the elderly. Older age is associated with the presence of concomitant disease making the therapy difficult. Concidering the results of earlier studies, age itself should not be a justification for a paliative care decisions or reduced intensity chemotherapy. However, elderly patrients as more fragile, do not always receive the right treatment. So, the best method for identifying non-fit patients is not known.
Aims: To evaluate if Age Adjusted Carlson score (AACS) at diagnosis can predict clinical outcome in older patients with DLCLB receiving the first– line chemotherapy.
Methods: Patients at the Institution for Oncology and radiology of Serbia between 2005 and 2015 who were diagnosed and received first-line chemotherapy for DLCLB, older than 65 years were enrolled. Clinical and treatment data were recorded including AACS at the diagnosis. Survival time was estimated using the Kaplan- Meier (KM) method, and Cox proportional hazard model was used to evaluate the risk factors significance for survival. A p-value <0.05 was considered significant.
Results: 87 patients were included in the study. 23 (26.44%) patients were older than 75 years, 52 (59.77%) were female, 38 (43.67%) were Ann Arbor stage 1 and 2, 28 (32.18%) were International prognostic index (IPI) score 0-1; and 38 (43.68%) were intermediate high or high risk. 44 (50.57%) patients were AACS≤5 and 43 (49.43%) patients were AACS≥6. Extronadal involvement was present in 65 (74.71%) patients at diagnosis. 60 (68.97%) patients were treated with Rituximab. 38 (43.68%) patients received CHOP, 27 (31.03%) mCHOP, 12 (13.79%) CVP and 10 (11.49%) CEOP regimen, with or without Rituximab. Statistically significant difference for overal responce rate (ORR) was registered between CHOP and CVP arm (p=0.004), as for mCHOP and CVP arm (p=0.001). CEOP was not inferior to CHOP (p=0.37) and mCHOP(p=0.47) in term of ORR. Complete responce rate (CRR) was significantly better in CHOP (73.68%), m CHOP (74.07%) and CEOP (80%) groups, than in CVP group (25%). ORR and CRR were better when Rituximab was used (ORR and CRR were 100% and 79.31%/78.26% for RCHOP/RmCHOP, but 88.89%/50% and 55.56%/50% for CHOP/mCHOP). There was no difference in toxicity among chemotherapy regimens. After a median follow-up of 43 months (range, 1-128), median overal survival (OS) times were 91 months (54-not reached) for CHOP, 8.5 months (2- not reached) for CVP. Median OS was not reached for mCHOP and CEOP arm. The CVP regimen was associated with a significantly worse OS and PFS than was the CHOP regimen (p=0.0002) or mCHOP regimen (p=0.0006). CHOP was uncommon in AACS≥6 group (p=0.003). There was no difference between AACS≤5 and AACS ≥6 groups in the frequency of other chemotherapy regimens. ORR was significantly better in AACS≤5 (p=0.005). Median OS were not reached for AACS≤5 and 43 months (20- not reached) for AACS≥6. Patients with AACS≥6 had significantly shorter PFS (p=0.006) and OS (p=0.001) than patients with AACS≤5.
Summary/Conclusions: AACS≤5 was associated with a significant reduction in the risk of death among older patients with DLCLB treated with different chemotherapy regimens. This study suggests that AACS may predict responce to chemotherapy and survival in older patients with newly diagnosed DLCLB. As predictive biomarker and clinical prognostic indicator for OS and PFS, AACS may be useful in defining the group of older DLCLB patients that would have more benefit from best supportive care instead of chemotherapy.

PB1697

THE PROGNOSTIC NUTRITIONAL INDEX AS A PREDICTOR OF PROGNOSIS IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: The prognostic nutritional index (PNI), an indicator of nutritional status and systemic inflammation, is associated with short- and long-term outcomes of various malignancies. The prognostic value of the PNI in diffuse large B cell lymphoma (DLBCL) remains unknown.
Aims: The aim of present study is to determine the prognostic value of baseline PNI in DLBCL patients.
Methods: We retrospectively analyzed data from 76 DLBCL patients treated with R-CHOP or R-CHOP-like regimens. We evaluated the significance of PNI as a predictor of response to treatment, overall survival (OS) and event-free survival (EFS).
Results: Lower PNI levels were found in patients with advanced Ann Arbor clinical stage (47.56±6.97 *vs* 53.28±5.43, *P*<0.001) and in those with poor response to therapy (41.63±6.1 *vs* 51.08±6.11, *P*<0.001). Patients with PNI≤44.55 (cut-off was calculated by receiver operating chararacteristics) had significantly worse 5-year OS (35% *vs* 88.8%, P<0.001, log rank test) and 5-year EFS (28% *vs* 81.3%, *P*<0.001, log rank test). Cox regression analysis showed that PNI≤44.55 was an independent prognostic factor for OS (HR 4.729, 95% CI 1.327-16.854) and EFS (HR 3.024, 95% CI 1.037-18.819).
Summary/Conclusions: PNI is an important prognostic factor in DLBCL, in addition to and independent of the International Prognostic Index. PNI may emerge as a simple, fast, easy to use and inexpensive new prognostic marker for DLBCL patients in routine clinical practice.

PB1698

ROLE OF PET/MRI IN HEMATOLOGIC MALIGNANCIES: PRELIMINARY OBSERVATIONS

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Background: Hybrid imaging using simultaneous PET/MRI could be a valuable diagnostic modality for the staging and follow-up of patients with hematologic malignancies. Since the availability of this technique is currently restricted to a small number of centers, data available in this setting is still limited.
Aims: Our objective is to analyze the results and potential advantages of PET/MRI in hematologic malignancies.
Methods: A total of 30 PET/MRI studies were performed at our institution using a Biograph mMR device. Informed consent, which included use of clinical data, was obtained before every procedure. PET/MRI was performed 45 minutes after injection of 18F-FDG. Images were obtained from orbital region to proximal third femur. Magnetic Resonance was obtained by HASTE sequencing.
Results: A total of 21 patients were studied. Median age was 62 years (range 35-89).Patients' diagnoses were: Diffuse Large B Cell Lymphoma (8 patients); Follicular Lymphoma (3); Multiple Myeloma (2); Primary Central Nervous System Lymphoma (2); Hodgkin Lymphoma (3); Plasmacytoma (1) and other Low grade Lymphomas (2). In 7 patients (23%) PET/MRI was used for initial staging, and in 11 patients (37%) it was used for follow-up, comparing previous results with other staging techniques (CT scan/PET-CT). In 3 patients (12%) the PET/MRI was used to study possible relapse of the disease. In 9 patients (30%) a second PET/MRI was performed for either follow-up (6 patients, 20%), or to evaluate potential relapse (3 patients,10%). Of all procedures performed, PET/MRI was useful for establishing initial stage (6 patients, 20%); confirm response to therapy (14 patients. 46%); and prove disease relapse (3 patients

10%). Finally, in 7 patients (23%) in whom conventional imaging had shown residual disease, PET/MRI was able to demonstrate complete remission.
Summary/Conclusions: Taken together, our results suggest that PET/MRI is a promising technique which may improve diagnostic accuracy, with a reduction of radiation dose in patients with hematologic malignancies. Further studies are needed in order to validate this technique for clinical use.

PB1699

DOSING OF CHEMOTHERAPY IN OBESE ADULT PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES: A SURVEY OF PRACTICE IN THE UK

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Background: With obesity levels increasing to epidemic proportions, treating obese patients optimally with chemotherapy is a significant issue. There is accumulating evidence of increased cancer risk in obese patients and obesity related co-morbidities can compromise relative total dose intensity, resulting in poorer overall survival in some tumour types.
In 2010 the American Society of Clinical Oncology (ASCO) convened an expert panel to review the literature on chemotherapy dosing in obesity. The panel concluded that up to 40% of obese patients received "capped" doses because of clinicians' concerns regarding toxicity. They concluded that there was no evidence of increased toxicity in such patients and released a Clinical Practice Guideline recommending that obese patients should be dosed on their actual (full) body weight.
Aims: To investigate UK clinical practice in this area and establish whether it has changed as a result of the ASCO Clinical Practice Guideline.
Methods: A questionnaire was completed by 81 specialist cancer services pharmacists from the British Oncology Pharmacy Association. The questionnaire assessed: 1) criteria used for defining obesity, 2) if dose adjusting for obesity was undertaken at individual hospitals and if so what methodology it was based on, 3) if practice had been influenced by the ASCO guideline and 4) what formulae/ methods were being used to assess body surface area (BSA), ideal body weight (IBW) and glomerular filtration rate (GFR).
Bone marrow transplant conditioning and treatment of leukaemia were excluded to reflect ASCO's guideline; treatment of Non-Hodgkins and Hodgkins Lymphoma were included.
Results: Of the 73 pharmacists who fully responded, just less than half (47%) stated that their hospitals follow ASCO's recommendations and dose on actual body weight. The most common reasons for non-adherence included the lack of robust evidence and a need for clinical teams to discuss the guidelines and reach a consensus of how to adapt locally. Of the 53% who confirmed that they reduce doses in obese patients, 30% dose reduced in all obese patients and 70% in selected patients where 'capping' decisions were based on treatment goal (100%) and the pharmacokinetics of the individual drugs (38%).
There was no uniformity in formulae used for BSA, IBW or GFR calculations. Methodology varied for defining obesity, adjusting the dose, assessing GFR and some centres used IBW in GFR formulae, some actual body weight. This lack of uniformity is not unique to the UK and is likely to be a widespread issue.
Summary/Conclusions: Further research is needed to clarify chemotherapy dosing in obese patients to aid clinicians and pharmacists in these decisions. Pharmacokinetic and pharmacogenetic studies would provide a more personalised approach to treatment, and can be undertaken in the context of large prospective trials. Until evidence is available for specific cancers, a unified approach to dosing in obesity based on current guidance should be encouraged.

PB1700

RITUXIMAB DURING INDUCTION THERAPY MIGHT NOT IMPROVE OVERALL SURVIVAL OF VERY ELDERLY JAPANESE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: As aging of population is advancing, the number of the elderly patients with diffuse large B-cell lymphoma (DLBCL), most common type of lymphoma, is increasing. In addition to difficulty to treat patients age 80 and over due to comorbidities and poor organ functions, the optimal treatment of these patients with DLBCL remains unclear since there was no well-controlled, randomized trial to confirm clinical benefits of rituximab during induction therapy for these patients, unlike younger patients.
Aims: In this study, we assessed clinical contribution of rituximab during induction therapy on survival of Japanese patients with DLBCL age 80 and over, especially in actual practical setting.
Methods: Previously, we reported that rituximab-containing remission induction therapy improved the outcome of Japanese patients with mature B-cell lymphoma newly diagnosed at 20 National Hospital Organization hospitals from January 2000 to December 2004. A retrospective study was conducted using this database, although it lacks detailed information on treatment, *e.g.* combinations of drugs, dose intensities of each drug, and the number of treatment cycles.
Results: From database, we identified 100 patients age 80 and over (12.5%) from 798 DLBCL patients. Only 15 patients (15.0%) didn't receive an anthracycline-containing regimen, and were excluded from further analyses. This ratio was statistically higher, compared to younger patients ($p<0.0001$). Among 85 patients who received anthracycline-containing regimens, 45 deaths were observed during follow-up period, and 8 deaths (17.8%) were due to other than lymphoma. This was not significant, compared to younger patients ($p=0.859$). Based on administration of rituximab in remission induction therapy, 85 patients were divided into two groups since patients didn't receive rituximab during induction until its approval for DLBCL in September 2003 in Japan. Sixty-two patients (72.9%) were treated without rituximab (R- group) and 23 patients (27.1%) with rituximab (R+group). Median follow-up periods were 38.7 months and 21.8 months, respectively. No significant difference of patient characteristics, age, sex, disease stage, extranodal involvement, B symptom, PS, LDH, IPI and AA-IPI, were observed between two groups. Better response was observed in R+group than R- group (CRR 56.5% *vs* 43.5%, ORR 82.6% *vs* 66.1%), but these differences were not significant ($p=0.3337$ and $p=0.183$ respectively). Estimated 2-year progression-free survival of R+group was significantly higher than R- group (50.4% *vs* 25.6% $p=0.030$), but 2-year overall survival was similar (45.3% *vs* 42.3% $p=0.344$). The same trends were seen when deaths of other than lymphoma were censored ($p=0.260$). R- group was further divided into 2 groups, patients who never received rituximab throughout their lifetimes (R-R- group n=48) and those who received rituximab during salvage therapies (R-R+group n=14). Median survivals of these groups were similar (19.2 months *vs* 21.8 months $p=0.900$), and there was no significant difference from R+group ($p=0.351$ and $p=0.486$ respectively).
Summary/Conclusions: Rituximab during induction therapy didn't improve overall survival of Japanese patients with DLBCL age 80 and over, unlike younger patients. However, later administration of rituximab during salvage therapies could improve prognosis. Large-scale studies are further needed to confirm these results.

PB1701

PROGNOSTIC ROLE OF NTPROBNP LEVELS AT DIAGNOSIS IN ELDERLY PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA (DLBCL)

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Background: Immunochemotherapy using the R-CHOP regimen (Rituximab, Cyclophosphamide, Doxorubcin, Vincristine and Prednisolone) represents the standard treatment of elderly patients (pts) with Diffuse Large B Cell Lymphoma (DLBCL), but treatment can be complicated by cardiac side effects often attributed to the cytotoxic drug doxorubicin. NTproBNP levels in plasma are typically increased in patients with left ventricular dysfunction and have been proposed as a marker for cardiac injury during cytotoxic treatment.
Aims: The aim of this study was to investigate the prognostic role of NTproBNP at diagnosis in elderly DLBCL pts.
Methods: We conducted a retrospective analysis, including 130 elderly (≥65 years) pts, median age 74 years (range 65-91), diagnosed with DLBCL from 2009 to 2014 in our institution for whom NTproBNP levels at diagnosis were available. The impact of NTproBNP levels on response and survival was evaluated in 126 patients who either received anthracycline-containing immunchemotherapy (92 pts) or immunochemotherapy without anthracyclines (34 pts). Anthracycline consisted in standard doxorubicin in 52 pts and liposomal doxorubicin in 40 pts.
Results: At diagnosis, 82/130 pts (63%) showed elevated NTproBNP levels. NTproBNP levels correlated positively with age (*p=0.0001*), and creatinine values (*p=0.001*) and negatively with levels of albumin (*p=0.0001*), and hemoglobin (*p=0.0001*). Poor performance status (ECOG ≥2) was associated with higher levels of NTproBNP (*p=0.0004*). Pts with a previous history of hypertension and ischemic heart disease had increased NTproBNP levels (*p=0.006*, and *p=0.02,* respectively). There was no correlation between NTproBNP and left ventricular ejection fraction at diagnosis (Spearman's rank coefficient p=0.7, p_s=-0.05). In a multivariate analysis, albumin levels <4 g/dl retained the significant association with elevated NTproBNP levels (*p=0.004*). Patients with normal NTproBNP levels at diagnosis had a higher probability of complete

responses (91 *vs* 70%, *p=0.02*). Higher NTproBNP levels were associated with inferior event-free and overall survival both in univariate (*p<0.001*) and multivariate analysis including IPI and anthracycline-containing chemotherapy as significant parameters (Table 1).

Table 1. Multivariate Cox regression analysis for event-free survival.

Parameter	Haz. Ratio	P-value	[95% Conf. Interval]
Anthracycline, No vs. Yes	4.200275	0.000	2.040647 8.645453
IPI, 3-5 vs 0-2	2.867061	0.008	1.318712 6.233381
NTproBNP, unit increase	1.000168	0.018	1.000029 1.000307

Summary/Conclusions: NTproBNP levels at DLBCL diagnosis are frequently elevated in elderly patients with DLBCL, predict poor outcome and are associated with a number of patient characteristics reflecting "host-related factors", such as age, history of cardiac injury, performance status and laboratory abnormalities as low hemoglobin and albumin values. Studies on mechanisms that lead to increased NTproBNP levels in addition to wall stress on myocardiocytes are warranted.

PB1702

AGGRESSIVE LYMPHOMA: DEMOGRAPHICS, DIAGNOSTIC PATTERNS AND OUTCOMES IN GROOTE SCHUUR HOSPITAL IN CAPE TOWN, SOUTH AFRICA

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Background: The high rate of HIV in South Africa led to an increasing incidence of aggressive non Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL), but also of infection with Mycobacterium tuberculosis (TB) which may mimic lymphoma. We have observed in Cape Town that lymphoma patients present with advanced disease and that their route to diagnosis is often markedly delayed.
Aims: This study describes the demographic distribution of aggressive lymphomas in our Cape Town institution as well as examine survival outcomes as impacted by documented time delays along the diagnostic pathway.
Methods: We studied 163 patients diagnosed and treated for aggressive lymphoma by Radiation Oncology and Haematology at Groote Schuur Hospital Cape Town in 2013/14. We collected diagnostic and outcome data from hospital records and patient interviews. We constructed a pathway to diagnosis as previously described by Howell DA *et al.,* in BMC Hematology 2013 Oct 31;13(1):9. Delays along the diagnostic pathway were divided into three time frames. 1. Patient delay (time from first symptoms until first contact with a medical practitioner) 2. Diagnostic delay (time from first medical contact to diagnostic biopsy). 3. Treatment delay (time from biopsy to referral to our unit and initiation of therapy).
Results: NHL was diagnosed in 122 (74.8%) and HL in 41 (25.2%). In the NHL cohort 29 patients (23.8%) were HIV positive and in HL 18 patients (43.9%), p=0.014. There was no significant association between HIV status and stage at presentation and HIV positive patients did not have a significantly poorer overall survival (OS) (59.6% *vs* 69%, p=0.251). There was, however, a significant association of HIV with progressive disease in the alive group at time of data analysis (8 patients (28.6%) *vs* HIV negative 10 patients (12.5%), p=0.05). HL had better OS compared to NHL (35 (85.4%) *vs* 73 (59.8%), p=0.003). The HL cohort was more likely to present with high-risk disease (80.5%), lymphadenopathy (82.9% *vs* 56.6% p=0.001) and B-symptoms (73.2% *vs* 54.1% p=0.032). Despite presenting at this advanced stage, they waited significantly longer for referral from first doctor's visit to diagnostic biopsy (125 days *versus* 43 days p=0.01) and from first symptoms to treatment (271 *versus* 97 days p=0.004). Investigations for the diagnosis of TB as well as empiric TB treatment had a major impact on this delay. Empiric TB treatment was given to 26% of the HL cohort prior to diagnosis of lymphoma, compared to 4.9% in the NHL cohort (p=0.00). Fine needle aspiration (FNA) was carried out in 63 (61%) prior to excisional biopsy and in 17 (27%) FNAs were repeated. Only 10 of 88 FNA studies (11%) suggested lymphoma. Time delay between FNA and diagnostic biopsy median 22 days (IQR 9.8-64). Longer delay from first doctors visit to referral for diagnostic biopsy was associated with an increased mortality (alive: median 40 days (IQR 23-69), dead: median 64 days (IQR 27-154), p=0.04). This is also true for time from first doctor's visit to treatment (alive: median 59 days (IQR 29-122), dead: median 97 (51-205), p=0.03) and for time from first symptoms until treatment (alive: median 103 days (61-178), dead: median 131 (IQR 73-299), p=0.04).
Summary/Conclusions: In aggressive lymphoma diagnosis the period between first patient contact with a health professional and the diagnostic biopsy is crucial. FNA examination is often the first step in the diagnostic pathway of lymphadenopathy in South Africa due to the high rate of TB and poor access to diagnostic biopsy. This led to an inappropriately long 3-week median delay from first FNA to diagnostic biopsy. These three key areas - use of FNA, poor access to excisional biopsy and treatment with empiric TB therapy – significantly delayed diagnosis resulting in severe consequences with increased patient mortality.

PB1703

TRANSGASTRIC ENDOSCOPIC ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION BIOPSY FOR LYMPHOMA DIAGNOSIS OF ISOLATED SPLENIC LESIONS

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Background: Isolated splenic lesions are relatively infrequent, but represent a diagnostic challenge, as tissue sampling for histologic analysis may be difficult, in particular if the size of the lesion is small. Percutaneous image-guided splenic biopsies are rarely performed due to the perceived high risk of hemorrhage that could lead to urgent splenectomy, and diagnostic splenectomy is often preferred as primary diagnostic procedure. Endoscopic ultrasonography (EUS)-guided fine needle aspiration (EUS-FNA) biopsy allows sampling of tissue which is adjacent to the stomach wall. EUS provides a good imaging of the spleen through the gastric wall. There have been only few cases of successful histopathologic diagnoses by EUS-guided splenic biopsies reported in the literature. We report our experience with trans-gastric EUS-FNA in the investigation of patients with isolated splenic lesions suspicious for lymphoma.
Aims: We retrospectively assessed the diagnostic performance and safety of EUS-FNA to elucidate the tissue diagnosis of splenic abnormalities suspicious for lymphoma.
Methods: Between 2009 and 2016, we evaluated nine patients with splenic lesions detected by CT, Positron Emission Tomography (PET) and US in our Institution. We used a linear echo-endoscope and 19/20 gauge needles for transgastric EUS-FNA biopsy. Patients were admitted to the hospital to guarantee an overnight monitoring after the procedure. Platelet counts were in the normal range and INR was <1.5.
Results: The age of the patients was 40–83 year (average 58.6 year); five patients were male and four female. All patients presented focal splenic lesions that varied in size from 17 to 100 mm (average 41 mm). Bone marrow biopsy was negative for lymphoma localization, and lymph node involvement was absent or minimal. There was no evidence of bleeding or other complications related to the procedure after splenic EUS-FNA biopsy. In seven patients tissue sampling was sufficient for a pathological diagnosis (78% sensitivity). Diagnoses were Hodgkin Lymphoma in two patients, and Diffuse Large B-cell Lymphoma (DLBCL) in five patients. Only two patients required further diagnostic procedures, including laparoscopic spleen biopsy in one patient and splenectomy in one patient. In both cases final diagnosis was DLBCL.
Summary/Conclusions: Transgastric EUS-FNA biopsy of splenic lesions is a safe diagnostic procedure with high sensitivity for the diagnosis of lymphoma, reducing the need for splenectomy.

PB1704

THE LYMPHOCYTE COUNT AND THE MONOCYTE COUNT AND THE LYMPHOCYTE TO MONOCYTE RATIO AT DIAGNOSIS WERE A PROGNOSTIC PARAMETERS IN DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS FROM A LARGE MULTICENTER STUDY

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Background: There is increasing evidence that tumor microenvironment and host immunity play an important role in lymphoma progression. The absolute lymphocyte count (ALC) and the absolute monocyte count (AMC), calculated from the complete blood count, were considered a surrogate for host immunity.
Aims: The aim of this collaborative multicenter study was to verify the prognostic significance of ALC, AMC and ALC/AMC ratio in a large cohort of newly diagnosed patients with DLBCL and we also examined whether ALC and AMC could be utilized as a simple Independent prognostic factors for survival.
Methods: It is a retrospective and multicenter study carried-out by seven hematology departments in the western of Algeria. 467 patients with DLBCL had been diagnosed during an eight years period (2007-2014). All patients, older than 16 years, were included in the study. The median age at the diagnosis was 54 years (16 to 89). There were 265 males and 202 females. The localized stage (I-II) was 43% and the advanced stage (III-IV) was 57%. The performans status (PS≥2) was 41%. LDH increased level in 47%. The number of extra-nodal involved sites was as follows: 0=26%, 1=43%, 2=19%, ≥3=12%. The IPI score were as follow: low risk=41%, intermediate low risk=23%, intermediate high risk=24% and high risk=12%. The IPI-R score were as follow: very good=11%, good=52%, bad=37%. The overall survival (OS) and progression free survival (PFS) were calculated according to Kaplan and Meier method and the survival curves were compared using the Log Rank test. The multi-factorial survival analysis was performed by the use Cox regression test. The end point date was the December the 31st 2015. The median of follow up was 24 months (6-109).
Results: The mono-factorial analysis of OS (comparison of the survival curves)

showed: Age (≥60 *vs* <60) (p=0.5); sex (M *vs* F) (p=0.04); Performans Status (0-1 *vs* ≥2) (p=0.06); the Ann Arbor (localized *vs* advanced stage (p=0.008). Bulky *vs* no Bulky (p=0.32); B symptoms (A *vs* B) (p=0.05); LDH (Normal *vs* Increased) (p=0.4); IPI score (p=0.046); IPI-R score (p=0.16- p=0.002 and p=0.001); ALC (>1000µl *vs* <1000µl) (p=0.082); AMC (>630/µl *vs* <630/µl) (p=0.001). ALC/AMC ratio (≥2.11 *vs* <2.11) (p=0.001). The mono factorial analysis of PFS showed: Sexe (p=0,247); PS>=2 (p=0,325); the Ann Arbor localized *vs* advanced stage (p=0,089); IPI score (p=0,709); IPI-R score (p=0,581); LDH>Normal *vs* Increased (p=0,465); AMC >630 (p=0,778); ALC>1000 (p=0,155) and the ALC/AMC ratio ≥2.11 *vs* <2.11(p=0, 03). The multi-factorial analysis of the overall survival showed that the most discriminative prognostic factors were: Age>60 years old (HR=1.77, p=0.002); Female sex (HR=0.58, CI 95% 0.41-0.82, p=0.002); PS<2(HR=0.58, CI 95% 0.41-0.81, p=0.002), ALC>1000 (HR=1.28 IC 95% 0.68-2.42, p=0.043), AMC>630 (HR=0.72 IC 95% 0.45-1.15, p=0.018), ALC/AMC ratio>11 (HR=1.54 IC 95% 0.72-3.02, p=0.02).
Summary/Conclusions: This study shows that a simple parameters like absolute lymphocyte count (>1000/mm3) and monocyte count (>630/mm3) and the lymphocyte to monocyte ratio at the diagnosis can easily be used routinely in the evaluation of newly diagnosed diffuse large B-cell lymphoma to identify high –risk patients with a worse survival in the rituximab era.

PB1705

THE ABSOLUTE MONOCYTE AND PLATELET COUNTS IN PRIMARY GASTRIC DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH RITUXIMAB AND CHOP

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Background: Primary gastric lymphoma (PGL) accounts for 3% of gastric malignancies and 10% of lymphomas. The most common histological subtype of PGL is the primary gastric diffuse large B cell lymphoma (PGDLBCL). The standard of care of PGDLBCL patients (pts) is the combination rituximab-CHOP immunochemotherapy (R-CHOP). Recently serum albumin (SA), β2-microglobulin (B2M), hemoglobin level (Hb), absolute neutrophil (ANC), lymphocyte (ALC), monocyte (AMC) and platelet counts (PC) etc have been introduced into the clinical practice for better pts' stratification. However the data regarding the prognostic significance of these markers are limited.
Aims: Therefore, we decided to access the possible prognostic impact of SA, B2M, Hb, ANC, ALC, AMC and PC in R-CHOP treated PGDLBCL pts.
Methods: We retrospectively reviewed the clinical outcome of 42 R-CHOP treated PGDLBCL pts with median age 56.7 years and 54.76% male. The laboratory levels of SA, Hb, ANC, ALC, AMC and PC were recorded. Serum B2M levels were measured radioimmunologically. The best cut off values of SA, B2M, ANC, ALC, AMC and PC to predict overall survival (OS) by Kaplan-Meier method were calculated by a receiver operating characteristic (ROC) curve analysis. Univariate and multivariate analyses were performed by the log rank and Cox proportional-hazards methods, respectively.
Results: The estimated 5-year OS of the whole group was 80.9%. The median values of SA, B2M, Hb, ANC, ALC, AMC and PC were 39.1 g/L, 2.95 mg/L, 121 g/L, 4.69 G/L, 1.79 G/L, 0.51 G/L and 324 G/L, respectively. By applying the best cut off values of SA, B2M, Hb, ANC, ALC, AMC and PC that were 33.9 g/L, 2.6 mg/L, 105 g/L, 6.12 G/L, 1.77 G/L, 0.518 G/L and 335 G/L, respectively, it was observed that only Hb, ANC, AMC and PC correlated with OS. The independent prognostic significance, however, was confirmed in multivariate analysis only for AMC and PC (hazard ratios 15.482 and 13.525; p=0.012 and p=0.017, respectively). The dichotomized AMC and PC generatedthe AMC/PC prognostic index (PI) and stratified patients into3 risk groups: very good (AMC <0.518 G/L and PC <335 G/L),good (AMC <0.518 G/L or PC <335 G/L) and poor-risk (AMC ≥0.518 G/L and PC ≥335 G/L) populations. For both the very good and good-risk groups median OS has not been reachedwith estimated 5-year OS of 100% and 86.4%, respectively whereas the median OS for poor-risk pts was 4 months with an estimated 5-year OS of 28.6% (p<0.001).
Summary/Conclusions: AMC and PC are independent prognostic markers to predict survival in PGDLBCL patients. AMC/PC PI could provide additional prognostic information for better stratification of these pts.

PB1706

TWENTY YEARS' EXPERIENCE OF TREATMENT OF ADOLESCENTS AND YOUNG ADULTS WITH NON-HODGKIN LYMPHOMA – A SINGLE CENTER EXPERIENCE

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Background: Adolescents and young adults (AYAs) with non-Hodgkin lymphomas (NHL) comprise a unique group of patients, under-represented in clinical trials. Furthermore, the prognosis of these patients is inferior to that of younger patients with hematological malignancies. Therefore, many questions regarding the biology and treatment strategies of this group remain unanswered.
Aims: We aimed to determine the characteristics of AYA NHL patients in the last 20 years in a single tertiary center. Also, we intended to identify risk factors for poor prognosis in this population.
Methods: We reviewed retrospectively the database of our NHL patients at the Rabin Medical Center between the years 1995-2014 and identified patients who met the following criteria: age 18-40 years old, a confirmed histological diagnosis of NHL and treatment in our institution, with at least one year follow-up. In order to define prognostic factors we used the Mann-Whitney U test to compare between the group of patients with poor outcome and those with better outcome. 3-year event free (EFS) and overall survival (OS) were calculated according to the Kaplan-Meier analysis.
Results: A total of 85 patients with confirmed diagnosis of NHL were included in this study. Median age was 32 (range, 18-40) years with 42% male. 74 patients (87%) were diagnosed with aggressive lymphoma and 11 (13%) with indolent lymphoma. The histologic subtypes included diffuse large B-cell lymphoma - 50%, primary mediastinal B-cell lymphoma - 28%, Burkitt lymphoma - 9%, follicular lymphoma - 12%, marginal zone lymphoma - 1%. Due to the small number of patients with indolent lymphoma, this group of patients was not analyzed. B symptoms were present in 32 patients (43%) and 48 had bulky disease (68%). More than half of the patients had advanced stage disease. The IPI score was low (0-1) in 40 patients (56%), low-intermediate (2) in 12 patients (17%) and intermediate-high (3) in 19 patients (27%). Half of the patients were treated with CHOP like regimen (CHOP, R-CHOP, CHOEP), 30% were treated with MACOP\VACOP-B with or without rituximab and 16% received intensive regimens (hyper-CVAD & GMALL). Most patients (74%) received rituximab as part of their treatment regimen. 92% of the patients achieved complete remission (CR), 19% of them relapsed. 14 patients (19%) underwent stem cell transplantation: 12 autologous and 2 allogeneic. Overall, 7 patients died during follow - up, 6 of them with DLBCL and 1 with PMBCL. All died due to progressive disease. 3-year EFS and 3-year OS for the whole group was 74% and 90%, respectively, for patients with DLBCL-67% and 86%, respectively and for patients with PMBCL- 90% and 96%, respectively (Figure 1). The results of Mann-Whitney U test revealed that the group of patients with worse outcome had higher IPI (p=0.03), higher aaIPI (p=0.03) and lower use of rituximab in the treatment regimen compared to those with better outcome (p=0.05).

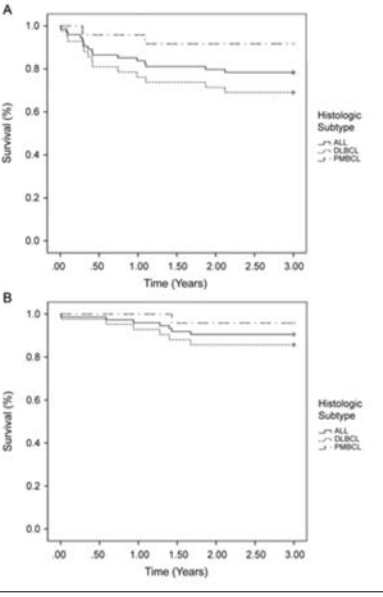

Figure 1.

Summary/Conclusions: In our cohort of AYA patients with NHL, DLBCL was the most frequent subtype of lymphoma followed by PMBCL. This is in accordance with previous reports. The 3 year EFS and OS of AYA patients with DLBCL in our cohort were lower or similar to other studies of AYA patients while results for patients with PMBCL were superior to those in the literature. The aaIPI/IPI score was validated in our cohort as a reliable prognostic index. As in adults, the inclusion of rituximab in a combined regimen was associated with better outcomes than chemotherapy alone.

PB1707

PRETREATMENT CRP LEVEL IN DLBCL PATIENTS: AN IMPORTANT PREDICTOR OF TREATMENT RESPONSE IN RESOURCE CONSTRAINT SETTINGS

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Background: The currently available prognostic model for Diffuse Large B Cell Lymphoma (DLBCL) is not sufficient to predict the outcome of this heterogenous group of DLBCL. There is a need to find better prognostic markers for optimal risk stratification of DLBCL patients.
Aims: This study aimed to evaluate the prognostic significance of proinflammatory markers in pretreatment peripheral blood sample of patients of DLBCL.
Methods: We measured levels of CRP and Th1 and Th2 cytokines (IL2, IL4, IL6, IL10, IFN gamma and TNF alpha) in the pretreatment serum of 46 newly diagnosed DLBCL patients and in 10 healthy controls. CRP was measured by nephelometry and cytokines were measured using flowcytometric bead array assay. We compared the levels of these proinflammatory markers between cases and control groups. We evaluated the correlation of levels of these proinflammatory markers with disease characteristics like B symptoms, Ann Arbor stage, IPI and with treatment response.
Results: The median age of cases was 55 years (range 13-80 years) while that of controls was 46 years. Among DLBCL patients 72% were males and 28% were females. According to the IPI scoring the low, intermediate and high risk groups comprised of 28%, 28%, and 44% patients respectively. Of the 41 patients who received treatment, 58% achieved CR, 12% achieved PR and 24% had progressive disease respectively after frontline treatment with chemotherapy. In this study we found that the levels of CRP, IL4, IL6, IL10 and IFN gamma were significantly elevated in DLBCL patients as compared to the control group as shown in Table 1. The pretreatment serum level of CRP in DLBCL patients was significantly elevated in those with B symptoms and high risk IPI group as compared to those without B symptoms and low risk IPI respectively. It was also noted that IL2 showed a significant inverse correlation with stage and IPI of DLBCL patients. The median serum value of CRP was 30.5mg/l and 81mg/l in patients who achieved complete remission (CR) and those who did not achieve CR respectively after the frontline chemotherapy (p value - 0.007). The median serum level of IL6 was 31.5pg/ml and 14pg/ml respectively in those who achieved overall response (OR) and those who did not achieve. Thus OR was significantly lower in those patients with elevated IL6 (p value - 0.05).

Table 1. Comparison of cytokine levels between cases and controls.

Cytokine	Cases(n 46) Median (Range)	Controls(n10) median	Cases Mean ± SD	Controls Mean +SD	P Value*
IL2 pg/ml	0 (0 – 8)	1.48 (0 – 5.29)	1.30 ± 1.82	2.34 ± 1.24	0.414
IL4 pg/ml	1 (0 -8)	0 (0 -2)	1.77 ± 2.04	0.16 ± 0.53	**0.002**
IL6 pg/ml	15 (0 -8625)	1.50 (0 -4.67)	220.11 ± 1268.96	1.96 ± 1.45	**<0.001**
IL10 pg/ml	4 (0 – 181)	0.63 (0 – 1.21)	19.88 ± 41.75	0.54 ± 0.50	**0.001**
TNF alfa pg/ml	0 (0 – 419	0 (0 -4.02)	10.85 ± 61.82	0.40 ± 1.27	0.175
IFNgamma pg/ml	1.45 (0 – 24.69)	0 (0 - 0)	2.30 ± 4.07	0	**0.001**
CRP mg/l	44.5 (1 – 338)	1.95 (1 - 5)	82.30 ± 87.13	2.34 ± 1.24	**<0.001**

*Mann Whitney U Test

Summary/Conclusions: Pro inflammatory markers can be used to predict treatment outcome in DLBCL patients. Our work concludes that there is alteration in cytokine profile of patients with DLBCL with inclination towards heightened Th2 response. CRP has shown a significant correlation with adverse disease characteristics and treatment response. It can emerge as a readily available and cost effective prognostic marker to predict treatment response in DLBCL patients especially in resource constraint settings.

PB1708

PROGNOSTIC IMPACT OF MYC/BCL-2 PROTEIN OVEREXPRESSION IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN THE RITUXIMAB ERA: A SINGLE-INSTITUTION EXPERIENCE

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Background: Protein overexpression and rearrangements of myc and bcl-2 genes have been related with an adverse prognosis in diffuse large B-cell lymphoma (DLBCL) patients in the rituximab era. But data are not always consistent and sometimes contradictory.
Aims: To analyze the incidence of MYC and BCL2 protein overexpression in DLBCL as well as to evaluate the prognostic impact in terms of time to progression (TTP) and overall survival (OS).
Methods: We carried out a single institution study with tissue biopsies obtained from patients diagnosed of DLBCL in the period 1994-2011. Tissue fixation and processing were performed using standard methods. Tissue microarrays (TMAs) that contained two representative 2-mm cores from each tumor were prepared. Immunohistochemical stainnings were performed using fully automated protocols. We used the following antibodies: MYC (clone Y69;Roche), BCL2 (clone 124; DAKO). The cut-off level for BCL2 and MYC expression was 50% and 10% respectively. MYC and BCL2 expression were evaluated by an pathologist expert as well as an hematologist. At the same time, MYC and BCL2 expression was evaluated using computerized image analysis with Image Pro Plus 6.0. A mean number of 1000 cells were analyzed per case. TTP and OS curves were built by the Kaplan_Meier method and compared by the log-rank test. Impact of TTP and OS was studied by the Cox regression test. Results: 140 DLBCL patients were included, median age was 70 (23-76), male/female ratio 73/67, Ann Arbor stage III or IV: 73 (52%), high LDH 72 (51%), high β2-microglobulin 62 (44%) low risk IPI 53 pt, int-low 26pt (19%), int-high 31pt (23%), high 28pt (20%) IPI ≥3: 59pt (42.1%). 45 patient were treated with CHOP-like regimens and 95 with rituximab-CHOP schedules. Median follow-up (alive pts) was 49 months(12,135).
Results: We observed MYC overexpression (≥10%) in 101pt (72%), BCL2 overexpression (≥50%) in 71pt (50%) and both in 56pt (40%). We did not observe any correlationship between MYC, BCL2 or both overexpression and clinical-biologic baseline characteristics. Patients with MYC and BCL2 overexpression had a lower complete response rate than the rest: 62% *versus* 76% (p=0.09). Patients with both markers had a worse 5 year TTP: 67% *versus* 83% (p0.09) and also a worse 5 year OS: 60% *versus* 79% (p0.05),. When we restricted the analysis to the rituximab-treated patients, patients with both markers had a worse 5-y TTP and OS than the rest (57% *vs* 73%, p=0.05) and (58% *vs* 85%, p=0.002). In the multivariate analysis including the IPI variable and myc/bcl-2, myc/bcl-2 overexpression showed an independent prognostic value on OS, HR 3,876(1.58, 9.51), p=0.003.
Summary/Conclusions: In our experience the protein overexpression of MYC and BCL-2 had a negative impact on the outcome, specially in the rituximab era with a lower CCR and a significant worse TTP and OS. Further studies on these area are required.

PB1709

DOUBLE - HIT NON- HODGKIN'S LARGE B-CELLS LYMPHOMAS IS MORE FREQUENT THAN WE THINK, BUT IPI SCORE IS NOT THE PREDICTOR GOOD ENOUGH TO RECOGNIZE THIS TYPE OF LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) exhibits various morphologies, immunophenotypes, genetic aberrations, and clinical courses. These features vary across geographic regions, suggesting geographic heterogeneity as a characteristic of this type of lymphoma. DLBCL constitutes 31–34% of all non-Hodgkin's lymphomas in western countries, but in poor countries up to 50%. DLBCL is classified as the lymphoma with different entity in the recently published classification of the World Health Organization (WHO classification). Double-hit lymphomas (DHLs), as currently defined by the World Health Organization classification, are those lymphomas expressing the co-occurrence of *MYC* and *BCL2* or *BCL6* rearrangement as detected by fluorescence *in situ* hybridization (FISH) or standard cytogenetics.
Aims: Is the IPI score good predictor for recognize Double Hit in DLBC lymphomas.
Methods: We studied 62 patients with *de novo* DLBCL over the last six years and investigated the prognostic relevance of BCL-2 and C-MYC rearrangements positivity found by FISH. The prognostic significance of BCL-2 and C-MYC expression was evaluated within the context of DLBCL different subtypes and IPI score, but all treated with R-CHOP/ 21 day. Statistical analysis was performed by *SPSS for Windows (22.0*) and significance of p<0,05.
Results: We studied 62 patients with DLBCL, male 32 and female 30, mean age was 59,76+14,38 year. 58% of patients had high and intermediate high IPI score, whilst 42% of patients had low IPI score. With CS IV and III was 82% and only 18% had CS I and II. Patients with BCL-2 and C- MYC positivity found by FISH (13/62) – double hit, had a bad outcome and median OS was only 14 months. ECOG performance status 0 and 1 had 71% patients. No statistically significant differences in the frequency of BCL-2+between the group with low *vs* high IPI risk, (χ2-test, p=0,492). There was no statistically significant difference in the incidence of C-MYC+between the group of high and low IPI risk, (χ2-test, p=0,426). Intergroup analysis of the group of high *vs* low risk IPI, both positive, both negative or at least one negative gene polymorphism did not show statistically significant difference,(χ2-test, p=0,884).
Summary/Conclusions: Although currently available techniques such as

immunohistochemistry may still be used, whole genomic analytic techniques like FISH will likely play a major role in the evaluation of patients in the future to determine optimal personalized treatment, but FISH is exspensive and IPI score is not good enough to recognize double-hit.

PB1710

EFFICACY OF GEMCITABINE AS SALVAGE THERAPY FOR RELAPSED/ REFRACTORY AGGRESSIVE NON HODGKIN LYMPHOMA PATIENTS

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Background: High-dose therapy with stem-cell support (ASCT) is the standard treatment for relapsed non-hodgkin lymphoma (NHL), but is not feasible for elderly or frail patients. Gemcitabine-based salvage regimens were previously shown to be an effective treatment option with response rates as high as 80% accompanied by a relatively benign toxicity profile. Our local policy is to use these regimens either for frail patients that are not eligible for ASCT, or for relapse post ASCT.
Aims: Since we had the clinical impression that our success rates are lower than the reported, we decided to retrospectively analyze the outcome of NHL patients that were treated with gemcitabine-based regimens in our institute.
Methods: Based on local pharmacy query we annotated 35 patients that were treated with gemcitabine-based regimens between 1/2007-1/2015 for relapsed/refractory aggressive NHL. Clinical features and outcomes were evaluated using thorough medical record review. Statistical analysis was done using Chi square test and Kaplan-Meier method.
Results: The study cohort included 23 males and 12 females. Median age was 67.2 (28-83). Median follow-up from gemcitabine initiation was 8.7 months (0.4-48.7). The most frequently used protocol was gemcitabine-oxaliplatinum (77%, n=27). Eighty percent of patients (n=28) received full dose while 20% (n=7) received reduced dose regimens. Twenty-seven patients had B cell and 8 had T cell lymphoma. All patients received a median of 2 prior regimens (1-5), of which at least 1 was anthracycline-based therapy. Seventeen patients (49%) had relapsed while 18 (51%) had refractory disease. Overall response rate (ORR) was 37%, with 29% (n=10) CR, 8% (n=3) PR, and 63% (n=22) PD or SD. Of the 13 responders, 8 patients (61%) experienced relapse or progression during follow up. Median PFS was 61 days (range 12-1318), median OS was 23.7 months (0.4-48.7). Sixteen patients (45%) deceased during follow up. Grade 3-4 hematological-toxicity was reported in 17 patients (49%). Hospitalization due to treatment induced toxicity occurred in 12 patients (34%). Eight due to infections, 2 due to bleeding, 1 due to trauma and 1 due to abdominal pain. Several factors were tested as predictors of PFS: number of previous treatement regimens, dose intensity (full *vs* reduced), cell of origin, length of prior remission and relapsed *vs* refractory disease. The only predictor for better PFS was relapsed *vs* refractory disease (median 151 *vs* 41 days p=0.017) (Figure 1).

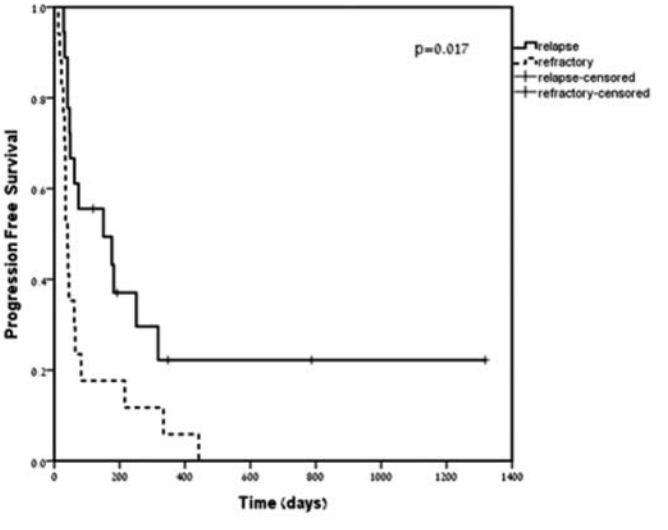

Figure 1.

Summary/Conclusions: compared to previous published data in relapsed/ refractory aggressive NHL, we observed less favorable outcome along with worse toxicity. Although ORR was 37%, the median PFS after gemcitabine-based regimens was dismal (61 days). The only predictor for better PFS was relapsed as oposed to refractoy disease. Notabely, the 4 long term survivors were all treated in first (n=3) or second relapse (n=1) and received full dose regimen. Our single institution results are inferior than the published literature. The admnistration of gemcitabine based therapy as salvage regimen for patients with relasped /refractory NHL was with limited success. Innovative therapies are urgently in need for this devastating patient population as well as randomized studies.

PB1711

EFFICACY OF ESHAP REGIMEN IN THE ABSENCE OF AUTOLOGOUS STEM CELL TRANSPLANTATION IN RELAPSED/REFRACTORY T-CELL LYMPHOMA

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Background: After relapse, prognosis of patients with peripheral T-cell lymphoma (PTCLs) was poor. Currently, there was no standard salvage regimen for PTCLs. ESHAP regimen, consisting of etoposide, methylprednisolone, high-dose Ara-C, and cisplatin, is one of the well accepted regimens for relapsed or refractory (R/R) lymphoma. However, the efficacy of ESHAP have been examined in the cohorts of various subtypes of R/R NHL, not specifically described the outcome in those with PTCLs.
Aims: The purpose of this study was to determine the efficacy and safety of ESHAP as first salvage regimen, not followed by stem cell transplantation (SCT), in R/R PTCLs.
Methods: According to institute's treatment plan, ESHAP was recommended as first salvage regimen for R/R PTCLs. We retrospectively evaluated the efficiency and safety of ESHAP in patients with PTCLs who progressed after one prior therapy and did not undergo salvage SCT. Disease status at first relapse/progression was categorized as early relapse (relapsed within 12 months after the completion of frontline therapy), late relapse (relapsed after 12 months of frontline treatment) and refractory disease (achieving less than partial response after frontline chemotherapy).
Results: From 2004 to 2014, 33 patients with R/R PTCLs received ESHAP as first salvage regimen at Chiang Mai University hospital. Overall response rate was 46% with complete responses (CR) of 39%. Median duration of response was 18 months with the longest duration of 131 months. Patients with PTCL-NOS, the most common subtype treated in this cohort (n=18), had ORR of 50% (CR/CRu 44%). None of the patients with HSTL and EATL type II responded to ESHAP. Reponses were independently influenced by disease status at first relapse/progression (Odd ratio; OR 2.7, 95%CI: 1.46-5.15) and secondary IPI (OR 2.12, 95%CI: 1.06-4.29). Median second progression-free (PFS) and overall survival (OS) were 8.0 and 11.0 months, respectively. Patients having late relapsed disease after frontline treatment had more favorable OS than those having early relapsed or refractory disease with a median OS of 21, 17 and 3 months, respectively (*P*=.001). The corresponding figures for median second PFS were 16, 8 and 2 months, respectively (*P*=.001). Patients achieving CR after ESHAP had significantly better median OS (39, 7 and 5 months, *P*<.0001) and second PFS (33, 2 and 2 months, *P*<.0001) than those achieving PR or having progressive disease. By multivariate analysis, the only factor affected OS was the response to ESHAP (HR 4.7, 95%CI 2.2-10.0, P<.0001) while second PFS was affected by the response to ESHAP (HR 5.9, 95%CI 2.4-14.6, *P*<.0001) and disease status at relapse/progression (HR 3.4, 95%CI1.2-9.7, *P*-value=.025). Grade 3-4 neutropenia (45.5%) and thrombocytopenia (33.4%) were common but manageable. Grade I-II transient rising of serum creatinine was observed in 36.4% of patients.
Summary/Conclusions: ESHAP has acceptable efficacy and toxicity for R/R PTCLs. It offers a long-term survival in patients with chemosensitive relapse who are not suitable for SCT.

PB1712

PRALATREXATE IN COMBINATION WITH BORTEZOMIB FOR RELAPSED OR REFRACTORY PERIPHERAL T CELL LYMPHOMA IN ELDERLY PATIENTS: A PILOT TRIAL

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Background: Peripheral T cell lymphoma (PTCL) is a heterogeneous group of aggressive lymphoma with poor prognosis and there is no efficacious standard regimen for the relapsed or refractory disease. Particularly, elderly patients generally have impaired bone marrow function, altered drug metabolism, comorbidities, and poor functional status. Therefore, the treatment of elderly patients with relapsed or refractory PTCL remains a challenge for clinicians.
Aims: A recent report demonstrated that the combination of pralatrexate and bortezomib enhanced efficacy compared with that of either agent alone *in vitro* and *in vivo* models of T cell malignancy. Hence, we performed a pilot study that evaluated the response rate, duration of response and safety profile of this combination regimen in elderly patients with relapsed or refractory PTCL.
Methods: Five elderly patients (age ≥65 years) with relapsed or refractory PTCLs, who progressed following one or more prior chemotherapy, were investigated. The histological subtypes were consisted of 2 PTCL-NOS, 2 AITL, and 1 NK/T cell. Two patients were previously treated with methotrexate-containing chemotherapy. Pralatrexate (15 mg/m^2) and bortezomib (1.3mg/m^2) were administered intravenously on day 1, 8 and 15 and repeated every 28 days for 4 cycles. Concomitant medications were included that vitamin B12 (1 mg) was

injected within 14 days before pralatrexate infusion and oral 45mg of leucovorin was taken during entire days of chemotherapy. The interim and final response were assessed according to the revised International Workshop Criteria (IWC).
Results: All 5 patients are men with median age of 71 years (range: 67 – 74). The number of prior systemic therapies ranges 1 to 5. Of 5 patients, two achieved partial response (PR) after 2 cycles and one achieved complete response (CR) after 4 cycles which have lasted 8 months until now. Another patient with interim PR progressed after 3 cycles. Two responders had no history of prior methotrexate-containing treatment and histologically all PTCL-NOS. Three other patients progressed after one or 2 cycles. The most common adverse events were mucositis and anorexia. Four patients complained of mucositis, but only 1 week delay of treatment was required for grade 3 mucositis in one patient. Generally most patients tolerated combination therapy. All patients were treated with planned pralatrexate without dose reduction or discontinuation due to toxicity. Only one dose of bortezomib was omitted due to peripheral neuropathy among all patients and one episode of herpes zoster occurred. None of the patients postponed treatment due to hematologic adverse events.
Summary/Conclusions: Combination therapy with pralatrexate and bortezomib could be used effectively and safely as a salvage therapy for relapsed or refractory PTCL in elderly patients. Larger studies are needed for evaluating benefit of combination therapy over single pralatrexate therapy.

PB1713

TREATMENT OUTCOME OF DIFFUSE LARGE B CELL LYMPHOMA IN RITUXIMAB ERA AND THE PROGNOSTIC VALUE OF R-IPI SCORE AT A CANCER CENTER IN TAIWAN: SINGLE INSTITUTION EXPERIENCE
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Background: The treatment outcome of diffuse large B cell lymphoma showed major improvement in rituximab era. The predictor of outcome is still under investigation.
Aims: To review and analyze the treatment experience of newly diagnosed diffuse large B cell lymphoma in rituximab era in our institution.
Methods: We retrospectively reviewed medical records from Jan 2005 to Dec 2014 from our institution. We figured out patients with newly diagnosed diffuse large B cell lymphoma. Clinical characteristics, treatment response, treatment modality, and survival were analyzed.

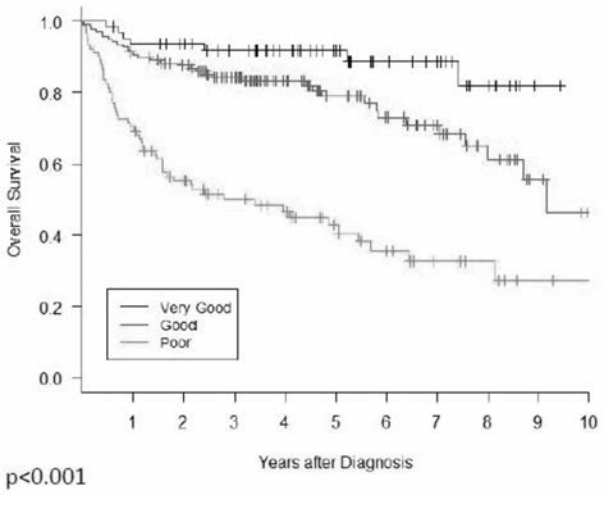

Figure 1. 2005-2014 overall survival of DLBCL by R_IPI.

Results: From Jan 2005 to Dec 2014, there were 292 newly diagnosed diffuse large B cell lymphoma in our institution. Median age was 57 years old. 50.7% of patients had advanced stage (stage III&IV), and 7.9% had poor performance status(ECOG2-4). According to revised International Prognostic Index score(R-IPI score), 21.6% had very good prognosis (score 0), 47.2% had good prognosis (score 1 or 2), and 31.2% had poor prognosis(score 3,4 or 5). According to primary site,62.3% had lymphadenopathy, 8.2% had mediastinum disease, 16.1% had gastrointestinal disease. Ki67 was available in 59.9% of patients. According to cell origin, germinal center B-cell-like(GCB) was 11.7% and non-GCB was 9.9%.78.4% of patients did not perform the test. HBsAg was positive in 25.3% of patients. 81.3% of patients were treated with RCHOP-like regimen. Complete remission(CR) was achieved in 79.4% of patients. 3-year event free survival(EFS) and overall survival(OS) were 70% and 75.3%, respectively. 5-year event free survival(EFS) and overall survival(OS) were 64.9% and 70%, respectively. The 5-year OS was 92.7%,77.5%,66%,47.3% in stageI/II/III/IV, respectively(p<0.001). The 5-year OS was 91.8%, 78.9%,42.8% in R-IPI very good group, good group and poor group, respectively(p<0.001). CR, treated with R-CHOP-like regimen, R-IPI score predicted the outcome of the patient. Primary site, Ki67, GCB were not associated with outcome in our cohort. Though Taiwan is in HBV endemic area, HsAg was not associated with outcome in our cohort (Figure 1).
Summary/Conclusions: Diffuse large B cell lymphoma has good prognosis for majority of patients in rituximab era. R-IPI score has significant prognostic value in Asian population. However, for patients with higher stage and higher IPI score, further investigation to improve outcome is still required.

PB1714

TREATMENT PECULIARITIES OF NON-HODGKIN'S LYMPHOMAS IN CHILDREN WITH NIJMEGEN BREAKAGE SYNDROME
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Background: Nijmegen breakage syndrome (NBS) is rare autosomal recessive disorder, typically characterized by increased chromosome fragility phenomenon, immune deficiency, hypersensitivity to radiation and high predisposition to malignancy.
Aims: To investigate the effectiveness of cytostatic treatment of non-Hodgkin lymphomas (NHL) in children with NBS.
Methods: 1992 through 2014 one hundred children in the Hematology Department of Lviv Children hospital were first diagnosed with, 9 (9%) out them with NBS. The median age of this children group was 8 years and 8 months (from 4 years 4 months to 14 years 11 months old). Male to female ratio was 3:2. The diagnosis of NBS was based on specific phenotype features, results of clinical, genetic (657del5 *NBN* gene mutation) and laboratory investigations. The diagnosis of NHL was based on clinical symptoms, morphological and immunological analysis of tumor substrate. Patients received treatment according to BFM-group protocols (NHL-BFM-90/95, NHL-DGLLU-2000).
Results: The NHL types were established as follows: lymphoblastic lymphoma (LBL) in 3 patients, diffuse large B-cell lymphoma (DLBCL) in 6 patients. In most cases the primary manifestations were nodal: peripheral lymph nodes - in 9 patients, mediastinal – in 7, abdominal lymph nodes - in 3, and spleen – in 7 children. Involved extranodal sites included liver (6 patients), lung and pleura (5), skull bones (2), kidney (1), CNS (1). Atypical cells (L1/L2 and L3-type lymphoblasts according to FAB-classification) in the bone marrow were found in 2 patients with LBL, 1 – with DLBCL. Cytostatic treatment was initiated in 8 patients. One patient was in the terminal state and died before the specific treatment. Three patients died upon the completion of first chemotherapy cycles, due to serious infectious complications (pneumonia, pneumothorax, enteropathy, necrotic stomatitis, palatal fistula). In a 4 year old boy with DLBCL, after 2 cycles, the tumor didn't go into regression. The salvage therapy (ICE+rituximab) was not effective; the child died of lymphoma progression. In 4 patients the cytostatic treatment was successful. Three children with LBL received a full course of protocol treatment without reducing the doses of cytostatic and are in remission at present (duration of remission in months – 215, 39, 30). In one girl with DLBCL the treatment was discontinued after the 5th cycle (AA N2) of NHL-DGLLU-2000 protocol, because of coccyx area soft tissue necrosis, thus the last BB cycle was not conducted. After 11 years of remission in this patient a secondary tumor (meningioma) was diagnosed, her treatment with the CyberKnife radiosurgery was effective.
Summary/Conclusions: The complexity of the clinical course of NHL in NBS patients was preconditioned by the presence of concomitant infections on the background of combined immunodeficiency, which caused death of 4 patients at the beginning of the treatment. One patient died from lymphoma progression. Specific NHL treatment in NBS patients is possible and effective, given the individual correction of doses and schedules of applied cytostatic agents, and if adequate supportive therapy is provided concomitantly. Nonetheless, after successful NHL treatment in children with NBS, the risks of secondary tumor development still run high.

PB1715

Abstract withdrawn.

PB1716

VARIATION IN R-CHOP REGIMENS FOR PATIENTS WITH STAGE II-IV DIFFUSE LARGE B-CELL LYMPHOMA IN THE NETHERLANDS
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Background: The 2014 Dutch national clinical practice guideline for diffuse

large B-cell lymphoma (DLBCL) recommends 8x R-CHOP21 or 6x R-CHOP14 followed by 2 additional cycles of rituximab (2R) as first line therapy for stage II-IV DLBCL patients with an age-adjusted International Prognostic Index (aaIPI) of 1-3. However, international guidelines tend to promote 6x R-CHOP21 as the preferred first line treatment.
Aims: The aim of this population-based study was to evaluate which treatment regimens were applied in the Netherlands.
Methods: We selected 840 patients diagnosed in 2014 with stage II-IV DLBCL and aaIPI 1-3 (median [range] age 69 years [18-99]; 55% male) from the nationwide population-based Netherlands Cancer Registry. All patients were treated outside clinical trials. In this study, first line treatment was defined as 6x or 8x R-CHOP14/21, and, in case of 6x R-CHOP14/21, with or without 2R. Overall response rate (ORR) was defined as achieving complete or partial response, *i.e.* CR(u) or PR. Data were analyzed for the total cohort, as well as stratified by age and treatment regimen.
Results: Treatment with R-CHOP14/21 was initiated in 651 (78%) patients, while 103 (12%) patients received other (chemo)therapeutic regimens. The remaining 86 (10%) patients received no therapy. Of those who received R-CHOP14/21, 508 (78%) completed 6x or 8x, 122 (19%) received less than 6x, and 21 (3%) received 7x. Of the patients who completed treatment (6x+/- 2R or 8x), 6x R-CHOP21, 6x R-CHOP21+2R and 8x R-CHOP21 regimens were provided in 21%, 20% and 45% of patients, while 6x R-CHOP14, 6x R-CHOP14+2R and 8x R-CHOP14 in 3%, 3% and 8% of patients, respectively. Of note, 8x R-CHOP14/21 was most commonly applied (53%; N=269). Patients aged <65 years (N=233) more often received 8x (82%), whereas patients aged ≥65 years more often received 6x (72%). As for patients aged 65-74 years (N=160), 39 (24%) patients received 6x R-CHOP21, 49 (31%) 6x R-CHOP21+2R and 55 (34%) 8x R-CHOP21. Only 17 (11%) patients received 6x R-CHOP14+/- 2R or 8x R-CHOP14. For patients aged ≥75 years (N=115), all but one received R-CHOP21 regimens, of which 43% received 6x, 39% 6x with 2R and 17% 8x. The ORR was 88% (448/508) for patients who completed treatment. Further, the ORR was 87% for 8x R-CHOP21, 83% for 6x R-CHOP21 and 94% for 6x R-CHOP14+2R and this was irrespective of age group.
Summary/Conclusions: Our results show that treatment with R-CHOP was initiated in 78% of patients with stage II-IV DLBCL and an aaIPI of 1-3. Three-weekly regimens were applied to 85% of patients who completed treatment. Although based on modest patient numbers, the ORR for 6x R-CHOP21, which is the internationally preferred R-CHOP regimen, was 83%. That value compares somewhat lower to the ORRs in the 8x R-CHOP21 and 6x R-CHOP14+2R groups, *i.e.* 87% and 94%, respectively. Collectively, these population-based analyses demonstrate that there is variation in the provision of R-CHOP regimens in daily practice, which could be related to the ongoing debate among clinicians in the Netherlands whether 6x or 8x R-CHOP21 should be the standard of care as first line therapy for stage II-IV DLBCL patients with an aaIPI of 1-3.

PB1717

DIFFERENT IMMUNOCHEMOTHERAPY REGIMENS FOR PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY ON 86 PREVIOUSLY UNTREATED PATIENTS

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Background: Primary mediastinal large B-cell lymphoma (PMBCL) arising from thymic medulla B cell, occurring more often in young females and characterized by a locally invasive anterior mediastinal bulky mass that often causes cough, dyspnea and a superior vena cava syndrome. Previous retrospective studies have suggested that it may respond better to more intensive chemotherapy regimens than to standard CHOP. The prognostic role of PET/CT scan and the feasibility of consolidation with radiotherapy (RT) on residual mediastinal mass still remain unclear.
Aims: To assess the impact of different immunochemotherapy regimens on survival outcomes in newly diagnosed patients with PMBCL.
Methods: We identified 86 pts with PMBCL treated in N.N. Blokhin Russian Cancer Research Center during last 10 years (2004-2014). The median age was 29 years (15-63), 56% were female, 62% had I/IIE stages, LDH elevated in 81% pts. Patients received three different regimens chemotherapy (CT): MACOP-B+R- 52 pts (group 1), R-CHOP -18 pts (group 2), R-EPOCH - 16 pts (group 3). After completion of CT, PET/CT scan was performed on 56 of 86 pts and in 20 of 56 (36%) it was abnormal. Radiation therapy to the mediastinum received 69 pts (80%).
Results: With a median follow 34 months, progression-free survival (PFS) was 80%, overall survival (OS) - 86%. There is no significant differences in PFS and OS depending on the CT regimens (p=0.1). It should be noted that none of the 16 patients treated on EPOCH-R, had relapse or disease progression. 4-year PFS was 91% in patients who were PET negative before RT regardless of the CH regimens,
Summary/Conclusions: Immunochemotherapy combined with local irradiation remains the standard treatment for PMBCL. Perhaps, more number of patients will be able to demonstrate significant benefit one chemotherapy regimens over the other and define the role of consolidation RT in patients with PET-negative mediastinal mass after standard chemoimmunotherapy.

PB1718

TREATMENT OF HIGH RISK AGGRESSIVE B CELL LYMPHOMAS WITH DA EPOCH R– A RETROSPECTIVE ANALYSIS

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Background: Promising results in patients suffering from high risk DLBCL, Burkitt`s lymphoma (BL), gray-zone lymphoma (GZL) and primary mediastinal B cell lymphoma (PMBCL) treated with DA-EPOCH R have been reported.
Aims: To evaluate retrospective toxicity and efficacy in DA EPOCH R treated patients.
Methods: In our centres high risk DLBCL - defined as double-hit/double hit score 2 or high/ high-intermediate risk NCCN IPI - BL, MGZL and PMCL are treated with DA EPOCH R. Retrospective analysis of toxicity and efficacy in DA EPOCH R treated patients.
Results: So far 39 previously untreated patients with a median age of 54a (28a – 76a) have been treated with a total of 190 cycles of DA EPOCH R: 16 DLBCL, 9 GZL, 8 PMBCL, 6 BL. 37 Patients have finished treatment, 2 are stilll on treatment. Targeted ANC <500/l occurred in 46%, thrombocytopenia <25.000/l in 20% and anemia <8g/dl in 12,5% of all cycles. Dose escalation was possible in 27 (73%) patients – but only in 5 (38%) of 13 patients >65a. 15 (63%) of 24 patients aged <65a received at least dose level 3 (144% increased dose of Etoposide, Doxorubicin, Cyclophosphamide). Due to peripheral sensory neuropathy, Vincristine had to be dose reduced in 52% of all cycles. Other CTCAE grade III/IV non-hematopoietic toxicities were infrequent and manageable. After a median follow up of 10 month (range: 1-25) overall survival (OS) rate is 74%. 2 patients in PR were (1 PMBCL, 1 GZL) bridged to allogenic stem cell transplantation, 1 patient in CR had to be switched to a less toxic regimen due to repeated febrile neutropenia after 3 cycles of. In 18 high risk DLBCL patients (8 DHS2/DHL, 8 high/high intermediate NCCN IPI) OS is 80% after a median follow up of 12 month. From 8 (4GZL, 2 DLBCL, 1 BL, 1 PMBCL) relapsed/ refractory patients 7 died. Causes of death were: 2 infectious complications (1 DLBCL HIV associated, 1 GZL) and 5 progressive disease
Summary/Conclusions: Although still preliminary data DA EPOCH R seems to be a feasible treatment with acceptable toxicity and a promising response rate. Dose escalation is age dependent. Especially in patients with high risk DLBCL DA EPOCH R is an alternative to insufficient induction therapy with R CHOP.

PB1719

SINGLE-HIT LYMPHOMA PATIENTS HAD MORE PRONOUNCED BENEFIT FROM DOSE-INTENSIFIED CHEMOTHERAPY THAN DOUBLE-HIT LYMPHOMA PATIENTS. SINGLE-CENTER EXPERIENCE.

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Background: B-cell lymphomas with *c-MYC* rearrangement are characterized by aggressive clinical behavior and poor prognosis when R-CHOP-21 is used. An efficacy of intensified chemotherapeutic regimens continues to be debatable.
Aims: To represent treatment results of *MYC*+agressive B-cell lymphomas with dose-intensified chemotherapy.
Methods: Since 2007 till 2015 years in National Research Center for Hamatology 23 cases of *c-MYC* rearranged non-Burkitt B-cell lymphomas (5 DLBCL and 18 BCLU intermediate between DLBCL and BL) were diagnosed. Ratio between men and women was 9:14. Median age was 51 years old (30-70). ECOG ≥2 was in 18 (78%) cases; 19 (83%) of pts had III-IV stage according Ann-Arbor classification; 17 (74%) had more than one extranodal site: 7 (30%) - bone marrow involvement, 2 (9%) - CNS; 18 (78%) pts had IPI 3-5. In 15 cases *c-MYC* translocation partner was locus of *IGH* gene, in 4 cases – locus of *IGL* or *IGK* gene, in 4 cases – undefined. In 8 cases with available karyotype were revealed complex abnormalities. *MYC+/BCL2*+double-hit lymphoma (DHL) was in 6 cases, *MYC+/BCL6*+DHL was in 3 pts, others 14 pts had single-hit lymphoma (SHL). In all cases dose-intensified treatment strategy was initially chosen: in 4 cases - R-DA-EPOCH and in 19 cases – protocol BL-M-04 consisted of A-C-A-C 4-6 courses (Course A: dexa 10 mg/m^2 1-5 days; Mtx 1500 mg/m^2 1st day12-hours infusion; ifosfamide 800 mg/m^2 1-5 days; vincristine 1 mg/m^2 1st day; doxorubicine 50 mg/m^{2}3rd day; AraC 150 mg/m^2 4-5 days bid; etoposide 100 mg/m^2 4-5 days, iv. Course C: dexa 10 mg/m^2, 1-5 days; Mtx 1500 mg/m^{2}1st day12-hours infusion; vinblastine 5 mg/m^2 1st day; AraC 2000 mg/m^{2}2-3 days bid; etoposide 150 mg/m^{2}3-5 days, iv. AraC 30 mg,

Mtx 15 mg, dexa 4 mg 1st day of each course, intrathecally). Rituximab was indicated in 12/19 cases. AutoSCT was performed in 5 cases. Kaplan-Meier method and log-rank test were used to estimate treatment results (SAS 9.3).
Results: 9/23 (39%) pts had a complete remission, 6/23 (26%) pts had a partial remission, 7/23 (30%) pts had disease progression (in one case after autoSCT), one pt (4%) died from infectious. In one case (4%) of DLBCL early relapse had occurred. 2-year overall survival (OS) and event-free survival (EFS) were 51% (median - 9,0 months (0,6-89,1)) and 40% (median 7,3 months (0,1-89,1)), respectively. DHL pts had worse treatment results compared with SHL pts: 2-year OS was 30% (median – 8,2 months (0,6-54,1)) *vs* 64% (18,6 months (1,1-89,1)) in DHL *vs* SHL groups, respectively (p=0,06) (Figure 1). 2-year EFS was 13% (median – 3,5 months (0,3-21,4)) *vs* 56% (median 17,4 months (0,1-89,1)) in DHL *vs* SHL groups, respectively (p=0,03) (Figure 1).

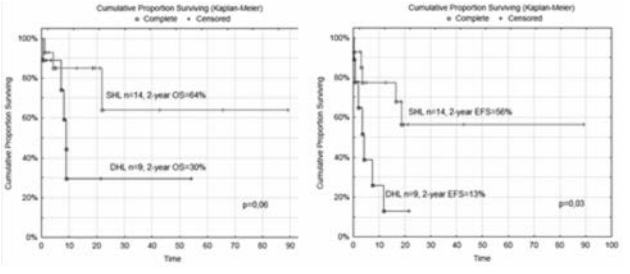

Figure 1.

Summary/Conclusions: Single-hit lymphoma patients have more pronounced benefit from dose intensification compared with miserly results in double-hit lymphoma patients.

PB1720

DICEP IS AN EFFECTIVE CHEMOTHERAPEUTIC REGIMEN FOR PATIENTS WITH RELAPSED OR REFRACTORY LYMPHOMAS WHO FAILED PRIOR SALVAGE CHEMOTHERAPIES

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Background: The outcome of patients (pts) with relapsed or refractory Hodgkin's (HL) and Non Hodgkin lymphomas (NHL) post failure of response to 1st salvage treatment tends to be very poor even after autologous stem cell transplantation (ASCT). Though several 2nd line salvage regimens have been used, the optimal treatment still remains an issue of clinical investigation and there is an unmet need for salvage chemotherapies, sufficiently strong to reduce the tumor burden with acceptable toxicities to allow safe yet successful ASCT.
Aims: We herein evaluated the DICEP regimen [Dose Intesified Cyclophoshamide (1,750 gr/m^2), Etoposide (350 mg/m^2) and Cisplatin (35 mg/m^2), days 1-3] in terms of safety and efficacy regarding disease response and stem cell mobilization and collection.
Methods: We retrospectively analyzed the data of 21 (10 HL, 11 NHL) pts with a median age of 26 (16-60) yrs who had refractory or relapsed disease after ABVD or R-CHOP regimens (6-8 cycles) and received as 1st line salvage chemotherapy at least 2 cycles of ESHAP (13 pts), ICE (3 pts), ESHAP+ICE (2 pts) and other regimens (3 pts). Two out of 21 achieved complete remission (CR) post 1st salvage, 17 partial remission (>50% response, PR) while 2 had stable or progressive disease. A single cycle of DICEP was administered either as mobilization chemotherapy (in 2 pts with CR), or as 2nd salvage treatment for patients with PR, stable or progressive disease.
Results: DICEP was well tolerated and no lethal or major organ toxicities were observed. Nevertheless, all patients experienced hematological toxicity (gr 3-4 WHO) and 18/21 developed febrlile neutropenia (3/18 experienced septicemia), however that completely resolved with the appropriate antibiotic therapy not requiring admission to intensive care unit. The hospitalization period was 20 (11-25) days. All patients were successfully mobilized and a medium of 7,9 (2,8 -33,5)x10^6/kg CD34+cells were collected after a median of 1 (1-3) days of apheresis procedure. Post DICEP administration, a further overall disease improvement of 42% was observed (6 additional CRs and 2 PRs), whilst in 1 pt the disease proved to be refractory. Finally 20/21 pts underwent ASCT. The 3,5yrs overall survival (OS) and progression free survival (PFS) from DICEP administration were 50% for the whole cohort of patients. Specifically, for HD pts the 2 yrs OS and PFS were estimated to be 75% and 65% respectively, while for NHL the 3,5 yrs OS and PFS were 45% and 50% respectively.
Summary/Conclusions: Taking into account the limitations of the retrospective nature of the present study, it seems that DICEP regimen is a sufficient salvage treatment demonstrating acceptable toxicity without negatively affecting the collection process. The promising overall response rates post DICEP in combination with the encouraging OS and PFS rates achieved post ASCT in that heavily pretreated group of patients, strongly support the rationale to use DICEP regimen not only as a "bridge" to a successful ASCT but also as 1st line salvage regimen in selected pts.

PB1721

THE COMPARISON OF THE PROGNOSIS SCORING SYSTEMS BETWEEN EVENT-FREE SURVIVAL AT FIRST 24 MONTHS AND OVERALL SURVIVAL IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL). R-CHOP (Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) is curative in the majority of DLBCL patients. However, about one-third of patients will have refractory disease or relapse following the therapy. Therefore, risk stratification at diagnosis for patients with DLBCL is of great clinical interest. Recently, it is shown that event-free survival (EFS) at first 24 months from the time of diagnosis in patients who received rituximab-based chemotherapy onwards the similar survival rates with healthy patients in their own age group and gender. Recently, the scoring system for prognosis called IPI24 is effective to predict the first 24-month EFS (EFS24) in DLBCL patients.
Aims: In this study, we aimed to compare the risk stratification anticipated by IPI24, IPI and NCCN-IPI for patients treated with RCHOP in the first EFS24 and overall survival in Trakya University Medical Faculty Hematology department and identify the prognosis scoring system which makes best risk stratification.

Table 1.

A Event free survival at first 24 months, Cox regression analysis

	P	HR	95%CI	
			Lower	Upper
IPI	0.135	0.715	0.461	1.108
IPI24	0.034	1.038	1.003	1.074
NCCN-IPI	<0.001	6.070	3.026	12.175

B Overall survival at first 24 months, Cox regression analysis

	P	HR	95%CI	
			Lower	Upper
IPI	0.143	0.709	0.448	1.123
IPI24	0.082	1.034	0.996	1.074
NCCN-IPI	<0.001	7.403	3.368	16.272

C Overall event free survival, Cox regression analysis

	P	HR	95%CI	
			Lower	Upper
IPI	0.148	0.725	0.470	1.118
IPI24	0.064	1.032	0.998	1.067
NCCN-IPI	<0.001	6.039	3.043	11.983

D Overall survival, Cox regression analysis

	P	HR	95%CI	
			Lower	Upper
IPI	0.132	0.718	0.468	1.130
IPI24	0.136	1.028	0.991	1.067
NCCN-IPI	<0.001	7.319	3.387	15.797

Methods: Clinical and laboratory data of 284 patients who have been diagnosed with NHL in our center since 2004 were retrospectively analyzed. The patients who were under the age of 50 and over the age of 85 and also receiving chemotherapy except for R-CHOP, apart from DLBCL were subtracted from the study and 103 patients (57 M, 46 F) were enrolled in the study. Clinical and laboratory data of the patients were obtained by scanning files. According to IPI and NCCN-IPI, patients were analyzed depending on 4 risk groups (low, low-intermediate, high-intermediate, high) which are created on using age, LDH, ECOG score, extranodal organ involvement and Ann Arbor stage. But the risk of events on the first 24 months calculated by IPI24 were calculated with the QX

calculator using age, sex, absolute lymphocyte count, bulky lymph node masses (>10cm), LDH, ECOG score and Ann Arbor stage. Data were analyzed with SPSS statistical software. Whether the prognostic scores were independent indicators or not in terms of survival and event-free survival was examined using Cox regression analysis. P<0.05 was considered significantly.
Results: The mean age of 103 patients in our study was 60±9 years and in%48 of them had extranodal disease,%64 of the patients had advanced disease,%24 of the patients had solid disease. In%37 of the patients ECOG score was ≥2.%38 of the cases were dead. NCCN-IPI was the most successful prognostic model to predict EFS24 in the patients (p<0.001). While IPI24 was statistically significant to predict EFS24 (p=0.03), IPI was not sufficient (p=0.13) (Table 1A). While the best prognostic model was NCCN-IPI to predict the overall survival in 24 months (p<0.001), IPI and IPI24 were insufficient (with order p=0.08, p=0.7) (Table 1B). To predict the overall EFS and overall survival in the patients, the best prognostic model was NCCN-IPI (p<0.001), but IPI and IPI24 were insufficient (p>0.05) (with order Table 1C-D).
Summary/Conclusions: In DLBCL patients before treatment, while NCCN-IPI was superior to IPI 24 as a prognostic scoring system to predict EFS24, IPI was insufficient. Similarly to predict overall survival, NCCN-IPI was the best prognostic model. Due to the mean age of our patients was high and the number of the patients was not enough IPI24 could be insufficient when compared to NCCN-IPI to predict EFS24 and overall survival. It would be useful to do more studies to show the effectiveness of IPI24 for determining the risk factors before the treatment on DLBCL patients.

PB1722

EFFICACY OF SPLENECTOMY IN DIFFUSE LARGE CELL LYMPHOMA
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Background: Diffuse large cell lymphomas (DLCL) are often associated with splenomegaly. Large-size spleen causes both abdominal discomfort, cytopenia, regional portal hypertension and a decreased efficacy of chemotherapy. This is why splenectomy is indicated in such cases.
Aims: To retrospectively analyze the efficacy of splenectomy in patients with diffuse large B-cell lymphoma (DLBCL) and diffuse large T-cell lymphoma (DLTCL).
Methods: 43 patients with DLBCL and two patients with DLTCL underwent splenectomy.
Results: Splenectomy was performed in 25 male and 18 female patients aged 25-76 years with DLBCL. Indications for splenectomy included splenomegaly (in all patients), hyperslenism (anemia, leukopenia, thrombocytopenia) – in 25 patients (58.1%), regional portal hypertension – in 20 (46.5%) patients; inefficacy of chemotherapy – in 18 (41.9%) patients, and concomitant autoimmune hemolytic anemia (warm type) – in two patients. Splenectomy proved effective in 40 (93%) patients with DLBCL: abdominal syndrome, anemia and leukopenia were reverted, hemolysis in AIHA relieved, and the platelet count was normalized in patients with concomitant thrombocytopenia. The following complications were observed postoperatively: bilateral pneumonia (1 patient), chronic adrenal insufficiency (3 patients), acute thrombophlebitis of superficial veins of the right leg (1 patient), acute pancreatitis (4 patients), and thrombosis of residual limb of the splenic vein (3 patients). One patient died immediately following splenectomy. This lethal outcome was caused by acute cardiovascular insufficiency, which developed within 4 days after operation. Splenectomy did not prove effective in patients with DLBCL. The surgery did not result in normalization of concomitant cytopenia in one female patient who died within 1 month after splenectomy due to both progression of illness and chronic adrenal insufficiency. Another female patient achieved a short-term recovery following surgery (hemoglobin level as well as leukocyte and platelet count normalized) but she developed a cytopenia after 30 days and passed away due to a rapid deterioration of illness and multi-organ insufficiency. As shown by a retrospective analysis of long-term results of splenectomy in patients with DLBCL, the overall survival post splenectomy reached 63.9 months, and the mean treatment-free survival reached 36.8 months. 11 patients did not require administration of chemotherapy following splenectomy at all. 17 patients with DLBCL (39.5%) developed a relapse of illness at different timepoints after surgery. Splenectomy was performed in two patients with DLTCL. In both cases, surgical treatment proved ineffective, and the patients died within 1 month following intervention due to a relapse of cytopenia, rapid deterioration of illness and multi-organ insufficiency.
Summary/Conclusions: Splenectomy remains to be an effective treatment option in non-Hodgkin lymphomas. In DLBCL, the surgery helps relieve both abdominal discomfort and hypersplenism; symptoms of regional portal hypertension alleviate; there is less or no need in administration of chemotherapy; and there is no more hemolysis in concomitant AIHA. In DLTCL, splenectomy is ineffective and should be thus avoided.

PB1723

DONOR-TRANSMITTED TRIPLE-HIT LYMPHOMA IN A RENAL ALLOGRAFT RECIPIENT
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Background: Donor cancer transmission is a rare long-term complication of kidney transplantation that carries an estimated risk of approximately 0.05%. Diagnosis and management of these malignancies are difficult because of their rarity and the need for an individualized approach to treatment. CM is a 30-years-old man with a medical history significant for chronic kidney disease who underwent kidney transplantation in May 2015 with his mother as the donor. In July 2015, the donor presented with an abdominal mass diagnosed as a triple-hit lymphoma (THL), a subset of highly aggressive B-cell lymphomas characterized by the overexpression of MYC, BCL2 and BCL6. Initial staging with PET/CT and bone marrow biopsy showed widespread disease with no marrow involvement, while circulating tumor DNA (ctDNA) assay confirmed a clonal B-cell proliferation. Analysis of ctDNA was performed on recipient's plasma, but ctDNA levels were undetectable. In September 2015, CM developed a perigraft mass that was identified as a THL as well. The peculiar clinical presentation and the histochemical similarities of the two THLs raised the suspicion that the same lymphoma had been transmitted from the donor to the recipient during the transplant procedure.
Aims: This case describes donor transmission of THL with kidney transplantation and points out difficulties in diagnosis and management.
Methods: Lymphoma biopsy specimens from both donor and recipient were obtained and prepared for histological and immunohistochemical (IHC) studies and stained for the identification of Ki67, CD20, BCL6, BCL2, MYC, CD10, MUM1/IRF4, CD5, cyclin D1, CD3, EBV-LMP1 and TdT. DNA was extracted from the biopsies of both donor and recipient THL with standard methods. Samples were assessed for nine microsatellite loci and a segment of the X-Y homologous gene amelogenin by PCR with use of a kit for chimerism determination. Fluorescent in-situ hybridization (FISH) studies were performed on specimen sections using probes for sex determination. B-cell clonality analyses targeting the IGH gene for rearrangements were conducted on ctDNA obtained from plasma of both donor and recipient and from the recipient's bone marrow.
Results: Histology and IHC showed identical findings in both donor and recipient. In particular, MYC, BCL2 and BCL6 were positive and the proliferative index was more than 90%. FISH recognized an XX pattern in both samples. Microchimerism analysis pointed out that the donor and the recipient biopsies had identical profiles considering discriminant alleles and amelogenin (see Figure 1). Moreover, the recipient sample profile was significantly different from his basal allelic profile obtained from peripheral lymphocytes at the time of the diagnosis. B-cell clonality in the donor sample was detected at the time of the diagnosis, however ctDNA levels assessed on recipient's sample were undetectable. The same clonal band was persistently detected by ctDNA analysis of the recipient plasma and bone marrow only after September 2015.

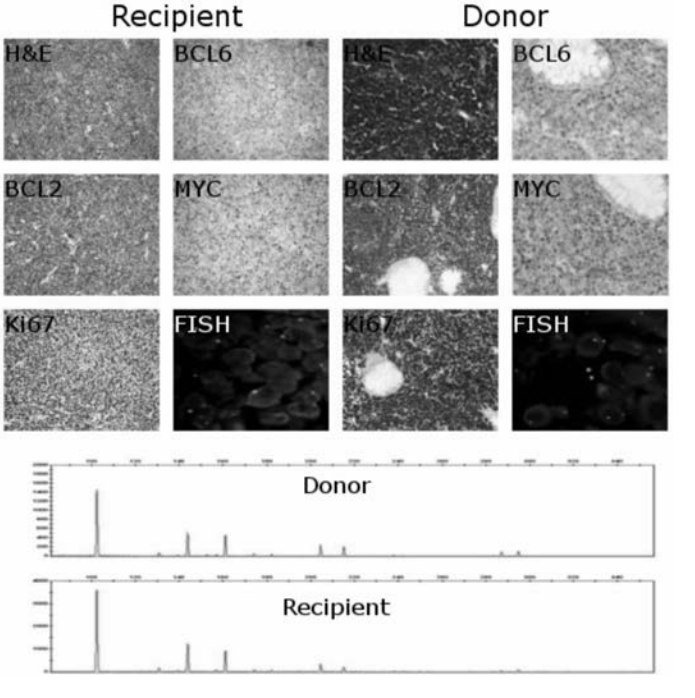

Figure 1.

Summary/Conclusions: We describe the transmission of a highly aggressive B-cell non-Hodgkin lymphoma by kidney transplantation. The THL was transplanted along with the renal graft, while immunosuppressive therapy and immunological impairment enabled its growth and expansion. The patient went on to complete the full course of 2 cycles of R CODOXM/IVAC and associated withdrawal of immunosuppression achieving a complete remission and preserving graft function. The donor underwent the same intensive regimen and achieved a complete response as well.

PB1724

TREATMENT OF C-MYC/BCL2 DOUBLE EXPRESSER DLBCL WITH R-CHOP CHEMOIMMUNOTHERAPY: AN AUSTRALIAN SINGLE CENTRE EXPERIENCE

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Background: Co-expression of c-myc and BCL2 in Diffuse Large B-cell Lymphoma (DLBCL), (increasingly known as 'double expresser' DLBCL), is associated with inferior outcome with standard-of-care R-CHOP immunochemotherapy.
Aims: To retrospectively identify patients with 'double expresser' immunophenotype within our patient cohort of DLBCL, and to analyze their outcomes following treatment.
Methods: Chart review of patients with DLBCL treated with RCHOP chemoimmunotherapy between 2007 and 2014 at our institution. We retrospectively performed immunohistochemical staining for c-myc and BCL2 on pre-treatment tumor tissue blocks to identify patients with 'double expresser' immunophenotype. Other prognostic factors including the revised international prognostic index (R-IPI) score, initial bulky disease (>7.5cm) and cell of origin were also recorded and analyzed. Progression-free survival (PFS) were analyzed using Kaplan–Meier curves and statistically tested with the Gehan-Breslow-Wilcoxon test. 2-tailed chi-square test was used to compare incidence of various prognostic factors in subgroup analysis.
Results: 89 patients were included in the study. Patients with high risk R-IPI score, bulky disease, and elevated serum lactate dehydrogenase level (LDH) showed reduced progression-free survival (p=0.03, p=0.04, p=0.002 respectively). In particular, LDH above 2 times of upper limit of normal (ULN) was associated with a 56% reduction in PFS. There was no significant difference in PFS between 'germinal center' and 'non-germinal center' cell of origin subtypes of DLBCL. 34 patients were found to have 'double expresser' immunophenotype. They showed a higher rate of disease progression (32% *vs* 16%, p=0.03), than the remaining DLBCL patients, with progression occurring predominantly within 24 months of treatment. The 'double expresser' subgroup also had a higher incidence of bulky disease (50% *vs* 13%, p=0.01), and LDH above 2xULN (35% *vs* 11%, p=0.005), at presentation. All patients with normal LDH levels and non-bulky disease (n=7) remained in remission at a median follow-up of 32 months (Figure 1).

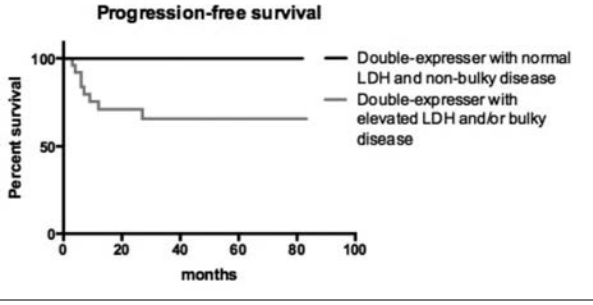

Figure 1.

Summary/Conclusions: We present a small single center series of 'double expresser' DLBCL. Patients with the double expresser phenotype had a higher incidence of bulky disease and markedly elevated LDH level, which was associated with higher risk of disease progression. Knowing this, it may be reasonable to adopt a more intensive treatment approach for these patients. Interestingly, having a normal LDH level at diagnosis and absence of bulky disease (known good prognostic factors) retained their protective influence even in patients with 'double expresser' phenotype.

PB1725

METRONOMIC CHEMOTHERAPY IMPROVES SURVIVAL IN RESPONDING PATIENTS WITH RECURRENT/REFRACTORY LYMPHOMA

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Background: Metronomic chemotherapy (MC) consists of continuous administration of oral chemotherapy at low, potentially less toxic doses without prolonged drug-free breaks. MC might be a useful for many patients with recurrent or refractory lymphomas that are unable to tolerate intensive therapies.
Aims: The aim of this study was to retrospectively analyze the efficacy and toxicity of MC in recurrent or refractory lymphomas in our Institution.
Methods: Retrospective analysis of patients with lymphoma treated with MC from 2009 to2014 in our Institution. The metronomic scheme consisted of oral 50 mg of prednisone, 50 mg of cyclophosphamide, 50 mg of etoposide, and/or 50 mg of procarbazine evenly distributed throughout the day. Clinical response, duration of response, progression-free survival (PFS) and overall survival (OS) was evaluated. Clinical response was defined as improvement of the symptoms of lymphoma and/or shrinkage of tumours (lymph nodes or affected organs) by physical examination and/or imaging.
Results: *Patient demographics and characteristics*: 28 lymphoma patients consecutively treated with MC were included. 10 patients had diffuse large B-cell lymphoma (DLBCL), 6 cutaneous T-cell lymphoma (CTCL), 5 peripheral T-cell lymphoma (T-NHL), 4 Hodgkin lymphoma (HL), 1 mantle cell lymphoma, 2 other types of non-Hodgkin lymphoma. Median number of prior regimens was 3 (range 1-8). 26 patients (93%) had refractory disease to prior treatment.
Efficacy: Clinical response was observed in 23 patients (82%) with a median duration of response 6 months (95% CI, 0-11 months). No differences were found in clinical response rate, duration of response, PFS and OS among the aggressive or indolent lymphomas. With a median follow-up of 14 months, median OS was 6 months. Of note, responders to MC showed a significantly increased OS (median OS of 11 months in responders *vs* 1 months in non-responders (p<0.001) (Figure 1). Remarkably, PFS was also significantly higher in responders, 42% of cases were progression-free at 6 months in responders *vs* 0% in non-responders (p<0.001). Twenty patients died: progressive disease (n=15), infection (n=4) and non-related (n=1). *Toxicity*: Seventeen patients (61%) had adverse events grade 3/4, mainly hematologic. A total of 6 patients had infections grade ≥3: urinary tract infection (n=2), pneumonia (n=2), multiresistant *Pseudomonas aeruginosa* bacteremia+*Cytomegalovirus* infection (n=1) and *Escherichia coli* sepsis (n=1). The main cause of treatment discontinuation was progressive disease. Only one gastrointestinal adverse event grade 4 led to MC discontinuation (3.6%). Dose modifications of MC drugs was performed in 15 patients (54%) and 12 patients required use of granulocyte colony stimulating factor (G-CSF). 54% of patients received cotrimoxazole as primary or secondary prophylaxis.

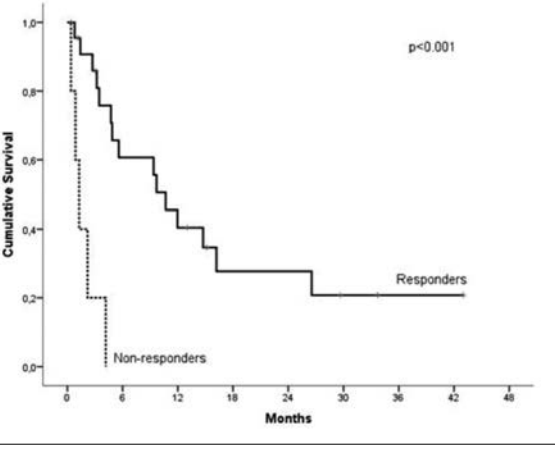

Figure 1.

Summary/Conclusions: Our study supports the anti-tumor activity of MC in patients with advanced lymphoma chemo-resistant and relapsed to multiple therapies with no other therapeutic alternatives. Clinical responses were observed in all lymphoma subgroups and the toxicity profile was acceptable, even in heavily pretreated patients. Even most of our cases had been considered refractory to prior treatment, responding patients to MC had an improved PFS and even OS.

PB1726

RELATIVE TOTAL DOSE INTENSITY OF TREATMENT WITH R-CHOP IN OBESE *VERSUS* NON OBESE LYMPHOMA PATIENTS DOSED ON ACTUAL BODY WEIGHT: A SINGLE CENTRE EXPERIENCE

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Background: The number of obese patients in the UK is growing to epidemic

proportions and due to an aging population the incidence of cancer is also increasing. To ensure good patient outcomes it is imperative that we optimally treat obese patients, particularly in the curative setting for diseases such as diffuse large B-cell lymphoma (DLBCL). In 2012 the American Society of Clinical Oncology (ASCO) released a Clinical Practice Guideline recommending that obese patients should be dosed on their full body weight. It is recognised however that clinicians in the UK continue to dose adjust (or 'cap' doses) in fear of excess toxicity.

Aims: This study aims to assess whether dosing on actual body weight in obese patients with R-CHOP chemotherapy for DLBCL affects relative total dose intensity of treatment (RTDI).

Methods: A retrospective analysis was performed of 77 consecutive patients who had received treatment with R-CHOP chemotherapy for DLBCL from 2006 to 2010. After exclusions for insufficient individual patient data or modifications to the regime *e.g.* addition of etoposide (R-CHOEP), the remaining number of patients for analysis was 66 (obese patients dosed on full body weight; n=18 and non-obese; n=48). Baseline characteristics were recorded. Weight and height measurements within 1 month of treatment initiation were used to calculate body surface area (BSA) and body mass index (BMI). Patients were defined as obese if BMI ≥30kg/m^2 as per WHO classification. IBW was calculated for obese patients using the BJ Devine formula. RTDI was calculated using previously published methods. It is the ratio of Actual Total Dose Intensity (ATDI) and Planned Total Dose Intensity (PTDI) expressed as a percentage *i.e.* RTDI (%)=ATDI/ PTDIx100. PTDI is the planned dose intensity over the entire treatment duration, averaged across the chemotherapy agents used. For permanent treatment discontinuation for a reason other than disease progression, relapse or death, the remaining cycles are calculated with planned length and zero dose. In cases of disease progression, relapse or death PTDI is calculated based on number of cycles completed. ATDI is the actual average dose intensity over the real treatment duration *i.e.* actual total dose (mg)/duration of therapy (weeks). RTDI therefore takes into account the effects of treatment delays as well as dose reductions, and premature cessation of therapy due to reasons other than disease progression or death. It is a surrogate marker of survival and multiple studies have demonstrated that achieving an average RTDI >90% is associated with improved long term outcomes. The chi squared test for trend was used to test for a trend in the number of patients being dose reduced at baseline between the two groups. Univariate analysis was performed for age, gender, performance status (PS), baseline characteristics including renal function, hepatic function, full blood count, line of treatment, and GCSF usage using chi-squared tests, t-tests and Mann-Whitney tests. Multivariable linear regression was performed to adjust for confounding factors.

Results: Before adjusting for confounding factors, there was a non-significant difference in average RTDI for R-CHOP between obese and non-obese patients of 7.22%. After adjusting for age, gender, performance status, and whether patients were dose modified in the 1st cycle, a multivariable linear regression showed a reduction in this difference to 5.67% . There was no evidence to suggest a difference in RTDI between the two groups (Table 1).

Table 1.

	Obese N=18	Control (non-obese) N=48	P-value
Average RTDI Mean (sd%)	94.05 (7.13)	86.83 (16.05)	0.071 (t-test)

Summary/Conclusions: These findings, although of limited value due to small patient numbers, would reassure clinicians and pharmacists that full weight based dosing in obese patients should not lead to excess toxicity. In conjunction with the broader published literature and ASCO's Clinical Practice Guideline they support dosing on actual body weight for R-CHOP for obese patients with DLBCL.

PB1727

OUTCOME OF OLDER PATIENTS WITH B-LARGE CELL LYMPHOMA (B-LCL) – AN OBSERVATIONAL STUDY OF KROHEM, THE CROATIAN COOPERATIVE GROUP FOR HEMATOLOGICAL DISEASES

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Background: Approximately half of the patients with diffuse large B-cell lymphoma (DLBCL) are older than 60 years and their outcome is inferior in comparison to younger patients.

Aims: We aimed to assess the impact of age, risk factors and the type of treatment on event-free survival (EFS) and overall survival (OS).

Methods: In this retrospective study, 304 patients with DLBCL older than 60 years or equal were included. A total of 218 patients were included in an observational study of patients treated with rituximab conducted at 15 general and university hospitals in 2007 and 2008. Additional patients were recruited from two clinical centers.

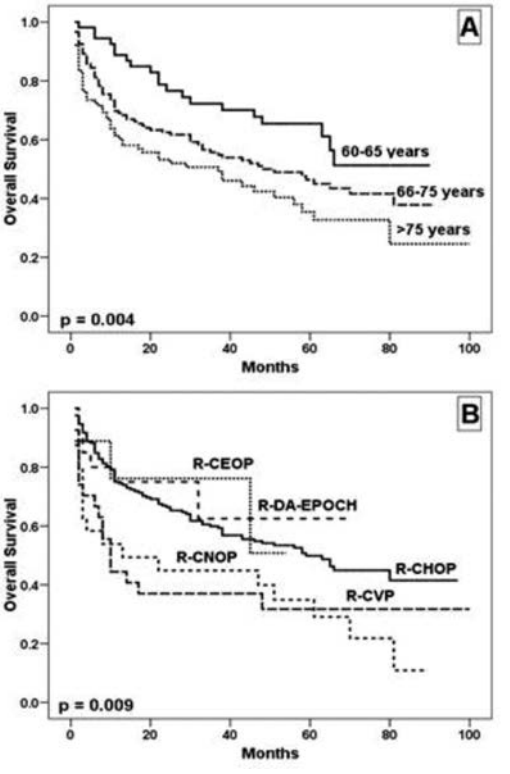

Figure 1.

Results: The median age was 73 years (range 60-90), 144 were men and 160 women. 205 patients were treated with R-CHOP, 27 with R-CVP, 24 with R-CNOP (mitoxantrone instead of doxorubicin), 20 with R-DA-EPOCH, 9 with R-CEOP (etoposide instead of doxorubicin), and 19 patients received other regimens or no chemotherapy. After a median follow up of 52 months for survivors, the estimated 5-year EFS and 5-year OS were 43% and 47%, respectively. Half of the patients are alive at the time of last follow up. Lymphoma, infections, and cardiac events were the leading causes of death. A total of 52% patients died during first-line treatment, 24% died in remission, and 24% died in relapse. There were 16 secondary malignancies reported. The aaIPI significantly correlated with EFS (p=0.002) and OS (p=0.001). Gender, bulky disease (>5cm), and extranodal involvement were not associated with survival, whereas B symptoms were significantly predictive of EFS (p=0.002) and OS (p<0.001). Age had a negative impact on survival: patients between 60 and 65 years fared well (5-year OS 65%), patients from 66 to 75 years of age worse (5-year OS 46%), and those older than 75 years the worst (5-year OS 38%); p=0.004 (Figure 1A). Treatment choice also influenced EFS and OS: R-CVP and R-CNOP had worst outcomes worst, whereas those of R-CEOP and R-DA-EPOCH were at least comparable to R-CHOP; p=0.025 for EFS, p=0.009 for OS (Figure 1B).

Summary/Conclusions: R-CHOP remains the standard of care in elderly patients with B-LCL. The aaIPI and presence of B symptoms influence prognosis. Survival decreases with age; cut-offs at 65 and 75 years are discriminative. R-CNOP has only modest efficacy, similar to R-CVP. Etoposide may serve as an alternative to anthracyclines for patients with cardiac comorbidities, and R-DA-EPOCH may represent a good option for high-risk patients.

PB1728

TREATMENT TOXICITIES AND OUTCOME OF AN INTENSIVE IMMUNOCHEMOTHERAPY REGIMEN (FAB LMB96) FOR ADULT AGGRESSIVE B CELL NON-HODGKIN LYMPHOMAS (BNHL) AT RISK FOR OR WITH CENTRAL NERVOUS SYSTEM INFILTRATION

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Background: The treatment of diffuse large B cell lymphomas (DLBCL) at risk for CNS infiltration is not well established but may benefit of regimens including systemic drugs crossing the blood-brain barrier. Furthermore, different studies have showed that patients with lymphomas with features intermediate between DLBCL and Burkitt (Int-BNHL) and MYC-rearranged lymphomas have inferior

outcomes when treated with R-CHOP compared to more intensive regimens. Treatment toxicity is a major concern in this setting where a superior efficacy has yet to be prospectively established.
Aims: We retrospectively reviewed the clinical and genetic characteristics, treatment toxicities and outcomes of DLBCL patients at risk for CNS infiltration and Int-BNHL selected for aggressive treatment at our institution between 2009 and 2013.
Methods: Selection was based on age, performance status, ≥2 extranodal sites (ENS) plus elevated LDH, CNS infiltration at diagnosis or MYC-rearrangement. Fab LMB96 regimen includes alkylator/anthracycline/high-dose methotrexate-based induction and high-dose cytarabine/etoposide or cytarabine/methotrexate-based consolidation, followed by maintenance; intrathecal chemotherapy is administered at induction and consolidation. Toxicities were graded according to CTCAE. Response was defined by conventional criteria and overall survival (OS) determined from diagnosis until death or last follow up. Treatment toxicities and outcomes of stage IV, intermediate-high and high-risk IPI DLBCL patients receiving R-CHOP during the same period were reviewed for reference.
Results: 15 patients with DLBCL (9) or Int-BNHL (6), median age 48 (23-65) yo, 6 men, with stage IV disease were treated with the LMB96 protocol to which Rituximab was added at the beginning of the induction and consolidation cycles. 10/11 evaluable patients had MYC rearrangements detected by FISH (MYC+). CNS involvement at presentation occurred in 3 patients and bone marrow infiltration in 6. 93% of the patients had ECOG performance status 0-1 and 12 had IPI 3-5; the other 3 patients had either CNS infiltration or MYC+. 74% and 43% of 94 treatment cycles were complicated by grade 3-4 hematological and infectious toxicities, respectively. These toxicities occurred mostly during induction and consolidation. All patients were admitted for induction/consolidation cycles and required transfusion support, myeloid growth factors and IV antibiotics. Serious neurological (1%) and gastrointestinal (13%) toxicities were also observed. Dose reductions/premature interruptions occurred in 6 patients Complete response occurred in 13/14 (93%) evaluable patients. With a median follow-up of 37.8 months, 3 patients (20%) died, 2 of toxicity (13%) and 1 with progressive lymphoma; median OS was 36 months. In 40 patients with stage IV DLBCL patients aged ≤65 yo diagnosed in the same period and treated with R-CHOP (75% IPI 3-5, 90% high LDH, 65% ≥2 ENS), CR rate was 67.5%. 18/40 (45%) patients died, 14 of NHL and 2 (5%) of toxicity; median OS was 27 months.
Summary/Conclusions: In this retrospectively analyzed, single-center study we show that an intensive immunochemotherapy regimen is feasible and highly effective in selected patients with high-risk BNHL. R-CHOP, although widely applicable, demonstrated poor disease control in the reference sample. Large prospective studies are vital to assess the impact of intensive regimens in the outcome of unselected patients with aggressive lymphomas.

PB1729

MANAGEMENT OF PRIMARY HEPATIC NON-HODGKIN'S LYMPHOMA (PHL)

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Background: Primary Hepatic (PHL) is a rare form of NHL, characterized by the exclusive involvement of the liver at the moment of the diagnosis, whereas other localizations, such as lymph nodes, spleen, bone marrow and peripheral blood, or other tissues are free of disease at least for 6 months after diagnosis. Even if its occurrence is rare, PHL should enter in the differential diagnosis of every space-occupying liver lesion, in particular if they are present in concomitance with normal levels of AFP and/or CEA and in absence of liver cirrhosis.
Aims: Only small series of PHL have been investigated in literature, nevertheless a non-fortuitous association with Hepatitis C Virus (HCV) infection has been reported among these series. The prognosis is believed to be dismal, with early recurrence and short survival.
Methods: Patients diagnosed with PHL at our institution between 1990 and 2014, among a population of 600 NHL, were retrospectively analyzed. 11 PHL were defined, 10 patients had a B-cell lymphoma, DLBCL in 6. The prevalence of HCV infection was 72%. Combination CHT was the mainstay of treatment for PHL. 10 patients were treated with CHT (10/11 : 90%). In 7 patients, the multi-drug regimen we used consisted of the CHOP or a CHOP-like scheme; in 2 of them (diagnosed after 2002) rituximab was added, at standard schedule. In the indolent types a fludarabine-based scheme was used. One patient with a single-focal lesion underwent to surgical treatment, with a CR. 11/11 patients achieved complete remission of the disease after the frontline therapy (six CHT courses/surgical treatment) (CR : 100%). Only one patient, 47 years old, Diffuse Large B-Cell Lymphoma, relapsed 144 months after CR: she underwent to retreatment with Rituximab+CHOP with the result of SD, so she underwent to third line treatment with R-Bendamustine, but, due to cardiotoxicity, she had to stop treatment and in the same month died for heart failure.
Results: At time, 10/11 (90%) of patients are alive with a median overall survival (OS) was 123 months (r. 40-228) and median disease- free survival (DFS) was 120 months (range 36-223), no statistically significant differences were found between PHL and other types of NHL in terms of OS and DFS. HCV infection did not appear to influence the results of therapy.
Summary/Conclusions: Our study confirms the rarity of PHL, shows a high prevalence of HCV infection, and demonstrates that the outcome of patients with PHL may be favorable.

PB1730

LEUKEMIZATION OF FOLLICULAR LYMPHOMA: DIAGNOSTIC FEATURES AND CLINICAL COURSE OF A RARE FORM OF THE DISEASE

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Background: Follicular lymphoma is a frequent lymphoid neoplasm, accounting for 22 percent of all non-Hodgkin's lymphoma in Russia. At diagnosis, some patients with FL manifest a detectable leukemic phase – leukemization (FL-LP), but this feature has been seldom described and has poorly prognosis.
Aims: to characterize a group of patients with FL with leukemization and evaluate the efficacy of therapy (R-CHOP /R-FMC/high dose therapy (HDT)).
Methods: Among 250 patients diagnosed with FL in National Research Center for Hematology, 18 (7, 2%) had characteristic FL-LP (by cytological blood smears and flow cytometry analysis). 8/18 patients had extranodal foci: lung, stomach, spleen, lumbar muscles, upper jaw, vertebrae. 17/18 patients had bone marrow involvement. Morphologically in biopsies of tumors in the majority of patients had a indolent FL (10/18 pts were diagnosed with I-II cytological grade of FL, nodular tumor growth and nodular-diffuse tumor growth in 14/18 pts). As first line therapy we used standard therapy (R-CHOP or R-FMC) or HDT with autoSCT.
Results: Median follow-up was 66 months (range 12–217). The 5-year overall survival (OS) and progression-free survival (PFS) were: 70% (standard error 10) and 35% (15), respectively (Figure 1A-B), Median OS was not reached, median RFS was 3 years.

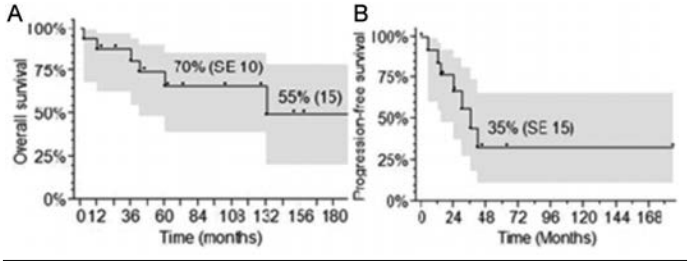

Figure 1.

Summary/Conclusions: FL with leukemization characterized by a low overall and disease-free survival. The most effective treatment regimens of this group are: R-CHOP with autoSCT or R-FMC. These courses allow a greater degree to achieve complete eradication of the tumor clone in the bone marrow. Given the recurrent course of the FL, the most expedient to conduct autoSCT in first-line therapy.

PB1731

DIFFUSE LARGE B-CELL LYMPHOMA IN HIGH RISK AAIPI 2-3 YOUNG PATIENTS: CLINICAL PRESENTATION AND SURVIVAL AFTER FIRST LINE STANDARD R-CHOP THERAPY

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Background: The outcome of diffuse large B-cell lymphoma (DLBCL) has substantially improved with the addition of Rituximab (R) to chemotherapy regimens although there is not a randomized trial comparing R-chemotherapy *versus* chemotherapy alone in high risk young patients.
Aims: We aim to present a retrospective study of the clinical, immunohistochemical features (IHC), and survival of patients≤60 years with aaIPI 2-3 treated with 6 cycles of standard R-CHOP in a single center.
Methods: Three hundred sixty seven patients were diagnosed of DLBCL from January of 2004 to June of 2015 in our centre, 119 (32.4%) were ≤60 years and 63 (52.9%) of them had aaIPI 2-3. Cases were classified as germinal center (GB) or activated B-cell (ABC) subtype according with the IHC Hans's algorithm.

Clinical and biological features, progression free survival (PFS) and overall survival (OS) were analyzed. Survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test.
Results: Sixty three young patients with aaIPI 2-3 DLBCL were included. Median age was 50 years (range 21-60), 29 (46%) were male. Clinical characteristics at presentation were: ECOG ≥2 in 45 (67.2%), Ann Arbor stage III-IV 53 (84.1%), B symptoms 27 (42.9%), Bulky >5cm mass 19 (30.2%), extra nodal disease 43 (68.3%), CNS involvement 3 (4.8%), high LDH 37/53 (69.8%), high B2M 12/25 (48%), aaIPI2 48 (71.6%) and aaIPI3 15 (23.8%).Thirty-six cases had data to be classified by IHC: 22 (61.1%) CGB and 14(38.9%) ABC. Sixty-two (98.4%) patients started treatment: 58 (92%) with 6 cycles of R-CHOP and 4 (8%) of R-CHOP-like (2 R-CNOP, 1 R-Bortezomib-CAP and 1 GA101-CHOP). Nine (14.2%) patients did not complete treatment, 5 due to adverse events (2 not recorded, 2 severe infections, 1 cardiac event) and 4 due to progression. Intrathecal prophylaxis was administered in 10 (16.1%) cases and as therapy in the 3 patients with CNS involvement. Response was evaluated with PET/CT in 41 (71.8%) patients. Intention to treat response rate was: CR 46 (73%), PR 4 (6.3%), refractory disease 9 (14.3%), not evaluable 4 (6.3%). With a median follow-up of 44 months PFS was 69% and OS was 70%. PFS was 75.2%in patients with aaIPI 2 and 46.7% in patients with aaIPI3 (p=0.02). OS was 77.4% and 38.7% respectively (p=0.01). Sixteen patients relapsed or progressed[AS1], 4 were lost of control and 12[AS2] were treated with a second line regimen in our center (9 R-ESHAP, 1 MTX-ARAC, 1 Burkitt regimen and 1 DHAP). Only 3 patients achieved enough response (2 CR and 1 PR) to[AS3] undergo an autologous stem cell transplant. After second line therapy, median PFS was 2 months and median OS was 4 months.
Summary/Conclusions: Six cycles of R-CHOP is an effective first line regimen for young patients with aaIPI2, but alternative therapies are needed for patients with aaIPI3. Moreover, only a small percentage of patients who fail first line therapy are rescued. Clinical trials with new drugs are needed especially for the very high risk population.

PB1732

ANALYSIS OF RISK FACTORS FOR CENTRAL NERVOUS SYSTEM RELAPSE IN DIFFUSE LARGE B-CELL LYMPHOMA: THE EXPERIENCE OF A TERTIARY HOSPITAL

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Background: Central nervous system (CNS) relapse is an uncommon (5%) unfavorable complication of diffuse large B-cell lymphoma (DLBCL). Independent predictors at diagnosis have been described to establish higher-risk groups that benefit from CNS prophylaxis: IPI ≥3, elevated serum LDH levels, B symptoms, bulky mass, CD5+, MYC rearrangement, >1 extranodal involvement, ECOG >1 and absence of Rituximab in systemic treatment. Many studies have demonstrated that the frequency of CNS relapse varies from 10% to 50%, depending on risk factors.
Aims: Analyze triple intrathecal therapy to prevent CNS relapse in DLBCL with high risk factors.
Methods: We analyzed retrospectively, from 2009 to 2015, 75 patients with newly diagnosed DLBCL. The decision to administer them all triple intrathecal therapy (TIT) (metotrexate, cytarabine, hydrocortisone) was based on the CNS relapsing risk factors in DLBCL described above. Systemic therapy was CHOP-*like*. Qualitative variables are described in percentages and quantitative variables are described with means and standard deviations. We use Chi square test to compare different categorical variables with CNS relapse. The overall survival is tested with Kaplan-Meier tables.
Results: Of 75 patients, 41 were male. Median age at diagnosis was 62 years. 6 (8%) patients experienced CNS relapse. The mean Overall Survival (OS) was 62 months CI 95% (55-69). OS at 6 months was 90.4%, at 2 years 80.5% and at 5 years 72%.Table 1 shows comparison between parameters described and CNS relapse.

Table 1.

Risk factors at diagnosis	Frequency (n)	CNS relapse (%)	P value
B symptoms (Yes/No)	37/38	10.8/5.3	0.430
Bulky mass (Yes/No)	19/56	10.5/7.1	0.640
Ann-Arbor Stage (I-II/III-IV)	11/64	9.1/7.8	0.999
Serum LDH levels (elevated/non-elevated)	46/29	8.7/6.9	0.999
Extranodal sites (0-1/>1)	42/33	4.8/12.1	0.395
IPI (0-2/3-5)	30/35	6.7/8.9	0.999
CNS involvement at diagnosis (Yes/No)	2/73	50/6.8	0.155
Rituximab (Yes/No)	73/2	8.2/0	0.999
CD5 (Positive/Negative)	4/53	0/9.4	0.556
MYC rearrangement (Negative/Not realized)	6/69	16.7/7.2	0.405

Summary/Conclusions: Spanish GELTAMO group has recently published the Guideline for diagnosis, prevention and therapeutic management of CNS involvement in DLBCL. In our experience, TIT was effective to prevent CNS relapse (8% compare to 10-50% described in literature in high risk patients). However, patients with very high risk factors could benefit from combination therapy recommended by GELTAMO based on inmunochemotherapy+TIT+ High Dose Metotrexate.

PB1733

ANALYSIS OF THE TOXICITY AND EFFICACY OF LIPOSOMAL CYTARABINE IN THE PROPHYLAXIS AND TREATMENT OF CNS LYMPHOMATOSIS: THE BALEARIC LYMPHOMA GROUP EXPERIENCE

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Background: CNS lymphomatosis is a generally fatal complication of aggressive NHL. Without specific CNS prophylaxis the risk of CNS relapse is higher (20-30%) in patients with very aggressive NHL such as burkitt or lymphoblastic lymphoma/leukemia. DLBCL has a lower risk (around 5%) but several factors have been identified that increase the incidence of CNS relapse to levels similar to very aggressive NHL. All above mentioned patients are candidates for CNS prophylaxis. However there is no consensus about which is the best way of administering CNS prophylaxis. Best results seem to be associated to the use of intravenous (iv) high-dose methotrexate (HDMTX) but with significant toxicity. Other options are intrathecal (IT) administration of MTX, cytarabine or liposomal cytarabine (ITLC). However no randomized trial has been performed to test which is the best option in terms of tolerance and efficacy.
Aims: We aim to analyse the experience of the centres of the Balearic Lymphoma Group about the tolerance, toxicity and efficacy of ITLC in the prophylaxis and therapy of CNS lymphomatosis.
Methods: We retrospectively reviewed all cases treated with ITLC in Son Espases and Son Llatzer Hospitals. Standard diagnostic, response assessment and follow-up data was obtained from medical records. All cerebrospinal fluid (CSF) samples were evaluated through 8-colours flow cytometry. Survival analysis was done using Kaplan-Meier curves and the Log-rank test.
Results: From 2005 to 2015, 58 patients received ITLC. They received a total of 180 ITLC injections. Toxicity was as follows: 33% headache, 20% neurological deficits, 11% nausea, 9% dizziness, 4% vomiting, 4% fever, 2% transient blindness and 2% photophobia. In the prophylactic cohort (n=26) with a median follow-up of 55 months (17-81) only 3 CNS relapses (11%) were observed in a testicular DLBCL, Burkitt and plasmablastic lymphoma. This represents a cumulative incidence of CNS relapse of 8%, 14% and 20%, respectively for DLBCL, Burkit/lymphoblastic and plasmablastic lymphoma. In the treatment cohort (n=32), complete clearance of CSF was obtained in 77% of cases (86%, 62% and 100% of respectively DLBCL, Burkit/lymphoblastic lymphoma and Primary CNS lymphoma). Median OS was 6 months (0-16). Causes of death were lymphoma progression in 19 patients (79%), treatment toxicity in 2 and other non-related in 3 (12%) (Table 1).

Table 1.

Characteristics	Global group(n=58)	Prophylaxis cohort (n=26)	Treatment cohort(n=32)
Median age (range)	53 (10-85)	47 (12-85)	55 (10-81)
Gender:			
·Male	44 (76%)	19 (73%)	25 (78%)
· Female	14 (24%)	7 (27%)	7 (22%)
Diagnostic:			
DLBCL	31 (53%)	17 (65%)	14 (44%)
Lymphoblastic or Burkitt	16 (27%)	7 (27%)	9 (28%)
Primary CNS Lymphoma	6 (10%)	1 (4%)	5 (16%)
Mantle Cell Lymphoma	3 (5%)	1 (4%)	2 (6%)
Peripheral T-cell lymphoma	1 (2%)	0 (0%)	1 (3%)
Follicular Lymphoma	1 (2%)	0 (0%)	1 (3%)
First line treatment:			
· Intensive treatment ALL-like	14 (24%)	7 (27%)	7 (22%)
·Intensive treatment for CNS	11 (19%)	1 (4%)	10 (31%)
·Conventional CHOP-like	23 (40%)	17 (65%)	6 (19%)
Other	10 (17%)	1 (4%)	9 (28%)

Summary/Conclusions: The toxicity profile was good especially when concomitant IT and oral dexamethasone was administered. In the prophylactic cohort the incidence of CNS relapse was low in the DLBCL group similar to previously reported for iv HDMTX and much better than IT MTX. ITLC may be a good alternative for CNS prophylaxis but randomized clinical trials are needed. Patients in treatment cohort had a high rate of clearance of CSF but survival is still poor in CNS lymphomatosis.

PB1734

CHARACTERIZATION OF HEPATITIS C VIRUS ASSOCIATED B-CELL NON-HODGKIN LYMPHOMA IN MANSOURA REGION (EGYPT), ANRS 12263 STUDY

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Background: The prevalence of Hepatitis C virus in Egypt is 14.7%, which is considered the highest in the world. Genotype 4 represents about 93.1% of cases which is considered among the highest prevalence areas of genotype 4 all over the world. Non-Hodgkin lymphoma is the 5th most common cancer in both male and female in Egypt. The association of HCV infection and development of B-cell lymphoproliferative especially the causative relationship not yet clear in Egypt.
Aims: the aim of this primary pilot study is to evaluate the incidence and clinical characteristics of HCV associated lymphoma in Egypt.
Methods: Between January 2012 and January 2013, we enrolled 110 adult patients with newly diagnosed B cell NHL. HCV infection was defined by the detection of anti-HCV antibodies by enzyme-linked immunosorbent assays with or without viraemia at initial diagnosis. Patients with Hepatitis B virus co-infection could be included but not those with HIV co-infection. Anti HCV positive samples, underwent HCV-RNA testing at disease diagnosis beside a rheumatoid factor screen.
Results: The incidence of B cell non-Hodgkin lymphoma associated with HCV infection was 60.9% (67/110 patients) which is considered the highest reported value in the literature. The incidence of cases presented with viraemia was 80% (32/40 patients). As regard histology, the majority of HCV associated lymphoma were DLBCLs (71.6%), SLL/CLL (13.4%), marginal zone (7.5%) and follicular (7.5%).
Summary/Conclusions: B cell lymphomas are highly associated with HCV infection (In Egypt incidence=60.9% *vs* 2.5% in France). The difference in genotype distribution may be responsible for different incidence and behavior of HCV associated lymphoma. Further studies is needed to have a molecular signature for this unique environmental and genetic background.

PB1735

PRIMARY BONE LYMPHOMA (PBL) - "REAL WORLD" EXPERIENCE OF A SINGLE CANCER HOSPITAL. CLINICAL CHARACTERISTICS, PROGNOSTIC FACTORS AND MANAGEMENT OF TWELVE PATIENTS.

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Background: PBL was first described as a distinct clinopathological entity on 'reticulum cell sarcoma of bone' in 1939 by Parker and Jackson. It is a very rare condition which constitutes less than 1% of all malignant lymphomas, less than 2% of all bone tumors and less than 5% of extra-nodal lymphomas. A number of studies have been reported, but most with a limited number of patients. Being an uncommon entity, there is a lack of experience in paramaters such as clinical characteristics and optimal management.
Aims: To determine patient characteristics, prognostic and treatment factors that could affect outcome measured by overall survival (OS) and complete remission (CR).
Methods: Herein, we retrospectively review 12 patients diagnosed with PBL and treated at our institution from 2001 to present.
Results: The demographic and clinical characteristics of the 12 patients at the time of diagnosis are summarized in Table 1. The median age of the patients was 49 years old (range, 31-81 years). Eight (70%) patients were female and 4 (30%) male. The histological type of all 12 patients (100%) was Diffuse Large B-Cell Lymphoma. Ten patients (83%) were presented with Ann Arbor Stage I or II disease. Two patients (17%) had high stage disease. The median follow-up after achieving CR (without relapse) to date was approximately 80 months (range, 0-170 months). Our analysis demonstrated that age less than 47 years normal LDH level, Ann Arbor Stage I or II and female gender were found to be favorable prognostic factors for achieving CR and OS. Notably, the pelvis was the most frequently primary involved site of the bone (5; 41.5%). All twelve patients underwent chemotherapy with most of them (10; 83%) receiving six to eight cycles of the RCHOP regimen. The majority (11; 92%) of them received radiotherapy. After front line chemotherapy followed by radiotherapy most patients (9; 75%) achieved CR. Among the remaining three patients, the one died before completing first line chemotherapy due to an infection while in chemotherapy-induced neutropenia. The other patient is undergoing autologous stem-cell transplantation due to refractory PBL. The third one achieved CR after he underwent autologous stem-cell transplantation due to refractory PBL. Both patients that had refractory disease were male and initially diagnosed with high stage PBL. The median OS was 89,5 months (range, 6-178 months).

Table 1. Patient demographic and clinical characteristics.

	Median	Range
Age	49 years	31 - 81
Gender M/F	4 (30%)/8 (70%)	
Serum LDH	250 IU/L	137 - 857
Clinical Stage I & II	10 (83%)	
Pelvis as primary involved site of the bone	5 (41,5%)	
Initial CTx: RCHOP	10 (83%)	
Response to initial treatment CTx+RT: CR	9 (75%)	
ASCT	2 (17%)	
Follow up after CR (without relapse)	80 months	0 - 170
OS	89.5 months	6 - 178

Summary/Conclusions: Although the total number of patients was relatively small, the data from our study supports that most PBL patients have had early stage disease (IE-IIE) on diagnosis which also was the most important favorable prognostic factor. Overall, patients with primary lymphoma of the bone with DLBCL have an excellent prognosis and only a limited number of patients underwent autologous stem-cell transplantation in order to achieve CR.

PB1736

PRIMARY BONE DIFFUSE LARGE B CELL LYMPHOMA: EXPERIENCE AT A SINGLE CENTER IN JORDAN

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Background: Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma, although there may be geographical and ethnic differences with respect to DLBL; these differences are associated with particular clinicopathologic features and outcomes. Primary bone diffuse large B cell lymphoma (PBDLBL) comprises 4–5% of all extranodal non-Hodgkin lymphoma (ENHL) cases and less than 1% of all malignant lymphomas. PBDLBL is so rare that many of its characteristics remain unknown, with no data at all available from Jordan.
Aims: To examine the epidemiology and clinical features of PBDLBL in Jordanian patients as a model for other Middle East countries in which such data are scarce.
Methods: Between September 2002 and December 2015, 112 patients aged 16 years and above were diagnosed with DLBL and treated at King Abdullah University Hospital.Patients with ENHL were included in the study only if they had a tissue diagnosis of DLBL at the site of involvement. According to the site of ENHL involvement at the time presentation Clinical stage was defined according to the Ann Arbor classification. Disease involving the lymph nodes, spleen, and Waldeyer's ring were defined as "nodal".
Results: This retrospective study included 114 patients with DLBCL (54 females and 60 males; age range, 16 to 94 years; mean (SD), 51.0 (18.7) years). Of these, 66 (57.9%) presented with heterogeneous extranodal organ involvement. Of all extranodal sites, bone was involved in nine (14%) cases. Of these cases, 33% were female and 67% were male. The mean age of patients with extra nodal involvement was 46.7 (20.6) years. There was no significant difference between those with nodal and those with extranodal lymphomas in terms of gender (p=0.656) or age (p=0.158). The spine was the most common site of extranodal involvement with DLBL in six patients, the pelvis in one patient, the clavicle in one patient, and the scapulae in one patient. Back pain was the most common presenting symptom (six (67%) patients). A pathological fracture was noted in one patient (11%), a shoulder mass in one patient, and lower limb paralysis in one patient. No B symptoms were present in any patient. Serum lactic dehydrogenase (LDH) levels were elevated in only one patient.
Summary/Conclusions: PBDLBL was the second most common extranodal manifestation of DLBL (14%), with the spine being the most frequent site.the most common extranodal site was the gastrointestinal tract (18%). Back pain was the main presenting symptom, with an absence of B symptoms. LDL levels were normal in most patients. The present study highlights geographic and ethnic variations in the primary disease site in patients with DLBL.

PB1737

THE INFLUENCE OF CARDIOVASCULAR COMORBIDITIES ON THE THERAPEUTIC RESPONSE AND PROGNOSIS OF PATIENTS DIAGNOSED WITH AGGRESSIVE NON HODGKIN LYMPHOMA

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Background: The NHLs are a heterogeneous group of lymphoproliferative malignancies with different patterns of behavior and responses to treatment. The aggressive type of NHL has a shorter natural history, but a significant number of these patients can be cured with intensive chemotherapy regimens.
Aims: The aim of this study was to determine to what extent cardiovascular comorbidities influence choice of therapy, treatment response and survival of patients diagnosed with NHL.
Methods: We have retrospectively analyzed clinical data of 47 aggresive NHL patients who were consecutively diagnosed at the Hematology Department of the Municipality Hospital of Timisoara,between 2009 and 2015. Disease stage and treatment were established after collecting clinical,laboratory, and pathological data. Survival analysis was estimated by means of the Kaplan-Meier curves and comparison of survival between subgroups was performed using the log-rank test. IBM SPSS Statistics 23 was the software that was used.
Results: There were 20 patients males and 27 females, averaging the age of 53.82 years, a median age at diagnosis of 56 years and a standard deviation of 15.49. The estimated overall survival at 5 years for the whole group of patients is 45% while the free survival disease at 5 years is 38%. In the case of patients diagnosed with high blood pressure (HBP), the overall survival is 45% at 5 years compared to the 43% for the patients who did not show HBP while the free survival disease for the patients diagnosed with HBP at 5 years is 45% compared to those who did not indicate the presence of HBP (37%). As for the coronary artery disease (CID), there were found differences both for the overall survival at 5 years (59% *versus* 41%) and for the free survival disease at 5 years (58% *versus* 32%). Also, for the patients diagnosed with heart failure the overall survival at 30 months proved to be 0% compared to the overall survival for those who did not show this pathology (65%), and this is the same for the free survival disease found at these patients (p=0.025).
Summary/Conclusions: All the patients were subjected to polychemotherapy of R-CHOP or R-CNOP type. Referring to the associated cardiovascular commomorbidty neither the high blood pressure nor the coronary artery disease prove to be factors of negative prognosis, neither did they influence the survival and nor required the diminution of the chemotherapy dosages. Only the heart failure influenced the survival.

PB1738

LATE MYELOTOXICITY OF INTENSIVE MODIFIED PROGRAM NHL-BFM-90 IN ADULT POOR-PROGNOSIS PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background:
High dose chemotherapy showed to be more effective compared with standart dose therapy in DLBCL patients with poor prognosis, but late myelotoxicity wasn't estimated enough.
Aims: To evaluate late myelotoxicity of intensive modified program NHL-BFM-90 (mNHL-BFM-90) in adult poor-prognosis patients with diffuse large B-cell lymphoma (DLBCL).
Methods: Results of complex clinical, laboratory and instrumental examination, including cytological, histological and standard cytogenetic bone marrow study of 40 patients with DLBCL which received mNHL-BFM-90 protocol in the Hematology Research Center in the period since 2002 till 2009 years were analyzed. Group consisted of 20 men, 20 women, median age – 57 (31-76) years at the time of survey. Comparison group included 19 patients (8 men and 11 women), median age – 70 (39-80) years at the time of survey, who received CHOP /R-CHOP-21 therapy. Median follow-up after completion of treatment - 6 years. Results of study of bone marrow were analyzed before chemotherapy and after 5-10 years after treatment. Cellularity of bone marrow, the amount of erythroid, granulocytic and megakaryocytic germs, signs of dysplasia, and dysplastic changes of stroma were determined by histologically and cytologically. Also performs standard cytogenetic bone marrow examination to identify karyological violations. As late myelotoxicity were considered signs of toxic damage of bone marrow, which appeared first time in late period after chemotherapy . Cases with initially presented and preserved after chemotherapy myelotoxicity signs, as well as presence and absence of baseline after chemotherapy – weren't taken into account.
Results: Cytopenia (with no signs of myelodysplasia and substitutive transfusions of blood components need) were detected in 52% of patients with high-dose group, 46% of cases are accounted for thrombocytopenia. Reduced cellularity of bone marrow in 15 (38%) patients, p=0.02, reducing of erythroid and megakaryocytic germs in 13 (33%), p=0.01, and 19 (48%), p=0.009, respectively, stroma changes in 17 (43%) patients, p=0.02, were revealed like signs of late myelotoxicity in patients after high-dose chemotherapy for mNHL-BFM-90 program. Standard cytogenetic study of bone marrow was performed for first six patients, all had a normal karyotype, without any karyological abnormalities, therefore further study wasn't performed.
Summary/Conclusions: Long-term myelotoxicity of high-dose program mNHL-BFM-90 exceeds significantly toxicity of standard therapy CHOP/R-CHOP-21. However, myelodysplastic syndromes or cytopenia requiring transfusions of blood components wasn't observed.

PB1739

ANALYSIS OF CLINICAL PARAMETERS, ABSOLUTE LYMPHOCYTE AND ABSOLUTE MONOCYTE COUNT IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA

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Background: The results of gene expression profiling (GEP) analysis of diffuse large B cell lymphoma (DLBCL) confirmed existence of two subtypes of DLBCL with different outcome. In order to reproduce GEP findings several studies have investigated the potential role of absolute lymphocyte count (ALC), absolute monocyte count (AMC) and ALC/AMC as surrogate markers. However, the contradictory data were reported.
Aims: The aim of this study was to evaluate prognostic significance of clinical parameters, laboratory parameters: ALC, AMC and ALC/AMC on the overall survival (OS) and event free survival (EFS).
Methods: A total of 583 patients (295 females/288 males) with the median age of 60 years (range 18-89) were included in the study. According to the Ann Arbor classification, stage I, II, III and IV had 74 patients (12.7%), 212 (36.4%), 110 (18.9%) and 187 (32.1%), respectively. Bulky disease was present in 190 patients (32.6%) and B symptoms in 370 patients (63.5%). Bone marrow involvement was present in 77 patients (13.2%). Low Revised International Prognostic Index (R-IPI) was presented in 97 patients (16.6%), intermediate in 307 (52.7%) and high in 174 (29.8%). Median ALC at diagnosis was $1.35x10^9$/l (range 0.07-$17.8x10^9$/l), AMC $0.60x10^9$/l (range 0.06-$8.58x10^9$/l) and ALC/AMC 2.5 (range 0.07-$37.0x10^9$/l). Cut off values for LAC, AMC and ALC/AMC were used as previously published and included $1.1x10^9$/l, $0.62x10^9$/l and $2.6x10^9$/l for ALC, AMC and ALC/AMC, respectively. Regarding cell of origin according to Hans algorithm, 45.3% of patients had GC (germinal center) type, while 64.7% had non-GC type. All patients were initially treated with rituximab plus CHOP or CHOP like protocols.
Results: Complete remission (CR) was achieved in 425 patients (72.9%), partial remission (PR) in 79 (13.6%), stable disease (SD) in 19 (3.3%) and progressive disease in 60 (10.3%). Disease relapse was confirmed in 88 patients (15.1%). The patients with B symptoms of disease had inferior OS (Log Rank=11.27, p=0.001) and EFS (Log Rank=12.62, p=0.001) compared to those without B symptoms. Furthermore, presence of bulky disease influenced OD (Log Rank=10.58, p=0.001) and EFS (Log Rank=11.35, p=0.001). The prognostic value of R-IPI was highly statistically significantfor both OS (Log Rank=62.3, p<0.0001) and EFS (Log Rank=56.83, p<0.0001). The patients with GC type had better OS (Log Rank=7.44, p=0.006) and EFS (Log Rank=6.34, p=0.012) compared to non-GC type. However, there was no influence of ALC, AMC and ALC/AMC neither on OS nor EFS. Multivariate analysis among significant parameters (presence of B symptoms, bulky disease, R-IPI and cell of origin GC *vs* non-GC type), has pointed out the major impact of R-IPI on OS (HR 1.75, p<0.0001) and (HR 1.44, p=0.0001) along with the presence of B symptoms (OS, HR 1.51, p=0.01; EFS, HR 1.58, p<0.008).
Summary/Conclusions: ALC, AMC and ALC/AMC failed to prove influence on survival of DLBCL patients indicating the need for new potential surrogate markers of GEP results. Moreover, R-IPI as clinical prognostic score may help to differentiate risk groups within DLBCL patients.

PB1740

OUTCOME AND PROGNOSTIC FACTORS IN DIFFUSE LARGE B-CELL LYMPHOMA: AN INSTITUTIONAL EXPERIENCE OF A TERTIARY CARE CENTRE FROM INDIA

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma(NHL), accounting for approximately 30% of all new diagnoses. We conducted the retrospective study in our institution to analyze the main clinical features at diagnosis, response to therapy and the outcome of patients diagnosed with DLBCL.
Aims: To analyze the prognostic factors, response to therapy and the outcome of patients with DLBCL.

Methods: This study enrolled 74 patients with histologically confirmed diagnosis of DLBCL treated from January 2003 to December 2014. Complete clinical patient and disease related details were recorded. All patients were treated with chemotherapy with or without radiotherapy. Clinical features, treatment response and impact of different prognostic factors on clinical outcome were analyzed. Bulky disease was defined as any mass greater than 10cm in diameter.

Table 1.

Characteristic:		Number(%)
Age	<=60	55(74.3)
	>60	19(25.7)
Site	NODAL	40(54.1)
	EXTRANODAL	34(45.9)
Nodal sites	<4	34(45.9)
	=>4	6(8.1)
B symptoms	NO	70(94.6)
	Yes	4(5.4)
Number of sites	ONE	40(54.1)
	TWO	22(29.7)
	THREE	7(9.5)
	FOUR OR MORE	5(6.8)
Bone marrow	NOT INVOLVED	70(94.6)
	INVOLVED	4(5.4)
Stage	I	36(48.6)
	II	20(27)
	III	13(17.6)
	IV	5(6.8)
IPI	LOW	58(78.4)
	INTERMEDIATE	9(12.2)
	HIGH INTERMEDIATE	7(9.5)
Treatment	CCT	31(41.9)
	CCT+RT	43(58.1)

Results: Median age of presentation was 50 years (range 18-85 years). 55(74.3%) of the patients were ≤60 years age and 19(25.7%) were >60 years age. Out of 74 patients, 53 were males and 21 were females. Ann Arbor clinical stage at diagnosis was 36(48.6%) stage I, 20(27%) stage II, 13 (17.6%) stage III, and 5(6.8%) stage IV respectively. Bulky disease was present in 6 patients (8.1%). Nodal disease was present in 40(54.1%) patients and 34(45.9%) had extranodal disease presentation. Supradiaphragmatic disease was seen in 44(59.5%) and 15(20.3%) had infradiaphragmatic as well as disease on both sides of the diaphragm. Most of the patients (93.2%) received either CHOP or R-CHOP chemotherapy. 43(58.1%) patients received consolidative radiotherapy. The median follow-up period was 22 months (range, 2 to 147 months). Complete response was seen in 51(68.9%) patients. With addition of radiation 9.4% improvement in local control was seen. Relapses was seen in 10(13.5%) patients, out of which 5(6.8%) had nodal and 5(6.8%) had visceral relapse. At 22 months, disease free survival (DFS) and overall survival (OS) was 66% and 81.5% respectively. Stage, International prognostic index (IPI), supradiaphragmatic disease, number of sites, extranodal diasease and number of nodal sites involvement were important prognostic factors having significant impact on response, DFS and OS (Table 1).

Summary/Conclusions: This study represents the largest Indian experience to treat DLBCL. Stage, IPI, supradiaphragmatic disease, number of sites, extranodal disease and number of nodal sites were the important prognostic factors for response, DFS and OS.

PB1741

STUDY OF DISPARITIES BETWEEN SERUM LDH AND SOLUBLE INTERLEUKIN-2 RECEPTOR (SIL-2R) IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) PATIENTS

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Background: Serum LDH or serum interleukin-2 receptor (sIL-2R) are two of the most important tumour markers of malignant lymphoma, and of diffuse large B-cell lymphoma (DLBCL), which is the most common histological types of non-Hodgkin lymphoma. We usually find elevated values of both in DLBCL patients; however, sometimes there are cases where, unexpectedly, only one value does not exhibit an abnormal level, while the other does.

Aims: To analyse the clinical characteristics of cases where serum LDH and sIL-2R are not correlated.

Methods: We retrospectively analysed 556 patients that visited our hospital from March 1994 to June 2012 and who were diagnosed for the first time as having DLBCL. We drew a distribution chart of the ratio of serum LDH and normal level (LDH/N) and serum sIL-2R. (Figure 1.) The median LDH/N was 1.04 (0.38-20.59), and sIL-2R was 1,420 (119-40,000). Then, we decided two groups; group I was LDH/N >2, sIL-2R <2,000U/ml, and group II was LDH/N <1, sIL-2R >3,000U/ml.

Results: The median age of the DLBCL patients was 67 years (19-97). Thirteen patients were in group I (six female and seven male), and a total of 13 patients were diagnosed as being at an advanced clinical stage of disease. There were five patients aged over 60 (38%), but there were nine cases (69%) in which Performance Status (PS) was over 2. Twelve patients (92%) belonged to the higher-risk group, in which International Prognostic Index (IPI) was over 3. As for incidence, there were four (31%), primary mediastinal cases, seven (54%) extra-nodal onset cases, and bulky mass was observed in nine (69%). We were able to review six cases for pathological diagnosis; three were of the GCB type and three were the non-GCB type. On the other hand, 15 patients were in group II (seven female and eight male), 10 cases (67%) were over 60 years of age, but there were only four (27%) cases in which PS was over 2, and finally there were three (20%) in the higher-risk group. Ten cases (67%) had retroperitoneal mass and seven (47%) showed bone marrow involvement. Pathological diagnosis could be reviewed again in six cases; two were the GCB type and four were non-GCB type.

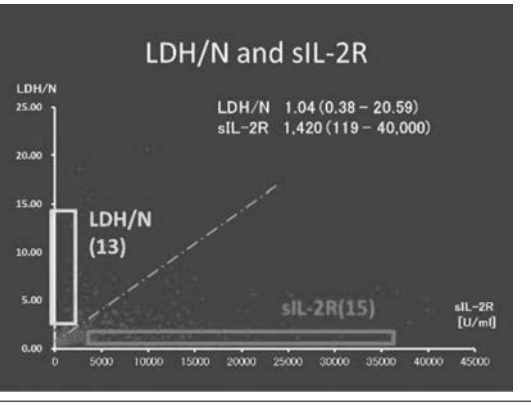

Figure 1.

Summary/Conclusions: DLBCL is a heterogenous disease. By analysing cases of disparity biomarkers between LDH and sIL-2R, we showed the possibility of identifying subgroups.

PB1742

DIFFUSE LARGE B-CELL LYMPHOMA IN THE ELDERLY: REAL-WORLD OUTCOMES OF IMMUNOCHEMOTHERAPY IN ASIAN POPULATION

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Background: The International Prognostic Index (IPI) has been the primary scoring system for predicting the survival in diffuse large B-cell lymphoma (DLBCL). However, the validity of IPI in the era of immunochemotherapy is somewhat uncertain, and the age cut-off for defining "elderly" patients remains especially controversial. Moreover, elderly DLBCL patients frequently do not receive adequate treatment, compromising the chance of complete remission.

Aims: We sought to evaluate real-life outcomes of immunochemotherapy in elderly DLBCL patients from a homogenous Asian population.

Methods: This was a single-center, retrospective study of 192 DLBCL patients over 60 years of age treated with first line rituximab – cyclophosphamide, doxorubicin, vincristine, and prednisone between May 2004 and July 2014. Treatment schedule, adverse events and survival outcomes were analyzed overall and by four age groups (over 60 to 64, 65 to 69, 70 to 74, 75 and above).

Results: Patients of 75 years of age and older were associated with significantly lower complete remission (CR) rate (86.5% *vs* 81.4% *vs* 82.0% *vs* 51%; P<0.001) and higher treatment related mortality (TRM) (5.4% *vs* 9.3% *vs* 13.1% *vs* 33.3%; P=0.001). Advanced age was also related to dose reductions (24.3% *vs* 39.5% *vs* 73.8% *vs* 100%; P<0.001) and less likelihood of completing planned chemotherapy cycle (73% *vs* 79.1% *vs* 78.7% *vs* 51%, P=0.005). Significantly poorer progression free survival (PFS) (3-year PFS rate, 45.9% *vs* 44.2% *vs* 44.3% *vs* 11.8%; P=0.043) and overall survival (OS) (3-year OS rate, 64.9% *vs* 58.1 *vs* 55.7% *vs* 23.5%; P<0.001) were observed for patients aged ≥75 years old. Multivariate regression analyses identified age ≥75 and initial ECOG performance status as potential risk factors associated with OS.

Summary/Conclusions: In conclusion, elderly patients <75 years old or those with better performance status were tolerable to standard immunochemotherapy, with acceptable survival profiles. In Asian elderly DLBCL population, 75 years seems to be judicious cut-off age for predicting the treatment outcomes. Treating patients aged ≥75 years requires more innovative therapy options and not just dose reductions.

PB1743

EXPERIENCE WITH R-MINICHOP IN CLINICAL PRACTICE IN THE OLDEST PATIENTS WITH AGGRESSIVE NON HODGKIN'S LYMPHOMA

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Background: About 40% of aggressive Non Hodgkin's Lymphoma (NHL) are diagnosed in patients older than 70 years. However, the experience in the treatment of this group of patients is very limited. The encouraging results of the GELA group (Peyrade *et al.,* Lancet Oncol 2011) with the R-miniCHOP regimen in patients older than 80 years suggest that a substantial proportion of them can be cured, and therefore it is recommended as the standard therapy in this population.
Aims: The aim of this study is to analyse our experience in using this regimen in elderly patients with aggressive lymphoma.
Methods: We retrospectively analysed patients consecutively diagnosed with aggressive NHL, older tan 70 years, treated with R-miniCHOP regimen (rituximab 375 mg/m2, cyclophosphamide 400 mg/m2, doxorubicin 25 mg/m2, vincristine 1 mg, prednisone 60 mg/day) in our center, from August 2010 to December 2015.

Table 1. Patient's characteristics

Characteristics	n=30
Age, median	81 yo (73-85)
Women	18
Comorbidities	
- Cardiovascular	15
- HBV	4
- HCV	3
- Previous neoplasia	5
Histology	
- Diffuse large B-cell lymphoma	25
- Mantle Cell	2
- Burkitt	3
Stage III-IV	21
IPI	
- Low	3
- Intermediate/low	4
- Intermediate/high	5
- High	18

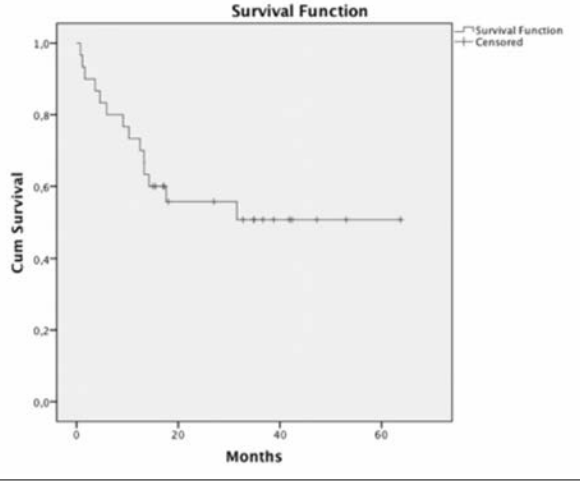

Figure 1.

Results: Thirty patients who received at least 1 course of treatment were included in our analysis. Clinical characteristics are summarized in Table 1. It is remarkable the poor prognosis of our patients, with median age of 81 years, the associated comorbidities and the high IPI. The median of treatment cycles received was 6 (1-8), 50% of cases received 6 cycles. Nine patients received prephase (Vincristine 1 mg and/or prednisone). Four patients received radiotherapy. Most frequent toxicity was hematological (grade 2 anemia and grade 3 afebrile neutropenia), ocurring mostly during the first cycle. Only one death occurred due to treatment toxicity (grade 4 neutropenia and sepsis). In seven patients, at least one cycle was postponed because of toxicity. The overall response rate was 63,3%, with 63,1% of patients achieving a complete response. During treatment, 11 patients experienced progression, 10 died and one reached partial response after salvage therapy with R-GEMOX (5 cycles). One death occurred because of other causes (arterial hemorrhage secondary to traumatism). During follow-up, three patients relapsed, with a median duration of response of 29.3 months. With a median follow-up of 17.15 months, median survival has not yet been reached (Figure 1).
Summary/Conclusions: R-miniCHOP represents an effective and safe regimen in this group of patients, confirming the previous experience of the GELA group. Toxicity observed in the first cycle shows the need of administering prephase in all patients.

PB1744

PROGNOSTIC FACTORS AND TREATMENT OUTCOME OF PEDIATRIC ANAPLASTIC LARGE CELL LYMPHOMA TREATED AT THE CHILDREN CANCER HOSPITAL EGYPT

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Background: Anaplastic large cell lymphoma (ALCL) belongs to the group of high-grade non-Hodgkin's lymphomas (NHLs) and typically presents as an aggressive systemic disease, with or without extranodal involvement. ALCL cells are characterized by the expression of the CD30/Ki-1 and more often by a T-cell phenotype. Frequently; ALCL is associated with the t(2;5)(p23;q35) chromosomal translocation, which gives rise to the fusion gene NPM–ALK. Recent data suggest that t(2;5) positive ALCL respond better to therapy and have a higher survival rate than translocation negative ALCL, thus implying that NPM–ALK expression may have a significant prognostic impact
Aims: The aim of the current study is to report the clinico-epinediologic data, prognostic factors and treatment outcome of pediatric ALCL treated at the Children Cancer Hospital Egypt during 8 years period.
Methods: A retrospective study including all patients diagnosed and treated as ALCL from july 2007 till July 2014 at CCHE. The diagnosis of ALCL was based on established morphologic and immunohistochemical criteria. ALK1 antibody directed to the NPM/ALK protein was used to detect the t(2;5) translocation.
Results: Forty-three patients were enrolled in our study, forming 5.4% of all NHL patients treated at CCHE within this period. They were 26 males (60.5%), and 17 (39.5%) females. Mean age was 11.5 years, median 11.7, range (3.7 to 17.2 years).The most common tumor primary site was generalized lymphadenopathy (62%). ALK status was available in 65% of the cohort (28 patients), of which 75% (21 patients) was positive and 25% negative. Bone marrow was free in all patients, while 5 patients (11.6%) had CNS involvement. Stage II and III were 37.2% each (16 patients), while stage IV was 14% (6 patients). Evaluation of tumor response post induction showed 25 (58%) of patients in complete remission (CR), 15 (34.9%) had partial remission (PR), 2 (4.7%) were progressive (PD), and 1 (2.4%) stationary disease (SD), while at the end of treatment 83.7% were in CR, 11.6% PD and 4.7% relapsed. At the end of the study period, 81.4% (36 patients) were alive, and (8 patients) 18.6% died. The mean FU period was 50months (range 12-96). The 4 years OS and EFS is 80.9% and 71.1% respectively.
Summary/Conclusions: Results of treatment of ALCL in our center are comparable to most of the reported studies. Salvage for relapsing and progressing patients is difficult, and the outcome is extremely poor, hence the need to identify biologic or clinical prognostic factors including minimal residual tumor to better evaluate chemotherapy response.

PB1745

CLINICAL OUTCOME AND PROGNOSIS IN 49 PATIENTS WITH MANTLE CELL LYMPHOMA TREATED WITH ROUTINE CLINICAL PRACTICE

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Background: Mantle cell lymphoma (MCL) is a distinct subtype of non-Hodgkin's lymphoma (NHL) that has commonly an aggressive clinical course and a poor prognosis. Current frontline intensive therapies have improved the outcome for patients (pts). Although these regimens have high response rates, most pts eventually have a relapse. There is not always possible to find a relationship between the intensity of treatment and survival outcomes of pts with MCL.
Aims: To assess the impact of treatment intensity on survival outcomes in newly diagnosed patients with MCL.
Methods: We conducted a retrospective analysis for pts with MCL diagnosed between 2004 and 2014 through our institutional databases. Frontline treatment modalities were categorized into 2 groups: high intensity (R-HyperCVAD or equivalent and/or stem cell transplant) and moderate intensity therapy (R-CHOP, R-B or equivalent). Median progression free survival (PFS) and overall survival (OS) for each treatment group were estimated using the Kaplan-Meier (KM) method and compared using the log rank test. Cox regression was used to identify univariate predictors of survival.

Results: A total of 49 pts MCL were identified. Median age was 58 yrs (31-78), 69% were male, 94% had advance stages and 59% bone marrow involvement; MIPI low (54%), int. (26%), high (20%). High and moderate intensity treatment accounted for 19 (39%) and 30 (61%) of pts. 53% pts in moderate group followed by R maintenance. Intensively treated patients were more likely to be young and have B symptoms (58% *vs* 20% respectively). With a median follow-up of 48 months, median PFS was 42 mos and OS not yet reached. Despite median duration of response with high and moderate intensity treatment was 57 mos *vs* 30 mos, there was no statistically significant difference in PFS (p=0,46) and OS (p=0,16) by treatment strategy. This lack of survival difference was true for all pairwise log-rank comparisons between treatment groups. No significant correlation with OS was observed with age (p=0,56), MIPI risk (p=0,13), or treatment intensity (p=0.30).
Summary/Conclusions: In our retrospective analysis, we were not able to validate the hypothesis, that frontline intensive treatment may improve OS in MCL. These data highlight the lack of a "standard of care" in MCL, and provide further rationale for the use of novel agents in newly diagnosed MCL.

PB1746

PREVALENCE OF LYMPHOPROLIFERATIVE DISEASES IN PATIENTS WITH AUTOIMMUNE DISORDERS

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Background: Lymphoproliferative disorders can be found in patients with previously diagnosed autoimmune disorder.
Aims: We have evaluated type of lymphproliferative disease in population of pts with autoimmune disorders and differences between genders.
Methods: Data from national registry for lymphoproliferative diseases were analysed. From 1992-2015. there was 45pts, male 9(20%) and female 36(80%), median age 57(33-76)years.
Results: 42(93%) of pts previously had autoimmune disesase with average 7,63(0,7-27) years. There were: systemic lupus erythematosus (SLE) 22/36(61%), reumathoid arthritis (RA) 8/36 (22%) and systemic sclerosis (SS) 6/36(17%) in women and SS 5/9 (56%), SLE 2/9(22%), RA 1/9(11%), polimiozitis 1/9(11%) in men.40(89%)pts had non Hodgkin lymphoma (NHL), multiple myeloma (MM) 3(7%) and Hodgkin lymphoma 2(4%). In NHL group 20 were DLBCL, indolent 18- NHL foll. and 2 very agressive.
Summary/Conclusions: The most frequent autoimmune disorder was SLE in women and SS in men.NHL DLBCL was the most common lymphorpoliferative disease. Pts with these condiotions had higher stage of disease at the diagnosis and there was no difference in survival between male and female pts.

Bleeding disorders (congenital and acquired)

PB1747

SUCCESSFUL SEQUENTIAL THERAPY USING RFVII WITH A NOVEL AGENT (PLASMA-DERIVED FACTOR FVIIA AND FACTOR FX MIXTURE) TO CONTROL PERIOPERATIVE BLEEDING IN A PATIENT WITH SEVERE HEMOPHILIA A AND INHIBITORS

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Background: The bypass therapy is usually performed to control bleeding in patients with hemophilia and inhibitors; however, approximately 10% cases are resistant to that using a single agent in the perioperative period. The plasma-derived factor FVIIa and factor FX mixture (pd FVIIa/FX), containing human serum-derived FVII and FX in a 1: 10 ratio, was recently developed in Japan as a novel bypassing agent. The bypass effect induced by FVII is enhanced under a high concentration of FX. We hypothesized that pd FVIIa/FX would induce a stronger bypass effect than conventional sequential therapy using aPCC and rFVII. Here we report our experience of sequential therapy using pd FVIIa/FX and rFVII as a bypass therapy during the perioperative period in a patient with severe hemophilia A and inhibitors.
Aims: To determin optimal dose of of rFVII and pd FVIIa/FX based on a preoperative PK study.
Methods: A pharmacokinetics (PK) study used by pd FVIIa/FX was performed a week before surgery. FX and FVII level was measured 15 minutes, 6 hours and 24 hours after pd FVIIa/FX (120 mcg/kg) was administered. FX level was measured at Kaketsuken Co. (Japan) by activated partial thromboplastin time assays under a high concentration of FVII. The Based on the PK study, FVII and FX activity levels were simulated to determine the optimal dose and interval of rFVII when rFVII and pd FVIIa/FX were administered. Thromboelastography (ROTEM^R) was used to rapidly allow to monitor the coagulation intra-operatively. Case: A 10-year-old male was diagnosed with severe hemophilia A at 8 months old. At 13 months old, inhibitor to factor FVIII (maximum 1625 BU/ml, the most recent: 19.2 BU/ml) was detected. He received immune tolerance induction therapy using rFVIII or VWF/FVIII for 2 years but with no response. At 4 years, he was started on prophylactic aPCC as a bypass therapy. At 10 years, he experienced frequent spontaneous intra-articular hemorrhage, particularly in the left knee and right ankle. Therefore, he was transferred to our institution to receive arthroscopic synovectomy of these joint. When the surgery started, the following sequential therapy was also initiated in accordance with the simulation: pd FVIIa/FX (120 mcg/kg) was administered three times every 36 h and rFVII (60mcg/kg) every 2 h. The interval of rFVII administration was gradually extended. Six days after surgery, the sequential therapy was switched to prophylactic aPCC administration.
Results: 1) Coagulation: The measured activity levels of rFVII and FX were almost the same as the simulation data. The INTEM^{R}assay, in the ROTEM^R, used to monitor the intrinsic pathway showed that coagulation was recovered by sequential therapy. 2) Safety: In accordance with the product document, pd FVIIa/FX (120 mcg/kg) every 36 h was administered and no thrombotic events were detected. 3) Efficacy: Little bleeding was observed during surgery, and no sign of intra-articular hemorrhage was observed on physical examination after surgery. 4) Cost: This regimen contributed to a 45% cost reduction compared with that with single agent bypass therapy using rFVII.
Summary/Conclusions: Sequential pd FVIIa/FX and rFVII as a bypass therapy during the perioperative period is feasible and effective for patients with severe hemophilia A and inhibitors. It is necessary to determine the optimal dose of rFVII based on a preoperative PK study.

PB1748

CONTINUOUS INFUSION FOR LIFE THREATENING HEMORRHAGE OF HEMOPHILIA PATIENT

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Background: Continuous infusion (CI) compare to bolus infusion has a merit of factor concentrate saving as much as 30% with same hemostatic effect. All biologic products will drcrease its activity even *in vitro*.
Aims: According to the instruction of inside package the factor concentrate recommend to administer within 3 hours of reconstitution at room temperature. Universally 24 hours' volume of CI were reconstituted at one time in almost all hospital. We applied this CI for life-threatening hemorrhage with a different method since 1996.
Methods: Thirty five of life threatening hemorrhage or major surgery from 28 patients were enrolled in this study for 15 years. All patients received FVIII con-

centrates with initial loading dose of FVIII 50U/kg and then the continuous infusion, 3 U/kg/hr for 3 days, and then gradually decreased the amount for 2 weeks. We empirically prepared the material of CI with every 2-4 hours reconstitution to keep a desired *in vivo* factor level. To verify of this we checked *in vitro* factor activity of 3 drugs as time goes on recently.
Results: Thirty five events were intracranial hemorrhage in 17, general surgery in 9 and orthopedic surgery in 9. Age distribution was 0-32 yr (mean; 24.8 yr). Severity was severe (16), moderate (7) and mild (5). We confirmed *in vivo* factor activity within permissible level in all patients. All recovered from hemorrhage or surgery and are healthy, but one had limping gate and one had mild neurologic sequelae for more than 10 years. The mean *in vitro* factor activity at 2, 4, 6, 8 and 24hours of reconstitution were gradually decreased to 97.7%, 95.3%, 92.9%, 90.6 and 73.0% respectively in all 3 drugs.
Summary/Conclusions: All biologic products decrease their activity as time goes on even *in vitro*. Empirically, we reconstitute the concentrate every 2-4 hours for CI since 1996. And we confirmed the *in vitro* activity will decrease as time goes on. In continuous infusion we have to reconstitute the concentrate every 2-4 hours to make a desired *in vivo* factor level.

PB1749

THE GENETIC ANALYSIS OF TWO PATIENTS WITH CONGENITAL COAGULATION FACTOR XI DEFICIENCY
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Background: Factor-XI deficiency (FXID) is a rare inherited autosomal recessive disord.
Aims: To analyze genetic mutation and explore its molecular pathogenesis for two patients with congenital coagulation factor XI deficiency
Methods: Two patients were found to have prolonged activated partial thrombin time (aPTT), then their activity of coagulant factors were tested and aPTT mixing test with incubation were performed. Low activity of the FXI (FXI: C) was found in both. Two patients' blood was collected, and their DNA was extracted. Polymerase Chain Reaction (PCR) was used to amplify all exons and exon-intron boundaries, and then the products of PCR were sequenced directjy. The mutation was determined through alignment of the normal FXI genomic DNA sequence by software chromas. The segment with mutation was sequenced backward.
Results: aPTT and FXI:C was 109.6s and 0.4% for patient 1 and 50.1s and 6.7% for patient 2,respectively.The results of aPTT mixing correction test and activities of other coagulant factors were all in normal range for both patients. The results of sequencing showed that a de novel homogenous nucleotide 18A deletion (18delA) mutation in exon2 was detected in patient1, leading frame shift mutation (Val-13TrpfsX15), and that combined heterogenous mutation, a nucleotide 738G>A transition in exon7 and a nucleotide 1021G>A transition in exon9, resulting a Trp228stop substitution and a Glu323Lys substitution,respectively, were detected in patient 2.
Summary/Conclusions: These mutations might be the molecular pathogenesis for two patients with congenital coagulation factor XI deficiency, respectively.

PB1750

A NOVEL FIBRINOGEN VARIANT: DYSFIBRINOGENEMIA ASSOCIATED WITH ΓASP185ASN SUBSTITUTION
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Background: Congenital dysfibrinogenemia is a qualitative congenital fibrinogen disorder characterized by normal antigen levels of a dysfunctional fibrinogen.
Aims: A patient with abnormal coagulation test was diagnosed as congenital dysfibrinogenemia. In this study, the causative mutations responsible for dysfibrinogenemia were detected in proband and her child.
Methods: The proband from Nanjing, China was presented with low fibrinogen and prolonged thrombin time (TT) during prenatal routine medical examination but with asymptoms. Her son also had low fibrinogen and prolonged TT. After extraction, genomic DNA was amplified by polymerase chain reaction. DNA sequencing was performed on the purified PCR products.
Results: Two heterozygous missence mutations including AαArg16His and γAsp185Asn were discovered in the proband. Her son only harbored the former mutation. AαArg16His had been reported by other teams and γAsp185Asn was identified firstly in our study as a novel variant. Endonuclease restriction digestion was performed to exclude polymorphism. The homologous sequences alignment was compared among different species to identify this new mutation site was a kind of conservative one.
Summary/Conclusions: The heterozygous AαArg16His and γAsp185Asn mutations identified in the study probably underlies the dysfibrinogenemia in this pedigree. The novel fibrinogen variant (γAsp185Asn) was named as fibrinogen Nanjing.

PB1751

INHIBITOR TO FIBRINOGEN WITHOUT PROPHYLACTIC THERAPY IN A PATIENT WITH CONGENITAL AFIBRINOGENEMIA. A CASE REPORT
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Background: Afibrinogenemia is a rare condition and has an estimated prevalance of 1:1000000. The more common manifestations of afibrinogenemia are umblical stump bleeding, and bleeding from mucosal surfaces, particularly menorrhagia, epistaxis, gastrointestinal bleeding and intracranial hemorrhage.
Aims: We report a case with congenital afibrinogenemia which had pulmonary and intracranial hemorrhage. Although the patient had not prophylactic therapy with fibrinogen concentrate nor cryoprespitate before admission to the hospital, laboratory investigation revealed mixed test positivity
Methods: A case of an 18 years-old girl with congenital afibrinogenemia admitted to our department because of dyspnea and tachipnea. Her chest X-ray and CT showed pulmonary hemorrhage. Therefore, she was administered with fibrinogen conscentrate in doses of 50 mg per kg and prednisolon in doses 1 mg per kg. Furthermore, she underwent a generalized convulsion on second day and neuroimaging tests revealed intracranial hemorrhage. Although she was treated with fibrinogen concentrate and cryoprespitate every day her laboratory investigation revealed prolonged PT, aPTT and TT tests and fibrinogen level in plasma lower than 0.5g /L. Hence, we performed mixed test and the result was positive. On follow up, she was given IVIG in doses of 2 g per kg once and plasma exchange was planned every day. Furthermore, she was treated with fibrinogen concentrate and cryoprespitate every day to raise fibrinogen level in plasma to 1 g/L. Unfortunately, she died at intensive care unite because of intracranial hemorrhage 17 days after admission to the hospital.
Results: Replacement therapy is generally effective in treating of bleeding episodes in congenital afibrinogenemia. Options for replacement include plasma-derived fibrinogen concentrate, cryoprespitate, and fresh frozen plasma; Antifibrinolytic agents may be used especially for dental procedures. Prophylactic therapy have been employed after life-threatening bleedings such as intrcranial hemorrhage. Acquired inhibitors have been rarely reported after replacement and prophylactic therapy.
Summary/Conclusions: We surprisingly revealed inhibitor to fibrinogen (mixed test positivity) although the patient did not have prophylactic therapy with fibrinogen concentrate nor cryoprespitate before admission to the hospital.

PB1752

ROLE OF HIGH DOSE ASCORBIC ACID IN TREATMENT OF METHAMOGLOBINEMIA
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Background: Methaemoglobin (MetHb) is an oxidized form of haemoglobin. MetHb has high affinity for oxygen, resulting decrease delivery of oxygen to the tissue. It can be congenital but in clinical practice drugs induced acquired form is more common. Methylene blue is used as first line therapy but the role of vitamin C in the treatment of methaemoglobinemia is yet to be established. Here we are presenting our experience with high dose of vitamin C used successfully to treated methamoglobinemia.
Aims: To study the role of high dose of ascorbic acid for treatment of methamoglobinemia.
Methods: This study was conducted over the span of 3 months (Nov 2015-Jan 2016). All patients visiting emergency services with suspected methamoglobinemia were included. In total we found three cases of the proven methamoglobinemia. Patients were given the best supportive therapy including haemodialysis, PRBCs, oxygen supplementation etc. All three patients were given high dose of ascorbic acid (2000mg/day).
Results: Table 1 mentions in detail the demographic details of patients included in the study. Two out of three patients had history of accidental consumption of inciting agent, while the third was prescribed dapsone for immune thrombocytopenia. Case 2 developed massive intravascular haemolysis leading to acute kidney injury requiring haemodialysis (5 sessions). All our patients were normal G6PD activity. Due to unavailability of methylene blue, it was decided to give high dose of ascorbic acid as an anti-oxidant (2000mg/day for 5 days followed by gradual tapering) to all these patients. All patients responded dramatically to this medication and had reduction of Meth-Hb levels.

Table 1. Treatment of cases of methamoglobinemia with 2000mg/day of Ascorbic acid

Age/Sex	Precipitating factor	Baseline Meth-Hb	Baseline disease	Outcome
45/M	Dapsone (300mg/day) for ITP	25	Immune thrombocytopenia	Alive
15/M	Naphthalene ball poisoning	25.5%	None	Alive
25/M	Paint thinner poisoning	46.4	None	Alive

Summary/Conclusions: Higher doses of ascorbic acid (2000mg/day) has proven to be beneficial in cases of methamoglobinemia.

PB1753

PROPHYLAXIS FOR HEMOPHILIA PATIENT IN KOREA

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Background: Prophylaxis compare to on-demand treatment for severe hemophilia patients has a great profit for not only life quality but also life itself.
Aims: We have started prophylaxis for 5 intracranial hemorrhage patients after recovery since 1996. Fifteen years later, prophylaxis for hemophilia A and B patients only under 15 y/o have been approved by National Health Insurance Review and Assessment from January, 2011.
Methods: Intracranial hemorrhage was observed in 16 episodes from 9 patients between 1996 and 2002 at our hospital. All patients received FVIII concentrates with initial loading dose of FVIII 50 U/kg and then the continuous infusion, 3 U/kg/hr for 3 days, and then gradually decreased the volume for 7-10 days. We persuaded the patient/parent for life long prophylaxis to prevent further life-threatening hemorrhage. Five among 8 patients followed the prophylaxis (30 U/kg, 3 days/week).
Results: Age distribution was 2 mo-26 yr (mean; 8.8 yr). Severity was severe (4), moderate (4) and mild (1). Trauma history was in only 1 case. Initial symptom was vomiting (8), headache (4), seizure (3), and irritability (1). Time interval between first symptom and arrival at ER were 15 min to 10 days (mean; 1.7days). Intracranial bleeding site was variety. Inhibitor was in 1 case and 2 cases were transient type. Previous life threatening hemorrhage history was observed in 5 cases (ICH in 4 and GI bleeding in 1). Family history was observed in 7 cases. Eight patients received FVIII concentrates with initial loading dose of FVIII 50 U/kg and then the continuous infusion, 3 U/kg/hr for 3 days, and then gradually decrease the volume for 7-10 days. Inhibitor patient was treated with by-passing agent. Craniotomy was done in 4 cases and no operation was in 4 cases. All recovered except one (who died within 30 minutes after arrival, bleeding site was cerebellar vermis & 3rd ventricle) and are alive without any neurologic sequelae except one with mild limping gate for more than 10 years.
Summary/Conclusions: Prophylaxis for severe hemophilia has to expand to all age, to moderate hemophilia with previous life threatening bleeding history and to inhibitor patients in Korea.

PB1754

VON WILLEBRAND DISEASE AND ACQUIRED VON WILLEBRAND SYNDROME – A DIAGNOSTIC ALGORITHM

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Background: Von Willebrand disease (VWD) is a common inherited bleeding disorder (prevalence of 1:10000) but acquired von Willebrand syndrome (AVWS) is a very rare bleeding state associated with lymphoproliferative, myeloproliferative, cardiovascular and autoimmune conditions, hypothyroidism, uremia, liver cirrhosis, pancreatitis and the use of ciprofloxacin, griseofulvin, valproic acid and tetracycline. More than 60% of the few reported cases of AVWS correspond to lymphoma, multiple myeloma, monoclonal gammopathy of undetermined significance, essential thrombocythemia, polycythemia vera, chronic myeloid leukemia and primary myelofibrosis.
Aims: To establish a diagnostic algorithm for VWD and AWVS, differential diagnosis must be done including other coagulopathies. Factor VIII:C (FVIII:C) and von Willebrand factor (VWF) are endothelium injury markers and their levels may increase in inflammatory diseases. Individuals with blood group O exhibit lower levels of VWF but there is a general raise of VWF with ageing.
Methods: The prothrombin time (PT) and the activated partial thromboplastin time (APTT) are not reliable tests in screening for diagnosis of VWD. Optional tests as thrombin time or fibrinogen are not relevant. So, a basic approach with PT, APTT, and an evaluation of platelet count, morphology and function, must be followed by WVF-specific tests, like a profile with VWF antigen (VWF:Ag), functional VWF (VWF:f) that corresponds to VWF activity (VWF:Act) or VWF ristocetin cofactor (VWF:RCo) and FVIII:C. For inhibitor screening the VWF:f is carried out with several dilutions of patient plasma with factor diluent (the parallelism loss suggests an inhibitor, which can be present in AWVS). Another procedure is the plasma mixing test with patient plasma and normal plasma. Low-dose ristocetin-induced platelet aggregation (RIPA), multimer analysis by agarose gel electrophoresis, molecular genetics, VWF binding to collagen (VWF:CB) and VWF propeptide (VWFpp) by enzyme-linked immunosorbent assay (ELISA) are other resources for specialized laboratories.
Results: The diagnostic algorithm (Figure 1) summarizes our methodology. The Table 1 shows our laboratory results of different types of VWD, AVWS and low VWF.

Table - Laboratory results of different types of VWD, AVWS and low VWF (levels in%)

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
VWF:Ag	35.0	34.0	30.2	9.0	49.1	53.4	13.4
VWF:RCo	33.0	8.0	<7.0 (4.8)	-	45.4	39.1	8.2
VWF:Act	36.0	<19.0 *(10.0)*	-	<19.0 *(1.0)*	40.9	-	<19.0 *(3.2)*
FVIII:C	64.0	53.0	37.7	4.0	54.8	53.5	18.1
VWF:CB	27.2	8.1	-	8.1	-	-	-
RIPA	-		-	Absent	-	-	-
Multimers	Normal	HIMWM	-	All absent	-	-	-
Genetics (mutations)	-	-	R1374H	-	-	-	-
VWF:f parallelism	-	-	-	-	-	-	Inhibitor
Defects	Type 1	Type 2A	Type 2M	Type 3	*Low VWF*	*Low VWF*	AWVS
Gender (M/F)	F	F	M	F	F	M	F
Age (years)	42	33	34	34	35	18	74

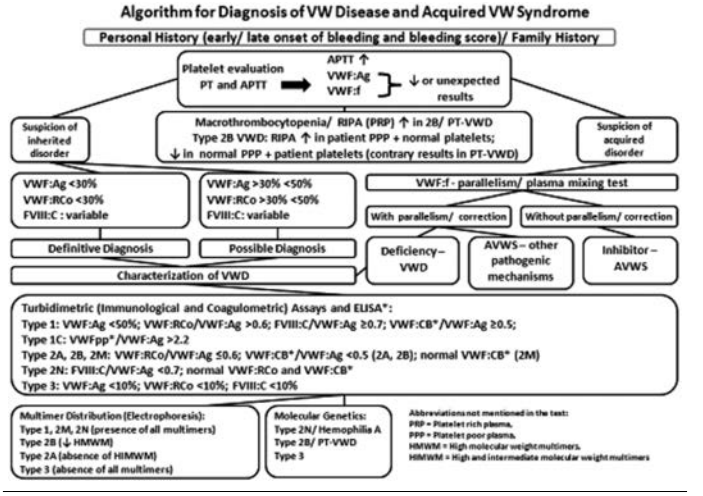

Figure 1.

Summary/Conclusions: 1) We propose an algorithm for the diagnostic approach of mucocutaneous bleeding with high clinical suspicion of VWD, platelet type pseudo-VWD (PT-VWD) and AVWS. 2) The main goal is to create a multistep screening based in threshold values. 3) The differentiation between type 2B VWD and PT-VWD is essential to avoid misdiagnoses and provide adequate therapy. 4) The clarification between type 1 VWD and low level of VWF:Ag and/or VWF:RCo (30-50%) is controversial: asymptomatic individuals don't need an extensive testing, only those with a past severe hemorrhages (a good predictor of recurrence) or presenting relatives with similar levels require an advanced assessment. 5) If VWFpp is available it may be helpful in the identification of type 1C VWD.

PB1755

DIAGNOSTIC CHALLENGES IN ACQUIRED VON WILLEBRAND DISEASE: A COMPLEX CASE OF PROSTATE CARCINOMA

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Background: Acquired von Willebrand disease (AVWD) is a rare bleeding disorder associated with a variety of underlying diseases and with clinical manifestations similar to congenital von Willebrand disease.
Aims: We present a case of prostate carcinoma in which AVWD was diagnosed.
Methods: The patient was an 80-year-old man who suffered from ulcerative colitis. He was diagnosed with a Gleason 7 prostate adenocarcinoma for which he received radiotherapy in combination with hormonal therapy. Due to melena he underwent a coloscopy during which biopsies were taken. The procedure was complicated by diffuse bleeding and a cardiac infarction. Urgent coronary artery bypass surgery (CABG) was performed but was also complicated by postoperative bleeding. Laboratory studies showed a normal platelet count, a prolongation of the activated partial thromboplastin time (aPTT) and low factor VIII levels. His von Willebrand factor (VWF) antigen level and ristocetin cofactor activity were low.
Results: The diagnosis of AVWD in this patient challenged us to look for the different pathogenic mechanisms of the disease. Several disorders have been described to be associated with AVWD, the commonest being hematoproliferative, cardiovascular and autoimmune disorders. Neoplasms are rarely documented and their pathogenic mechanisms are not well known. The underlying mechanisms differ among these disorders and may even overlap. These include, among other things, mechanical destruction of VWF under high shear

stress and development of autoantibodies. Selective and/or non-selective absorption of VWF on or inside malignant cells is believed to be the main mechanism in hematoproliferative and non-hematologic malignancies.
Summary/Conclusions: In this report we give a concise overview of the mechanisms of the diseases that we encountered in this complex case.

PB1756

TAR SYNDROME - DIAGNOSTIC AND TREATMENT. CASE REPORT
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Background: Thrombocytopenia absent radius syndrome (TAR) is a rare genetic disorder that is apparent at birth. TAR syndrome is inherited as an autosomal recessive trait. This disorder is characterized by low levels of platelets in blood (thrombocytopenia) resulting in potentially severe bleeding episodes primarily during infancy.
Aims: Presentation of the case with (TAR) syndrome which was admitted to Hematology Oncology Unit at Pediatric Clinic with petechia and hematomas skin.changes,
Methods: : A 2.5 month old male child, was admitted in our unit with petechial and hematoma's skin changes and epistaxis. Diagnosis of the patient was made with help of the clinical examinations, laboratory findings, radiological images and genetic reaserch.
Results: At the radiological imaging examination of the upper limbs, radius was absent in both shoulders.. Complete blood count: hemoglobin, 11 g/dL; mean corpuscular volume, 95 fl; mean corpuscular hemoglobin, 34 pg; reticulocytic count, platelet count, 20,000/uL; prothrombin time, 12.8 seconds; partial thromboplastin time, 28.0 seconds. Liver function tests had the following results: bilirubin (total), 6.20mg/dL; (direct), 0.25 mg/dL; glutamic pyruvic transaminase, 35 U/L; and glutamic oxaloacetic transaminase, 34 U/L. Electrolytes and kidney function were: Na, 146 mmol/L; K, 3.8 mmol/L; serum creatinine, 0.9 mg/dL. TORCH screens were normal Radiography of the forearm showed bilateral absence of radii. Abdominal and cranial ultrasonography were normal. The genetic analysis was positive.
Summary/Conclusions: TAR syndrome is a rare genetic disorder characterized by low platelet level and radial aplasia. The tranfusions with platelet concentrate is needed when patient is presented with low platelets count.

PB1757

PROPHYLAXIS IN RARE FACTOR DEFICIENCIES OF CHILDREN
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Background: Rare factor deficiencies (RFD) cover the deficiencies of fibrinogen, Factor II, Factor V, Factor V+VIII, Factor VII, Factor X, Factor XI, Factor XIII, and vitamin K associated factors. Many national and international study groups conducted studies regarding the diagnosis and treatment of rare factor deficiencies in 2000s. The existence a mortality and morbidity risk associated with severe bleedings has brought the search for prophylaxes related to these disorders into the agenda. In addition, the good results obtained with primary and secondary prophylaxes in haemophilia cases have attracted the attention to the possibility that the same consideration could also be applied to rare factor deficiencies. Prophylaxis is recommended in these patients if such severe bleedings as central nervous system bleedings, gastrointestinal bleedings, and articular hemorrhages recur.
Aims: Our prophylaxis application related to 13 congenital rare factor deficiency cases being followed at our clinic comprising of five patients with Factor VII deficiency, four patients with Factorxdeficiency, three patients with afibrinogenemia, and one patient with FV deficiency, were evaluated in our article.
Methods: Information concerning our patients was compiled from patient files and the data contained in the electronic information processing environment created after 2005.
Results: Presenting ages of our patients ranged between one week and 7 years of age. Seven of them were boys and 6 were girls. Prophylaxis was applied due to intracranial bleeding to three of our Factor VII deficiency patients, for gastrointestinal system bleeding to one of them, and for the development of chronic hemarthrosis and hemarthrosis that requires the application of radioisotope synovectomy to one patient. 20 mcg/kg active recombinant FVII was used once a week in the prophylaxis of our FVII deficiency cases. Transition to two doses a week was required for one patient. Prophylaxis is being applied with 20 IU/kg prothrombin complex concentrate two days a week to our patients with Factorxdeficiency for central nervous system bleedings and with 50 mg/kg fibrinogen every two weeks to our patients with afibrinogenemia for recurring intramuscular bleedings causing severe pain and difficulty of walking. Our patient with FV deficiency to whom prophylaxis is applied due to intracranial bleeding, on the other hand, receives 20 ml/kg fresh frozen plasma once a week. The prophylaxis periods of our patients range between 2 months and 9 years.
Summary/Conclusions: The observation as well as clinical and laboratory findings of our patients together with the details related to their prophylaxis processes were presented in our proceedings accompanied with the relevant literature.

PB1758

TWO IMPORTANT FACTORS WHICH AFFECT HEMOSTASIS IN HEMOPHILIA PAITENT WITH SURGERY
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Background: The severity and hemostasis in hemophilia is based on one's factor level. In surgery of hemophilia patient appropriate hemostasis is very important for prognosis. Individual pharmacokinetic is quite different especially in recovery rate (RR).
Aims: We evaluate the prognosis of surgical cases according to different recovery rate recently. And we infused the concentrate in bolus followed by continuous infusion method during and after surgery. Also we checked *in vitro* factor activity as time goes on.
Methods: Twenty seven major surgeries were done in 20 patients (severe; 12, moderate; 4 and mild; 4). The mean age was 32 yr. The patient was divided into 3 groups. Group A (A) composed of 12 patients whose RR was above 0.8 with no further correction. Group B (B) composed of 10 patients whose RR are below 0.8 with further correction to make 1.0 before surgery. Group C (C) composed 5 patients whose RR was below 0.8 without further correction because of emergency operation. In continuous infusion we reconstitute the concentrate every 2-4 hours volume because *in vitro* factor activity will decrease as time goes on.
Results: The hospitalization periods of A, B and C were 14.5+/-12.1, 13.9+/-4.0, and 45.8+/-15.7 respectively (p=0,015 and 0.006 in A & C and B & C). The consumption of factor A concentrate of A, B and C were 481+/-195 U/kg, 1,311+/-283 U/kg and 3,502+/-1,529 respectively (p<0,001 in A & B and p=0.006 in B & C). Complications such as hematoma, sepsis CNS infection and hydrocephalus were observed in C. The mean *in vitro* factor activity at 2, 4, 6, 8 and 24 hours of reconstitution were gradually decreased to 97.7%, 95.3%, 92.9%, 90.6 and 73.0% respectively in all 3 drugs. Best activity at 24 hours was observed in light shield sample.
Summary/Conclusions: Although the recovery rate will be changed in different condition and time preliminary check is important for the emergency. In continuous infusion we recommend to reconstitute the concentrate every 2-4 hours to make a desired *in vivo* factor level.

Bone marrow failure syndromes incl. PNH - Biology

PB1759

INTERLEUKIN-27 RS153109 POLYMORPHISM AND THE RISK FOR ACQUIRED APLASTIC ANEMIA IN A CHINESE POPULATION

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Background: Acquired AA, characterized by pancytopenia in peripheral blood (PB) and bone marrow (BM) hypoplasia, is a BM failure syndrome attacked by autologous T cells on BM hematopoietic progenitors. Interleukin-27 (IL-27), as a novel heterodimeric cytokine of the IL-12 family, has been shown to play a vital role in the pathogenesis of aplastic anemia (AA) .The present studies demonstrated that IL-27 enhanced the production of TNF-α and IFN-γ in both $CD4^+$and $CD8^+$T lymphocytes from AA patients, and the elevated IL-27 and IL-27-induced TNF-α and IFN-γ overproduction might be involved in the pathogenesis of AA. Among the IL-27 polymorphisms identified, the SNP of IL-27 rs153109 polymorphism has been reported to be associated with the risk of asthma, chronic obstructive pulmonary disease and inflammatory bowel diseases. To our knowledge, little is known about the role of the SNP of IL-27 in individual susceptibility to AA. To address this question, we have detected the IL-27 rs153109 polymorphism with an aim of identifying site(s) of variation, which may be helpful for better understanding the genetic influences of this gene. We herein performed a case-control study to investigate whether the IL-27 rs153109 polymorphism is associated with AA in Chinese people.
Aims: The aim of the present study was to investigate the association of single nucleotide polymorphisms (SNP) of IL-27 gene with the risk for aplastic anemia (AA).
Methods: *Patients Studied* - The subjects comprised 205 AA patients (109 male and 96 female) with age range of (14–76 years) referred to our hospital, and 196 healthy controls from the employees of our hospital. The characteristics of the subjects are shown in Table 1A. *Extraction of Genomic DNA* - Genomic DNA was extracted using DNA Purification Kit (promega Cat. #A1125 Beijing, China) according to the manufacturer's protocols. *IL-27 rs153109 Genotyping by Polymerase Chain Reaction-restriction Fragment Length Polymorphism (PCR-RFLP)* - The genotyping of the TRAF1/C5 rs10818488 was performed by PCR-RFLP. The upstream primer 5´- ACCAAGAAACCCCATCCTCT -3´ and the downstream primer 5´- TCAGTCAGTGACCAGGATCG -3´ were used to generate a region of 224 bp of IL-27 gene. Genotyping for IL-27 rs153109 SNP was performed by restriction analysis using the PaeR7I restriction enzyme (New England Biolabs), which digests specifically DNA amplified from the G (but not the A) allele into 179-bp and 45-bp fragments. Both undigested and digested PCR products were visualized in 2% agarose gel stained with ethidium bromide. Genotypes were scored blindly, and analyses of all ambiguous samples were repeated. *Statistical Analysis* - SPSS16.0 (SPSS Inc., Chicago, IL) was used for statistics analysis. A *p* value <0.05 was considered to be statistically significant.

Table 1.

A The characteristics of AA patients and the controls.

	AA	Control
Number of patients(%)	205	196
Gender (male: female)	109:96	105:91
Age at diagnosis median (range)	35(14-76)	
Severity of disease		
NSAA, No.(%)	108(52.7)	
SAA, No.(%)	60(29.3)	
VSAA, No.(%)	37(18.0)	
Clinical response, No.	196	
No Response, No.(%)	76(38.8)	
Partial response, No.(%)	67(34.2)	
Complete response, No.(%)	53(27.0)	

B Comparison of IL-27 rs153109 polymorphism between A patients and the controls.

	AA (n=205)	Control (n=196)	*P* value
Genotype frequency (%)			
AA	81(39.5)	75(38.3)	0.653
AG	99(48.3)	98(50.0)	
GG	25(12.2)	23(11.7)	
Allele frequency (%)			
Allele A	261(63.7)	182(46.4)	0.908
Allele G	149(36.3)	198(50.5)	
Genotype frequency			
AA or AG	180 (87.8)	173 (88.3)	0.887
GG	25 (12.2)	23 (11.7)	
Genotype frequency			
AA	81 (39.5)	75 (38.3)	0.799
AG or GG	124 (60.5)	121 (61.7)	

C Comparisons of distributions of the genotype and allele frequencies of IL-27 rs153109 polymorphism between vSAA, SAA, or NSAA patients and the controls.

	vSAA (n=37)	SAA (n=60)	NSAA (n=108)	Control (n=196)	*P* value
Genotype frequency (%)					
AA	15(40.5)	23(38.3)	43(39.8)	75(38.3)	0.967[a]
AG	17(45.9)	30(50.5)	52(48.1)	98(50.0)	0.969[b]
GG	5(13.5)	7(11.7)	13(12.0)	23(11.7)	0.875[c]
Allele frequency (%)					
Allele A	47(63.5)	76(63.3)	138(63.9)	248(63.3)	0.968[a]
Allele G	27(36.5)	44(36.7)	78(36.1)	144(36.7)	0.989[b]
					0.879[c]
Genotype frequency					
AA or AG	32(86.5)	53(88.3)	95(88.0)	173(88.3)	0.761[a]
GG	5(13.5)	7(11.7)	13(12.0)	23(11.7)	0.989[b]
					0.938[c]
Genotype frequency					
AA	15(40.5)	23(38.3)	43(39.8)	75(38.3)	0.795[a]
AG or GG	22(59.5)	37(61.7)	65(60.2)	121(61.7)	0.992[b]
					0.792[c]

a vSAA compared with controls
b SAA compared with controls
c NSAA compared with controls

D Association of IL-27 rs153109 polymorphism in AA patients with response to treatment.

	NR (n=76)	PR (n=67)	CR (n=53)	Control (n=196)	*P* value
Genotype frequency (%)					
AA	30(39.5)	29(43.3)	21(39.6)	75(38.3)	0.903[a]
AG	36(47.4)	31(46.3)	25(47.2)	98(50.0)	0.499[b]
GG	10(13.2)	7(10.4)	7(13.2)	23(11.7)	0.991[c]
Allele frequency (%)					
Allele A	96(63.2)	89(66.4)	67(63.2)	248(63.3)	0.946[a]
Allele G	56(36.8)	45(33.6)	39(36.8)	144(36.7)	0.512[b]
					0.991[c]
Genotype frequency					
AA or AG	66(86.8)	60(89.6)	95(86.8)	173(88.3)	0.748[a]
GG	10(13.2)	7(10.4)	13(13.2)	23(11.7)	0.776[b]
					0.771[c]
Genotype frequency					
AA	30(39.5)	29(43.3)	21(39.6)	75(38.3)	0.703[a]
AG or GG	46(60.5)	38(56.7)	32(60.4)	121(61.7)	0.470[b]
					0.858[c]

a NR compared with controls
b PR compared with controls
c CR compared with controls

Results: In our study, the IL-27 rs153109 polymorphism genotypes between AA patients and healthy controls were first determined and compared. The frequencies of AA, AG and GG genotypes, and A and G alleles were 39.5%, 48.3%, 12.2%, 63.7% and 36.3%, respectively, in AA patients. There was no significant differences in terms of genotype and alleles distributions between AA patients and healthy controls (*P*=0.653 and 0.908, respectively).(Table 1B). Furthermore, AA patients were further subdivided into three groups according to the disease serevity: vSAA, SAA, and NSAA. The distribution of this polymorphism genotype was compared between each of these three groups and the controls, respectively. No statistical differences were identified (Table 1C). We also investigated whether the IL-27 rs153109 polymorphism could influence response to treatment. Nine of 205 (4.4%) patients were lost to follow-up. Responders included complete and partial responders. No significant correlations between the genotype and allele frequencies and response of treatment in this cohort of AA patients were observed (Table 1D).
Summary/Conclusions: In conclusion, IL-27 rs153109 polymorphism may not play an important role as a genetic risk factor in the pathophysiology of AA in a Chinese population. More studies with larger sample are warranted to further confirm the role of IL-27 rs153109 polymorphism in determining the risk of AA.

PB1760

THE DYSFUNCTION OF PLATELETS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Thrombosis is a dangerous complication of paroxysmal nocturnal hemoglobinuria (PNH), with a high mortality rate. However, the mechanism underlying the development of thrombosis in PNH remains unclear.
Aims: To explore the function of platelets in PNH.
Methods: Serum concentrations of the terminal complement complex (sC5b-9) were determined by ELISA and the levels of C5b-9, CD61 and CD62p on platelet membranes by flow cytometry. Clinical parameters were assessed, including D-dimer and platelet aggregation induced by adenosine diphosphate (ADP) and arachidonic acid (ARA).
Results: Serum sC5b-9 concentrations were significantly lower in the PNH/PNH-AA than in the control group ($p<0.01$). C5b-9 deposition was significantly higher on $CD59^-$ platelets than on $CD59^+$platelets in PNH/PNH-AA patients and healthy controls ($p<0.01$ each). D-dimer concentration was significantly higher in PNH/PNH-AA patients, especially those with lactate dehydrogenase concentrations >1000 U/L, than in controls ($p<0.05$). CD61 ($p<0.05$) expression was lower on $CD59^+$platelets in PNH than controls and $CD59^-$ platelets in PNH. CD62p($p<0.01$) expressions were lower on $CD59^-$ and $CD59^+$platelets ($p<0.01$) in PNH than controls. Platelet aggregation stimulated by the agonists ADP and ARA was lower in the PNH/PNH-AA than in the control group ($p<0.05$). Interestingly, CD61 expression on $CD59^+$platelets was higher in PNH patients than in patients with higher type II PNH clones.

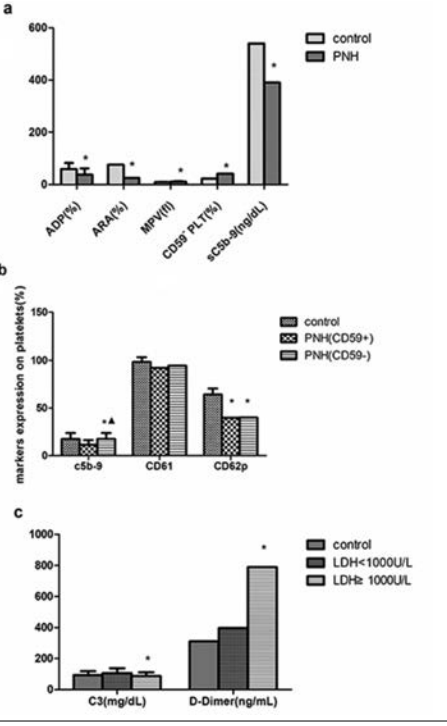

Figure 1.

Summary/Conclusions: Platelet function of platelets, especially of CD59+platelets, was inversely inhibited in PNH/PNH-AA even in the presence of continuous hypercoagulation.

PB1761

IDENTIFICATION OF HAEMOSTASIS SYSTEM PATIENTS, SUFFERING FROM APLASTIC ANEMIA

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Background: Since the first description by Paul Arliam acquired aplastic anemia (AA) over 100 years ago, but today this disease remains in the spotlight hematologists and physicians in adjacent fields. AA - a serious disease of the blood system, resulting from damage to the stem cells (precursors of all blood cells), resulting in a profound inhibition of normal hematopoiesis with the development of pancytopenia. One of the main clinical manifestations is hemorrhagic syndrome caused by thrombocytopenia and impaired activity of plasma coagulation factors: reduction factors of the prothrombin complex, decreased fibrinogen, lack of blood clot retraction. However, some patients with AA, there is a tendency to hypercoagulability, which is not as widely described in the scientific literature as hemorrhagic manifestations.
Aims: The search for diagnostic markers hypercoagulation status in patients with AA.
Methods: The research material was venous blood 25 patients (17 men and 8 women, mean age 38±0,5), with a diagnosis of «aplastic anemia». Patients received standard immunosuppressive therapy without platelet transfusions. Platelet count ranged from 8 to 30x10 9/l. Plasma level of hemostasis was evaluated in the following coagulation tests: activated partial thromboplastin time (APTT), prothrombin time Quick (PT), thrombin time (TT), the concentration of fibrinogen (Fg), the activity of the factor VIII, activity of antithrombin (AT). The control group consisted of 40 clinically healthy men and women of similar age.
Results: Compared to the normal performance of screening tests (APTT, PT, TT, FG) in 18 (72%) of 25 patients reported a significant increase in the activity of factor VIII, which is usually regarded as an indicator hypercoagulation state of hemostasis (189,8%±96,5% against normal value 119,0%±30.5%, p <0,001). In 8 (44%) of 18 patients with elevated factor VIII revealed reduced levels of antithrombin, which amounted to 68% (fluctuations from 74% to 62%). However, clinical manifestations hypercoagulation syndrome (thrombosis and thromboembolism) single patient not were observed.
Summary/Conclusions: Thus, in patients with AA showed increased activity of factor VIII and decreased levels of anti-thrombin. Whether these indicators markers hypercoagulation state, or is it a manifestation of compensatory functions, which prevent severe hemorrhagic complications in this category of patients. Research in this direction continues. Our further studies aimed at clarifying roles in the body of the identified changes

Bone marrow failure syndromes incl. PNH - Clinical

PB1762

ACQUIRED HLA-B*40:02 MUTATION LEADING TO FRAME SHIFT IN DIOPATHIC APLASTIC ANEMIA

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Background: Idiopathic aplastic anemia(AA) has been reported to be associated with autoimmunity to hematopoietic stem/progenitor cells (HSPCs). Specific human leukocyte antigen (HLA) alleles are the target antigens of autoreactive cytotoxic T cells (CTL). One study reported that Japanese patients with idiopathic AA typically inherited only those four HLA alleles such as HLA-A*02:01, HLA-A*02:06, HLA-A*31:01 and HLA-B*40:02. (Katagiri *et al.,* 2011). AA acquired copy number neutral loss of heterozygosity of the 6p arms (6p CN-LOH), leading to loss of one HLA haplotype. Moreover, the missing haplotype was concentrated in the same particular alleles : HLA-A*02:01, HLA-A*02:06, HLA-A*31:01 and HLA-B*40:02. Therefore, 6p CN-LOH may arise from selective pressure to escape from autoreactive CTL through loss of HLA alleles. In the other hand, nonsense mutation in HLA-B*40:02 (CAG to TAG) at codon 54 in exon 2 of HLA-B*40:02 with no involvement of 6p CN-LOH in a case with AA was first reported by Osumi T *et al.,* (Br J Haematol 2013:162:706).
Aims: In this study, we report a patient with idiopathic AA who acquired a mutation in HLA-B*40:02 allele (4 nucleotide deletion, leading frame shift). Our case is the second report of HLA-B*40:02 mutation, to our best knowledge.
Methods: HLA high resolution typing was performed by sequence based typing (Biowithus HLA PCR-SBT, Korea).
Results: A 54-year-old man was diagnosed with severe acquired AA with normal karyotype. Although he received immunosuppressive therapy (cyclosporine and anti-thymocyte globulin) for 8 months, he didn't achieve remission and regular blood transfusions and G-CSF had been required. Therefore, stem cell transplantation was scheduled. His HLA results identified by high resolution sequencing based typing in a peripheral blood sample was HLA-A*26:01, HLA-A*33:03, HLA-C*03:04, HLA-C*07:01, HLA-DRB1:07:01, HLA-DRB1:15:01, HLA-DQB1:02:02, HLA-DQB1:06:02. However, HLA-B could not be easily typed by sequencing based typing. To resolve the problem, we performed the low resolution HLA typing using SSP(sequence-specific primer) method and the result showed HLA-B*40 and HLA-B*44. Furthermore, HLA-B type of one sibling having HLA-A, HLA-C and HLA-DRB1 matching was HLA-B*40, HLA-B*44. We reanalyzed HLA-B high resolution typing using DNA from patient's buccal epithelial cells, patient's peripheral blood and the sibling's peripheral blood. The HLA-B typing of patient's buccal epithelial cells and the sibling's blood showed the same results : HLA-B*40:02, HLA-B*44:03. By a closer examination of sequence electropherogram of patient's peripheral blood, we identified that that 4 nucleotides (ACAC deletion) were deleted between codon 187 and 188 in exon 4 of HLA-B*40:02, leading to a frame shift. The mosaicism of wild type and mutant HLA-B*40:02 alleles was restricted to patient's blood cells, not buccal epithelial cells.
Summary/Conclusions: Acquisition of mutation could not be an accidental event, but is caused by immunological escape and selection, because HLA-B*40:02 is a possible target antigen of T cells in idiopathic AA. Activated CTLs might kill HSPCs that expressed HLA-B:40:02, whereas HSPCs carrying the mutation might survive with a growth advantage over other HSPCs expressing HLA-B:40:02. Our case suggests that mutation (ACAC deletion) of HLA-B:40:02 could be a possible mechanism to restore hematopoiesis via immune escape with no involvement of 6pLOH.

PB1763

HEMATOPOIESIS FEATURES OF APLASTIC ANEMIA PATIENTS WITH PNH CLONES

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Background: One of the reasons for the development of aplastic anemia (AA) is considered immunopositive inhibition of hematopoiesis – cytotoxicity of antigen-specific T lymphocytes and over-production of inflammatory cytokines type I. In addition, AA may be associated with defective hematopoietic stem cells (HSC) and a violation of the regulatory effect of stromal microenvironment. It is known that colony-forming units (CFU) HSC of AA patients drastically reduced and may be absent at all. At the same time in AA patients often recorded the emergence of PNH clones. However, the functional potential of HSCs of the indicated category of patients is characterized by little

Aims: Study of CFU HSC bone marrow of AA patients depending on the presence of PNH clones.
Methods: The research is based on the bone marrow aspirates of 55 AA patients with no PNH clone and 25 patients with PNH clones. CFU HSC was studied using the method of culturing cells in complete nutrient medium Metho-Cult HYY35 on the basis of methyl cellulose. This environment contains all the necessary growth factors and is able to support the growth of erythroid, granulocyte-monocyte and multilinear colonies. The cultivation was carried out in CO2-incubator at t – 37° C for 14 days in Petri dishes with a diameter of 40 mm.
Results: Revealed a sharp decline CFU HSC of AA patients with primarily established diagnosis prior to treatment. The total number of colonies in patients without PNH clones amounted to 57,0 (0-252) 1x105 of myelokaryocytes. At the same time, the number of colonies in PNH patients amounted to 130,0 (8-293) for the same volume of myelokaryocytes. The results suggest that CFU bone marrow HSC in AA patients with PNH clones is significantly higher than in patients without PNH clones. On this basis we can conclude about the relative safety of the proliferative potential of HSC in AA patients with PNH clones.
Summary/Conclusions: Analysis of CFU HSC confirms a defect in the development of progenitor hematopoietic cells in AA. However, in patients with AA with PNH clones the level of violations of the proliferative activity of HSC is expressed to a lesser extent. It is known that the effectiveness of therapy significantly higher in AA patients with PNH clones. Given our data on the functional status of HSC, it is possible to conclude that higher efficiency of treatment of AA patients with PNH clones may be associated with relative preservation of proliferative potential of HSC in this category of patients.

PB1764

COMPREHENSIVE LONG-TERM FOLLOW-UP OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: A REPORT FROM A SINGLE CENTRE
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare life-threatening acquired clonal hematopoietic stem cell disorder characterized by chronic complement activation associated with intravascular hemolysis, thrombosis, and bone marrow failure. Before the availability of anti-complement therapy with eculizumab, treatment remained largely supportive, since the only curative procedure available is allogeneic hematopoietic stem cell transplantation (HSCT), which is associated with substantial morbidity and mortality.
Aims: This study was planned to report on the management and clinical course in a series of PNH patients in a tertiary reference centre reflecting trends in diagnosis and management through the last four decades.
Methods: This descriptive, observational, single centre study evaluated patients diagnosed with PNH between January 1979 and January 2016. An electronic case report form was completed for each patient using clinical data collected from digital or paper medical records. Patients were divided into three groups according to the Parker classification: classic, associated with another bone marrow failure syndrome (BMFS) or subclinical.
Results: A total of 37 patients (mean age 37.2 years±20.6, 20 male (54%)] were included. A large number of diagnoses were made after 2008 (16 patients, 43%), related to improvements in flow cytometry (FC) analysis. The median follow-up time was 6.9 years [IQR: 3.4-15.2]. Nine patients (24%) were diagnosed with classic PNH, 26 (70%) with BMFS; and 2 (5%) with subclinical PNH. Median values for laboratory parameters at diagnosis were: serum lactate dehydrogenase (LDH) 489 UI/L [IQR: 404-1123]; hemoglobin 9.8 g/dL [IQR: 9.1-10.9]; haptoglobin 10 mg/dL [IQR: 7.8-57]; and serum creatinine 0.8 mg/dL [IQR: 0.6-1.1]. At diagnosis 68% of patients presented with pancytopenia. All 20 patients with FC data had GPI-deficient granulocytes (median 5.5% [IQR: 1-31]), as well as GPI-deficient monocytes (median 3% [IQR: 0.5-39.5]). Seven patients had type III erythrocytes (median 2.7% [IQR: 0.4-12]). As expected, FC analysis showed that the GPI-deficient cell clone size was higher in classic PNH than BMFS. A total of 15 patients (41%) required transfusion support. The most frequent therapy was immunosuppression (70%). Twelve (32%) patients, 3 of whom had classic PNH, underwent allogeneic hematopoietic stem cell transplantation (HSCT) at a mean age of 30 years (range 23–37 years). The stem cell source was peripheral blood in eight patients (67%), bone marrow in three (25%) and umbilical cord blood in one. Only two patients received eculizumab (5%). Two patients with classic PNH had a history of severe thrombotic complications (Budd-Chiari syndrome, pulmonary thromboembolism). At last follow-up, eight (22%) patients had died (3 with classic PNH, 5 with BMFS). The leading causes of death were hemorrhage and HSCT-related complications.
Summary/Conclusions: PNH is a rare disease with a highly variable clinical outcome. Improvements in FC techniques have contributed substantially to detect new cases in recent years. Long-term follow-up studies like ours facilitate a better understanding of the natural history of PNH and contribute to improve the management of our PNH patients.

PB1765

CLINICAL CHARACTERISTICS OF CHINESE PAROXYSMAL NOCTURNAL HEMOGLOBINURIA DIAGNOSED BY FLAER
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare bone marrow failure disorder caused by the absence of glycosylphosphatidylinositol (GPI) on the membranes of blood cells. It was claimed that FLAER gave a more accurate assessment of the GPI anchor deficit in PNH .It can detect as few as 1% PNH monocytes and neutrophils in aplastic anemia, that were otherwise undetectable using CD55 and CD59 on RBC's. Therefore, the clinical characteristics of PNH patients diagnosised by FLAER method might be different from previous CD55/CD59 and Ham test used before in China.
Aims: To find the clinical characteristics of Chinese paroxysmal nocturnal hemoglobinuria diagnosed by FLAER.
Methods: The clinical data of 98 cases diagnosed by FLAER methods from September 2011 to March 2014 were analyzed retrospectively, including demography, clinical features, laboratory examination results and complications.
Results: 48 were male and 50 were female, median age 30.5 years (1~74 years), the median follow-up time were 24 months (12 ~30 months). 11 patients with PNH less than 18 years of age belong to adolescents, 6 patients of them were male, median age was 13 years (1~16 years). There were 43 cases of classic PNH (43.9%), 45 of PNH combined with other specific bone marrow disorders (45.9%), 10 cases of subclinical PNH (10.2%).
The clinical features (Table 1) showed many patients with bone marrow failures. Fatigue (71.4%), hemoglobinuria (41.8%), headache/dizziness (38.8%), bleeding (22.4%), and dyspnea (19.4%) were commonly encountered symptoms. Thrombosis in 9 cases (9.18%), 3 cases of deep vein thrombosis, 1 case of pulmonary thromboembolism, 2 cases of mesenteric venous thrombosis, and 2 cases of hepatic portal thrombosis happened in group of classic PNH. Only one case of hepatic portal thrombosis was found in PNH with aplastic anemia. 17 cases cooccurrented with renal impairment respectively, there were 14 patients (14.5%), 16 patients (16.3%) and 1 patient (1%) at stage of CKD1, CKD2, and CKD3 respectively. Only 2 cases of PNH were suffered with pulmonary hypertension. The estimated OS at 3 years after diagnosis as 82.0%, 11 patients who died during follow-up period. Comparison of OS among the three subcategories was no statistical significance(P=0.978). Univariate analysis revealed that thrombocytopenia (P=0.048),abdominal pain(P<0.001), thrombotic events (P<0.001),and recurrent infections(P<.001) were of risk factors affecting survival. Multivariate analysis further confirmed that thrombotic events and recurrent infections remained to be statistically significant risk factors affecting survival (Table 2).

Table 1. Clinical features of patients with PNH.

Characteristic	Classic PNH	PNH SBMD	Subclinical PNH	Total
Hemoglobinuria	30(69.8%)	11(24.4%)	0 (0%)	41 (41.8%)
Abdominal pain	4 (9.3%)	2 (4.4%)	0 (0%)	6 (6.1%)
Dysphagia	4 (9.3%)	0 (0%)	0 (0%)	4 (4.1%)
Headache/ dizziness	13(30.2%)	25(55.6%)	0 (0%)	38 (38.8%)
Pectoralgia	0(0%)	1(2.2%)	0(0%)	1 (1.0%)
Back pain	3(7.0%)	0(0%)	0 (0%)	3 (3.1%)
Dyspnoea	9(20.9%)	10(22.2%)	0(0%)	19 (19.4%)
Bleeding	2 (4.7%)	19(42.2%)	1 (10.0%)	22(22.4%)
Fatigue	30 (69.8%)	34 (75.6%)	6 (60.0%)	70 (71.4%)
Infections	0 (0%)	9(20%)	1(10%)	10 (10.2%)

PNH with another specified bone marrow disorder PNH SBMD

Summary/Conclusions: Bone marrow failure is frequently found in the Chinese Patients with PNH. Renal, liver impairment and thrombosis in rare sites was relatively common. But pulmonary arterial hypertension happened seldom. Thrombotic events and recurrent infections influenced survival of patients with PNH.

PB1766

PREVALENCE AND PATTERNS OF TREATMENT OF APLASTIC ANEMIA IN KOREA
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Background:
Aplastic anemia (AA) is a relatively rare bone marrow failure syndrome and

has the wide variety of cytopenia spectrum from transfusion-independent mild cytopenia to severe pancytopenia that needs for allogeneic hematopoietic cell transplantation (HCT). However, the exact incidence of AA in whole-nation-wide survey and the whole picture of treatment pattern is extremely rare.
Aims: We aimed to figure out the real incidence and treatment pattern of AA in Korea as a nation-wide database analysis. Also we wanted to know whether incidence and treatment had regional variation.
Methods: We collected AA patients from Korea National Health Insurance Service (KNHIS) database. The minimal requirements for the diagnosis of AA were registation to a rare/difficult to treat disease as AA and performance of bone marrow biopsy at the time of registration. Newly diagnosed patients were defined as a new entry to registration as AA. Treatment were collected from the each drug prescription. Region was categorized into 4 areas according to city size; capital city, metropolitan city, samll city and rural area.
Results: Data between year 2008 and 2012 were collected from KNHIS database. The annual prevalance of AA was 8.6, 15.3, 20.5, 22.9 and 23.6 (average 17.5) patients per million (PPM) in years 2008, 2009, 2010, 2011, 2012, respectively. Prevalence of male (average 17.5 PPM) and female (average 18.9 PPM) were similar. Prevalence increased as the age increased: average 12.9 PPM in younger than 10y old; average 25.5 PPM in older than 80y old. Reginal differences were found: average 17.3 PPM in capital city, 17.7 PPM in metropolitan cities, 12.9 PPM in small cities and 24.2 PPM in rural areas. The crude incidence rates (CIRs) were similar in recent 5 years: 6.8 PPM in 2008, 8.9 PPM in 2009, 9.5 PPM in 2010, 9.4 PPM in 2011 and 8.4 PPM in 2012. There were age differences in CIRs. CIR in elderly population was more than 2 times higher when comparing with younger population: average 5.4 PPM in population younger than 10y; average 13.2 PPM in elderly population older than 80y. Average CIRs in reginal categories were 8.6 PPM in capital city, 8.3 PPM in metropolitan cities, 6.1 PPM in small cities and 10.8 PPM in rural areas. Treatments of newly diagnosed AA patients were follows: transfusion in 74.1%, cyclosporin in 34%, thymoglobulin in 26.4%, G-CSF in 44.6% and HCT in 12.5% . Thymoglobulin and HCT were received 34.5% and 21.5% of newly diagnosed AA youger than 40y, but the rates were droped to 16.8% and 4.5%, respectively in older than 40y patients.
Summary/Conclusions: Korea has high CIR of AA than wetern countries. CIR increases as age increases. Rural areas has higher CIR than small cities. The treatment pattern was different between younger age group and older age group as expected.

PB1767

APLASTIC ANEMIA IN CENTER TUNISIA
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Background: Aplastic anaemia is a rare but very serious blood disease, combining a central pancytopenia and bone marrow hypocellularity. Its etiology remains mostly unknown. Treatment is either allogeneic hematopoietic stem cell or on immunosuppressive therapy.
Aims: 1- To analyze the epidemiological, clinical and laboratory patients. 2-. Evaluate the terms and therapeutic results. 3- To study the different responses and survival depending on the treatment administered.
Methods: This is a retrospective study conducted over 14 years (January 2000 - December 2013) which included 95 patients with acquired aplastic anemia, diagnosed and treated at the hematology department of the University Hospital Farhat Hached hospital Sousse.
Results: The median age of patients was 32 years. The sex ratio was 1.26. At diagnosis, all patients were symptomatic. An anemic syndrome associated with hemorrhagic syndrome was the main factor of discovery (44.2%). At the initial blood count, pancytopenia was observed in 92.6% of patients. The myelogram showed a poor marrow in 85.2% of patients. A bone marrow biopsy, bone marrow was wealth 0-1 95.8% of patients. Myelosuppression was, moderate in 33 patients (34.7%), severe in 26 patients (27.4%) and severe in 36 patients (37.9%) by the score of Camitta. Fourteen patients underwent a bone marrow allograft. One patient received the anti-lymphocyte serum in combination with ciclosporin. Immunosuppressive treatment with cyclosporin alone was undertaken in 51 of our patients. Forty-two patients received androgen therapy, prescribed alone or in combination with ciclosporin or corticosteroids. At the end of our study: 35 patients (36.8%) were in CR, 6 patients (6.3%) in PR, 14 patients (14.7%) and RM 39 patients (41.1%) in Failure . One patient was lost to follow just after diagnosis. The factors that significantly influenced the response were the severity of myelosuppression, sex, hemoglobin, the rate of white blood cell, the neutrophil rate and allograft. The overall survival rate at 2 years was 47.3% at 5 years and 44.9%. The allograft is the most significant prognostic factor for survival.
Summary/Conclusions: Despite progress regarding treatment protocols, changes in the Aplastic anaemia is often fatal. The overall mortality rate in our series is due to the problem of access to specific therapies, namely the allogeneic hematopoietic stem cell and lymphocyte serum.

PB1768

MONITORING OF SMALL PAROXYSMAL NOCTURNAL HEMOGLOBINURIA CLONE IN PATIENTS AFFECTED BY SEVERE APLASTIC ANEMIA OR APLASTIC ANEMIA
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Background: Flow cytometry is the gold standard method for screening of paroxysmal nocturnal hemoglobinuria (PNH). The most reliable marker to detect and monitor small granulocyte or monocyte PNH clones is FLAER (fluorescent aerolysin), especially in conditions such as myelodysplastic syndromes or bone marrow failure, when traditional GPI-linked surface marker expression can be significantly altered. More than 50% of aplastic anemia patients have a PNH clone detectable by highly sensitive assays, although the underlying mechanism is yet to be unveiled.
Aims: Monitoring of small PNH clone in patients followed at our Institute for severe aplastic anemia or aplastic anemia.
Methods: Between May, 2011 and Feb, 2016, sixteen patients with severe aplastic anemia (sAA)/ aplastic anemia (AA) provided 52 blood samples for PNH flow cytometry at different time points in the course of their evaluation and treatment. We performed serial quantification of the PNH clone size by multiparameter flow cytometry using a Navios cytometer (Beckman Coulter) and Navios software. The immunophenotype evaluation is performed on EDTA whole blood samples. Granulocytes, monocytes and red blood cells (RBCs) were gated using forward and side scatter as well as lineage-specific markers. The GPI-linked markers FLAER and CD59 were comparatively evaluated. Neutrophils and monocytes were screened with FLAER/CD24/CD14/CD33/CD45 combination. The sensitivity of detection allowing identification of PNH clone sizes as small as 0.01% for RBCs and 0.03% for granulocytes.
Results: Eight of 16 sAA/AA patients were detected to have PNH clone positivity. All of them were treated with standard immunosuppressive therapy such as antithymocyte globulin and cyclosporine (ATG/CsA) or CsA alone and two non responder patients received allogeneic stem cell transplantation (alloSCT) after myeloablative conditioning. Granulocyte PNH clone was <1% in 4 patients, 1-5% in 3 patients and >30% in one patient. Lactate dehydrogenase (LDH) values were normal in all patients except the one with PNH clone >30%, who had clinical evidence of hemolysis. Haptoglobin level was normal in 5 patients. Two patients with PNH clone 1-5% had very low concentration of haptoglobin, without altered LDH values. These were patients who did not respond to ATG/CsA and received alloSCT. Direct Antiglobulin Test (DAT) were negative in all patients. PNH clone sizes were stable during immunosuppressive therapy courses with a median follow up of 42 months (3-60), while PNH clone became undetectable after alloSCT and haptoglobin levels returned to normal values (respectively 6 month and 36 month follow up).
Summary/Conclusions: Whether the presence of a small PNH clone in the setting of hypocellular marrow failure has clinical significance or predicts response to treatment and outcomes is still controversial, an accurate monitoring of PNH clone with high sensitive assays and hemolysis parameters might be useful to clarify the pathophysiology of these complex and rare diseases.

Chronic lymphocytic leukemia and related disorders - Biology

PB1769

RARE RECURRENT CHROMOSOMAL ABNORMALITIES IN CLL DETECTED BY CONVENTIONAL CYTOGENETICS AFTER STIMULATION WITH CPG-ODN & IL-2 AND FISH

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Background: Characteristic chromosomal abberations (*i.e.* 11q, 13q, 17p, and+12) play crucial role in chronic lymphocytic leukaemia (CLL) as important idenpendent prognostic factors. Combination of FISH and conventional chromosome banding (CBA) after stimulation with CpG oligonucleotides (CpG-ODN) and interleukin-2 (IL-2) allowed to capture increased number of chromosomal anormalities. We focused on rare recurrent chromosomal aberrations (RCA) that can provide new prognostic information in CLL.
Aims: Metaphase cytogenetic and molecular-cytogenetic analyses were performed in 766 CLL-patients from 2008 to 2015 (293 female, 473 male, median age at the time of analysis: 67 years) to evaluate the incidence of RCA.
Methods: CBA was performed on peripheral blood or bone marrow samples cultured in medium with stimulants. FISH was performed on unstimulated cells for detection of trisomy 12, del(11q), del(13q), del(17p). RCA were confirmed by interphase FISH (I-FISH), multicolor FISH or multicolor banding.
Results: A total of 714/766 (93,2%) cases were succesfully stimulated for metaphase analysis. Chromosomal aberrations were detected in 600/714 (84,0%) of patients by combining both methods. RCA were detected in 144 cases (144/714, 20,2%) and they were as follows: 3,5% rearrangement of *IGH* (translocations partners 2, 4, 8, 9, 11, 12, 13, 16 and 19), 3,4% gain of 2p, 3,4% del(6q), 2,5% del(14q), 1,7% gain of 8q (*MYC* gene), 1,3% del(8p), 1,3%+18, 1,1%+19, 0,6% del(5q), 0,6% t(18;22), 0,4% gain of 12q, 0,4%+21 and 0,1% t(2;18).
Summary/Conclusions: Cytogenetic results are highly relevant in defining the prognosis of CLL patients. I-FISH as gold standard for detection of important chromosomal aberrations and CBA after stimulation with cocktail of CpG-ODN and IL-2 are efficient tools (methods) in routine diagnostic (analysis) of chromosomal aberrations. The importance of RCA in CLL will be discussed in the poster.

PB1770

OXIDATIVE STRESS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Oxidative stress (OS) is an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. Extensive evidence has shown that disturbances of oxidative metabolism are a common feature of transformed tumor cells. However, the effect of OS levels in chronic lymphocytic leukemia (CLL) is largely unknown.
Aims: To investigate the oxidative stress status in CLL and analyze the relationship with the disease status and prognostic factors evaluated at diagnosis.
Methods: We analyzed peripheral blood samples from 76 patients with untreated CLL evaluated at diagnosis (48 male and 28 female; mean age 67 years; range 36-90 years; 57 patients with A Binet stage, 15 patients with B stage, and 4 patients with C stage) and 20 healthy individuals as matched control group. Serum total antioxidant capacity was determined by performing the d-ROMs test, whose chemical principle is based on the ability of a biological sample to oxidize N,N-diethylparaphenilenediamine (normal range 250-300 U CARR, where 1 U CARR is equivalent to 0.8 mg/L H2O2), while serum OS was assessed by means of BAP test, which measures the ability of a serum sample to reduce iron from the ferric to the ferrous ionic form (optimal value >2.200 micromol/L reduced iron).
Results: A significant oxidative damage was showed in CLL patients (d-ROMs test: 407 U CARR±194; range 194 - 794; BAP-test: 2.033 micromol/L±310.6; range 1.024 – 3.300) with respect to normal controls (d-ROMs test: 259.6 U CARR±87.5; range 158 - 425; BAP-test: 2.505 micromol/L±306; range 1.876-3.045) (p 0.0014 and 0.0001, respectively). However, no correlations were found with absolute lymphocytosis, haemoglobin and platelet levels, Binet disease stage and cytogenetics abnormalities (evaluated only in 52 patients) detected by FISH (normal *vs* del17p *vs* del11q *vs* trisomy12 *vs* del13q14). Unmutated IgVH CLL patients displayed significantly higher d-ROMs test values than mutated IgVH patients (p 0.0057). On the contrary, no differences were found in BAP test according to mutational IgVH status. At a mean follow-up of 50 months (range 6-211 months), 16 patients progressed needing treatment. d-ROMS test was found significantly higher at diagnosis in this cohort of patients with respect to non progressed patients (501 U CARR±135 *vs* 385±149; p 0.006). No differences were also found with respect to BAP test. Finally, no correlations were found between time to treatment and both d-ROMS and BAP test levels.
Summary/Conclusions: CLL patients show oxidative stress features (increased oxidative damage, evaluated by d-ROMs test, and reduced antioxidant capacity, measured by means of BAP test) which are usually irrespective of clinical and biological characteristics detected at diagnosis, reflecting constitutive CLL findings, rather than disease burden and activity. In particular, differently from a previous observation (Collado R *et al.,* 2014), no correlations were found in our cohort of patients between increased oxidative damage and unfavourable cytogenetics subgroups. Our data support the investigational use of antioxidants in the clinical setting.

PB1771

THE ROLE OF CD38, CD23, CD200 AND CD43 EXPRESSION IN DISCRIMINATION OF CHRONIC LYMPHOCYTIC LEUKEMIA AND MANTLE CELL LYMPHOMA

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Background: Chronic lymphocytic leukemia (B-CLL) and mantle cell lymphoma (MCL) share many features and their differential diagnosis may be challenging, especially when a leukemic picture alone is present. Monoclonal antibody panels are often useful, with CD23 being the most reliable. In the CD5+/CD10- subgroup cases with CD23+cells are usually classified as CLL, whereas CD23 – cells are diagnosed as MCL. However, the immunphenotypic profile of MCL cells is a quite heterogeneous, and cases of CLL and MCL can frequently overlap to a significant degree. MCL diagnosis should be confirmed by immunohistochemical cyclin D1 detection, sometimes with equivocal or even negative results. Other cytofluorimetric, cytogenetics or molecular techniques are reliable but not widely available.
Aims: B-CLL leukemic cells express CD23, CD22, CD38, CD79b, CD200 and CD43 antigens may improve the distinction between CLL and MCL cases. Both CD200 and CD43 are used in clinical cytometry laboratories for a long time now,but need for harmonization
Methods: We investigated by 5 color multiparametric flow cytometry 357 patients (241 males and 116 females; median age of 68±10,6 years) diagnosed with B-cell LPD were studied. According to the WHO criteria (R), patients were grouped as follows: 308 cases CLL,31 cases MCL, 18 cases HCL . Median PB lymphocyte counts at diagnosis were of 19.8×10^9 lymphocytes/l (range: 0.8–274×10^9 lymphocytes/l). Diagnosis of all MCL was confirmed by immunohistochemisrty detection of cyclin D1 or detection of t (11:14) by FISH. The monoclonal antibodies (MoAbs) used for flow cytometry labeling were obtained from the Beckman Coulter Company (BC, Florida, USA). CD45, CD79b, CD3, CD5 FITC, CD10 PE, CD11c, CD19, CD20, CD22, CD23, CD25, CD38, CD103, FMC7, CD43 (clone DFT1), Kappa, Lambda, CD200 (clone OX2, Becton/Dickinson Biosciences-BD, USA) MoAbs were used for this purpose
Results: We found in one CLL case CD200 negative, and one case CD43 negative. However we have not observed any CD23 negative in all CLL cases. We observed 10 MCL cases CD23+, 3 cases CD200 positive and 2 cases CD43 positive. CD200 appeared a valuable marker in the differential diagnosis of CLL and MCL (p<0.001). As for CD43 expression in CLL, it was positive in 297 (96.4%) cases but negative in only 11 (3.6%) cases while it was negative in 20 (64.5%) and positive in 11 (38.5%) cases. CD43 was significantly different between CLL and MCL. In the MCL group, the proportion of the CD43 cases was significantly higher (p<0.001), CD38,CD22,CD79b are not significantly.

Summary/Conclusions: We concluded that although CD 23, CD22, CD79b, CD38 is not a useful marker in discrimination of CLL and MCL, CD43 and CD200 used together in routine panels could be of diagnostic utility in excluding MCL diagnosis. However, as with other markers, the use of these markers may also be flawed by heterogeneous distribution and variable expression. Therefore, standardized sampling and staining for flow cytometry and specific techniques should be primarily defined, and then followed by the evaluation of these markers and harmonization studies in larger and more diverse patient populations.

PB1772

CD38 AND INTERLEUKIN 6 GENE POLYMORPHISM IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA- CORRELATION WITH CLINICAL AND LABORATORY PARAMETARS

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Background: CD38 receptor and interleukin 6 (IL-6) level increases proliferation and survival of chronic lymphocytic leukemia (CLL) cells and increased CD38 expression as well as IL-6 level are a negative prognostic marker CLL patients. CD38 and IL-6 have a genetic polymorphism characterized by a C>G variation.
Aims: This study was designed to examine the influence of CD38 (184C/G; rs6449182) and IL-6 (-174 G/C; rs1800795) gene polymorphism on laboratory and clinical parameters in chronic lymphocytic leukemia (CLL) patients.
Methods: Genotyping polymorphism is performed using restriction fragment length polymorphism-polymerase chain reaction for CD38 (184C/G) and IL-6 (-174 G/C) in 44 CLL patients and 5 healthy subjects. We separated CLL patients in three groups: 1) the G allele for IL-6 and C allele for CD38; 2) the C allele for IL-6 and CD38; 3) the full fragment for either IL-6 or CD38. The plasma level of VEGF was measured using immunoassay. Kaplan-Meier method was used for estimating survival rates.
Results: A higher prevalence of the C allele was found both in CD38 (184C/G, 84%) and IL-6 (-174 G/C, 66%) gene polymorphism of CLL patients. Furthermore, we found a lower level of CD38-positive cells in group 1 of CLL patients. The group 3 has significantly higher level of thrombocytes and lactate dehydrogenase (425 U/l, p<0.05) than group 2. The group 1 has increased beta 2 microglobulin in comparison of group 3. According to previous reports for a strong correlation between CD38 and VEGF expression, we found increased levels of plasma VEGF (med 50, range 16 - 438 ng/mL) in CLL patients *vs* control subjects and in group 1 of CLL *vs* other two groups (med 79 *vs* 50 and 53 ng/mL in groups 2 and 3, respectively). According to the pattern of bone marrow infiltration, the diffuse type was the most present in group 1 (33%), while the nodular/interstitial was dominant in group 3 (91.7%). Furthermore, patients in the group 3 demonstrated the most favorable clinical stage according to Binet (A - 91.7%) and Rai (0 – 75%). Regarding CD38 expression, there was no significant difference between groups, although patients with C allele exhibited reduced CD38 expression (12% *vs* 25%). Mean overall survival was 97, 101 and 82 months in group 1, 2 and 3, respectively without statistical significance. Median survival in group 1 and 3 was 65 and 79 months, respectively, while in group 2 was not reached . No statistical significant difference in survival among three groups was noted (p=0.90).
Summary/Conclusions: Presented results revealed that CLL patients with the full fragment for either IL-6 or CD38 genes tend to have more favorable clinical and laboratory parameters at diagnosis, but do not exhibit survival advantage.

PB1773

THE EXPRESSION OF TOLL-LIKE RECEPTORS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA.

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Background: B-cell chronic lymphocytic leukemia (B-CLL) presents with progressive accumulation of monoclonal B-cells in the peripheral blood, bone marrow and lymphoid organs. B-CLL is characterized by heterogenous clinical outcome. Some patients have an indolent disease and the others have an aggressive clinical course of B-CLL. Toll-like receptors (TLRs) play an important role in B-cell activation and maturation and may be involved in the pathogenesis of B-cell lymphomas. TLRs are essential receptors of the innate immune system and key regulators of the acquired immune system. TLRs are mainly expressed in human immune related cells such as monocytes, neutrophils, macrophages, dendritic cells, T cells, B cells and NK cells. The expression of TLRs and their association with other prognostic factors in B-CLL patients remains unclear.
Aims: The aim of our study was to evaluate the expression of TLR2, TLR4 and TLR9 and their significance as biological markers in patients with B-cell chronic lymphocytic leukemia.
Methods: 60 patients with newly diagnosed B-CLL were evaluated (29 females and 31 males). The median age of patients was 68 years (range: 52-88 years). The diagnosis was performed according to the IWCLL/NCI criteria for CLL. 40 patients (67%) had early-stage CLL (Rai stage 0-II) and 20 patients (33%) had advanced-stage CLL (Rai stage III and IV). All patients were either untreated. The healthy control group included 14 age-matched individuals (7 females and 7 males). Using quantitative reverse transcriptase PCR, the mRNA expression of genes TLR2, TLR4 and TLR9 was measured. The relative quantitation was indicated by cycle threshold (Ct) values. The Ct value of the target genes was normalized (ΔCt) to the Ct value of the GUS gene of the samples. The results were statistically analysed using 'STATISTICA 8,0'. Statistical analysis was performed by means of Mann-Whitney's U-test and p<0,05 indicated a significant difference. Overall survival (OS) was determined using Kaplan-Meier method. The long-rank test was used to compare the curves.
Results: In comparison to control group TLR2 and TLR4 mRNA expression was higher in B-CLL patients than in healthy individuals (ΔCt TLR2 6.46±9.58 *vs* 0.98±0.43 and ΔCt TLR4 11.91±70.22 *vs* 2.21±0.32)(p<0.05). TLR9 mRNA expression was higher in control group than in patients with B-CLL (ΔCt TLR9 23.65±16.29 *vs* 3.35±1.93) (p<0.05). We found that patients with higher mRNA expression of TLR9 had significantly longer OS than patients with lower mRNA expression of TLR9 (p<0.05).TLR2 mRNA expression was higher in patients with advanced-stage CLL (Rai stages III and IV) than in patients with early-stage disease (Rai stages 0-II) (ΔCt TLR2 19.10±17.76 *vs* 7.94±6.25) (p<0.05). Moreover we observed that expression of TLR2 in patients with anemia was significant higher than in patients without anemia (ΔCt TLR2 17.18±14.29 *vs* 7.10±5.98) (p<0.05) (Figure 1).

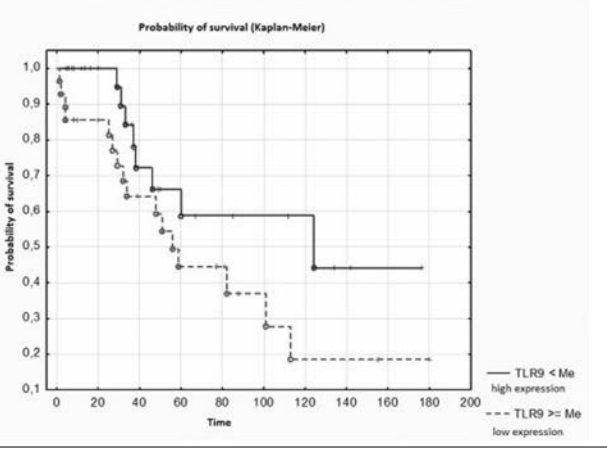

Figure 1.

Summary/Conclusions: In conclusion, our results suggest that TLRs could become new potential biological markers for the clinical outcome in patients with B-cell chronic lymphocytic leukemia. However, this observation should be validated by a larger study.

PB1774

AGING AND PD-1 & PD-L1 GENE EXPRESSION: MARKERS OF IMMUNOSENESCENCE?

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Background: Aging is characterized by a progressive decline in immune surveillance that favors infection and cancer development. Cancer cells can escape immune surveillance by upregulating inhibitory immune checkpoint such as PD-1 and PD-L1. High surface expression of PD-1 was reported in association with T-cell exhaustion. The PD-1/PD-L1 axis inhibits the immune response leading to anergy of lymphocytes.
Aims: In this study, we examined the expression of PD-1 and PD-L1 genes in lymphocytes subsets according to age.
Methods: Lymphocyte subsets (CD3+CD4+; CD3+CD8+, CD19+) were isolated using the MACS isolation system from PBMC of healthy donors (HD) and Chronic Lymphocytic Leukemia (CLL) patients. PD-1, PD-L1, IL4 and IFNɣ were quantified by qRT-PCR for each purified lymphocyte subset.
Results: PD-1, PD-L1, IL4 and IFNɣ gene expression was studied using qRT-PCR to evaluate 28 HDs [14 HDs under 50y (median-: 38) and 14 HDs above 50y (median: 62)] compared with 23 untreated CLL patients (median age: 69). The purity of each lymphocyte subset was 95%>99%. PD-1 was significantly

up-regulated in most lymphocyte subsets in the older group: CD4+, CD8+T cells and CD19+B cells (p=0.015; 0.02 and 0.01, respectively). Compared with a similar age group of HDs, PD-1 expression was strongly upregulated in CD4+, CD8+and CD19+subsets of CLL patients (p=0.001; 0.002 and <0.001). High PD-L1 expression was correlated with age in the normal B lymphocytes (p=0.046). The leukemic B cells also expressed higher levels of PD-L1 than circulating normal B cells as compared with the older group of healthy donors (p=0.001). In order to further investigate the PD-1/PD-L1 axis contribution to T cell dysfunction, we have checked for the expression of IL4 and IFNɣ genes in CD4+and CD8+lymphocytes subsets, respectively. A significant down-regulation of IL4 and IFNɣ (p=0.008 and 0.001) in CLL patients was also demonstrated.
Summary/Conclusions: 1) PD-1 and PD-L1 gene expression is correlated with aging 2) Upregulation PD-L1 is even more pronounced in leukemic B cells, suggesting a potential benefit of anti PD-1/PD-L1 drugs in CLL patients 3) In CLL patients, upregulation of PD1 and lower expression of IL4 and IFNɣ gene are observed in CD4+and CD8+T cells.

PB1775

NEXT GENERATION SEQUENCING (NGS) ION TORRENT AMPLISEQ-TM TECHNOLOGY FOR THE IDENTIFICATION OF TP53 MUTATIONS SCREENING OF 39 CASES OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
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Background: NGS technology allows to study the molecular genetics of CLL through the comprehensive detection of genetic lesions.
Aims: We describe the application of a NGS panel based on Ion AmpliSeq™ technology to analyze the full coding region of *TP53* (ex 2–11) in a single workflow.
Methods: Purified B-cells, from 39 therapy-free CLL patients (pts) visiting our outpatient clinic. Library preparation and sequencing were performed on the Ion Torrent™-PGM platform (Thermofisher) according to the manufacturer's protocols. For each run, 10 pooled samples were loaded on a 316chip and sequenced (flow rate 500x). Data processing, filtering and base calling were performed using the Ion Torrent server v4.4.2. Based on Variant Caller Parameters we accepted all variants with an allele frequency (VAF)>5% with high quality, rejected variants had VAF<3% and low quality. Genomic sequences were compared to the IARC TP53 database and confirmed using Sanger sequencing.
Results: ION-PGM sequencing data showed a high coverage in 90% of sequenced amplicons with average coverage uniformity of 89%, average mean depth of 13130 (range 8812–17960), and 97% of mapped reads on target. We identified 84 variants in 39 pts (including synonymous [N=2/2 pts] and non-synonymous [NS] mutations [n=44/26 pts]) in TP53 regions. A total of 84 variants were identified in 30/39pts (77%). Overall, of the 39 pts analyzed, 9 pts were $TP53^{WT}$, while 30/39 76.9% presented variants along the entire TP53 sequence. 3/39 presented only intronic mutations and were considered $TP53^{WT}$; 1/39 pts with a 3'UTR mutation with VAF=4.5%. In the remaining 26 pts NGS identified 46 exonic mutations (range, 1-5 variants/patient): 6/26 with clonal mutations only, defined as VAF>10%, (23.1%); 16/26 (61.5%) clonal and subclonal; and in 4/26 (15.4%) only subclonal variations were identified. Exon mutation hotspots were located in ex-7 (domain IV), ex-11 (C-terminal) and the 3'UTR, with 13, 11, and 10 variants, respectively. Overall, a notable% of mutations were identified having a VAF between 3–5%(60/84 variants, 71.4%) and between 5–10% (6/84, 5.9%), which may be considered subclonal variants defined as below the detection limit of Sanger sequencing. Of the 46 exon variants (26 pts) identified, 44 were NS mutations with the following effects at the protein level: 28 deleterious, 3 frameshift, 12 neutral, 1 stop/loss and 2 unclassified mutations and were indicated as alterations likely producing nonfunctional protein according to *in silico* analysis. The mutations in the 3'UTR (10) and 5'UTR (1) could potentially affect regulation of TP53 gene expression by target miRNA and/or transcription factors. Correlation with FISH analysis showed that of the 4 pts with del17p, 3 pts presented a clonal TP53 mutation (with VAF range 22.5–94.7%) in the remaining allele, while the 4th presented a subclonal mutation with a VAF of 3.1%, of note this was the only del17p patient alive in this cohort.
Summary/Conclusions: The ION PGM-TP53 panel offers an easy to use platform for the evaluation of small clonal TP53 mutations. A total of 67/84mutations were detected as small TP53-mutant subclones having a VAF<10%, that would likely be considered WT by Sanger sequencing, demonstrating the high sensitivity of the technique. The presence of subclonal mutations could anticipate the development of a chemo-refractory phenotype among CLL pts requiring treatment. We are currently evaluating TP53 clonal alterations identified by NGS screening in these CLL pts prospectively. Special thanks to Celegene and AIRC-CARICALRegional Grant 16695.

PB1776

GOLD NANOPARTICLES CONJUGATED WITH RITUXIMAB FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia (CLL) is a monoclonal disorder that is characterized by a continuous accumulation of malignant lymphocytes. Despite progress in targeted therapy options for CLL, relapse or progression to a Richter syndrome still appears. Thus, a more focused and targeted therapeutic option is required. This can be achieved by increasing the concentration of a cytostatic drug in the tumor while reducing its' systemic toxicity.
Aims: In the continuous effort toward the development of more efficient therapeutic approaches for the treatment of CLL, in the current study we report the conjugation of rituximab antibody drug onto spherical gold nanoparticles. Their effective trans-membrane delivery inside CLL cells and their validation as real-potential therapeutics with increased efficacy, in comparison with drug alone.
Methods: The internalization of Rituximab-nanocarriers was proved by the strongly scattered light from gold nanoparticles and was correlated with the results obtained by transmission electron microscopy and dark-field microscopy. The therapeutic effect of the newly designed drugs was investigated by several methods including cell counting assay as well as the MTT assay.
Results: The therapeutic effect of the newly-designed drugs was investigated by several methods including cell counting assay as well as the MTT assay and was found to be superior when compared with the drug alone, data confirmed by state-of-the-art analyses of internalization, cell biology (flow cytometry, apoptosis and autophagy assay), genomics (RT-PCR for MS4A1) and proteomics (confocal microscopy and western blotting for CD20).
Summary/Conclusions: The efficient formation of drug-nanocarriers was proved by spectroscopic characterization of the particles. The internalization of rituximab-nanocarriers was proved as a result of the strongly scattered light from gold nanoparticles and was correlated with the results obtained by TEM and dark field microscopy.

PB1777

THE ROLE OF TUMOR NECROSIS FACTOR (TNF) AND IT'S SOLUBLE RECEPTORS, STNFRI AND STNFRII, IN MYELOSUPRESSION DEVELOPING IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)
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Background: Myelosupression with severe anemia or thrombocytopenia refers to the adverse factors in clinical course of B-CLL. The production of TNF, a pleiotropic cytokine, and it's soluble receprors (sTNFRs), sTNFRI and sTNFRII, in B-CLL patients (pts) in blood are broken, but the final role of these proteins in development of myelosupression in B-CLL pts is studied poorly.
Aims: To determine the role of TNF, sTNFRs in myelosupression developing in B-CLL pts.
Methods: Determination of TNF, sTNFRs was performed using blood plasma of 74 pts with B-CLL at different stages of the disease before treatment. Determining the concentration of TNF was carried out by biological methods, using mouse fibroblasts cell culture line L929. The level of sTNFRI and sTNFRII was performed by immunological methods using the kits of BioSource Europe S.A., Belgium. These parameters were analyzed in relation with complete blod counts of the pts with B-CLL. The control group consisted of 15 healthy donors. Probability statistics was evalueted using Student's *t*-test. The reletionship between the concentration of TNF, sTNFRs and hemograms of pts at different stages (A,B,C by Binet) of B-CLL was established by correlation coefficient (*r*), which was calculated by Excel program.
Results: The significantly higher levels of TNF (0,938±0,124 ng/ml), sTNFRI (2,740±0,277 ng/ml) and sTNFRII (32,180±4,350 ng/ml) were established in plasma of B-CLL pts at all stages of the disease compared with healthy donors (0,089±0,017 ng/ml; 1,210±0,014 and 4,170±0,231 ng/ml, accordingly). On the other hand, appreciably lower levels (p<0,001) of TNF (0,482±0,184 ng/ml) and sTNFRII (17,080±1,294 ng/ml) were determined in plasma of B-CLL pts at early stages in comparision with more advanced stages, B-C (1,011±0,260 and

33,300±5,690 ng/ml, accordingly). An increased reticulocytosis (>10 $^{0}/_{00}$), as a sign of hemolytic syndrome, was accompanied by significantly higher levels of TNF in plasma comparing to pts with a normal number of reticulocytes (1,266±0,114 *vs* 0,752±0,188ng/ml). The concentrations of TNF and sTNFRII correlated negatively with hemoglobin level (r=-0,37) but positively with absolute lymphocytic count (r=0,42) at early stages of the disease. In addition, any changes in concentrations of these proteins were not detected in plasma of B-CLL pts with thrombocytopenia.
Summary/Conclusions: These studies suggest that increasing of the concentration of TNF and sTNFRs in B-CLL, possibly as a result of their production by leukemic cells, may adversely affect hematopoiesis, promote hemolysis and development of anemia of different origin in B-CLL pts. In advanced stages of B-CLL an augmented secretion both of TNF and sTNFRII comparing with early stages may contribute to the leukemic progression. Determination of TNF and sTNFRs in blood of B-CLL pts may be used to predict the development of anemia as well as to initiate treatment, especially at early stages of this disease.

PB1778

IS CD200 EXPRESSION A SIGNIFICANT MARKER FOR DIFFERENTIATION OF B- LYMPHOPROLIFERATIVE DISEASES? IN A SINGLE TUBE ANALYSIS
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Background: B cells have specific antigen patterns and flow cytometric techniques have been used for discrimination of chronic lymphoproliferative disorders (CLDs). Despite CD23 is considered a reliable marker, because of its positivity in chronic lymphocytic leukemia (CLL) and negativity in mantle cell lymphoma (MCL), there are some cases where CD23 does not discriminate. CD 200 is a trans-membrane glycoprotein belonging to the immunoglobulin super-family mostly expressed by B cells.
Aims: Our aim was to evaluate CD200 expression in the diagnosis and classifications of B-cell CLDs in a single tube by using flow cytometric techniques.
Methods: This is a retrospective study utilizing data on consecutive patients with B CLDs in a single center. The study was performed on erythrocyte-lysed whole peripheral blood samples, after staining with directly conjugated monoclonal antibodies. A single tube was designed to include CD45, CD19, CD5, CD23, CD22, CD10, CD20 and CD200. Samples were analyzed using 8-color MFC (FACS Canto II; BD, Bioscience), and FACS DIVA was used for data analysis. An antigen was considered positive when at least 20% of the cells expressed that antigen.
Results: A total of 59 B-CLD patients - 42 male (71.2%) and 17 female (28.8%) - were evaluated between January 2014 and January 2016. All patients expressed CD19 and while CD20 was found to be positive in all but one CLL case. 36 patients were diagnosed as CLL and 4 patients diagnosed as atypical CLL (aCLL). A total of 19 patients were diagnosed as lymphoma; 3 of them were mantle cell lymphoma (MCL), 2 of them were marginal zone lymphoma (MCL), 2 of them were follicular lymphoma (FL), 2 of them were diffuse large B cell lymphoma (DLBCL) and 2 of them were small lymphocytic lymphoma (SLL). Also 8 patients were unclassified and they were defined as other B lymphoproliferative diseases. While CD200 was positive in all CLL and aCLL patients, it was negative in MCL, MZL, DLBCL patients. CD200 was also positive in unclassified form of B lymphoproliferative diseases in 5 patients. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CD200 for diagnosis of B-CLL are 100%, 52.2%, 76.6% and 100% respectively. These values were 100%, 47.8%, 75% and 100% respectively for CD5. CD 23 was positive in 32 of 36 B-CLL patients (88.9%), and weak positive in the remaining 4 B-CLL patients (11.1%). CD 23 was also found to be positive in 1 (4.3%), weak positive in 4 (17.4%) and negative in 18 (78.3%) patients without B-CLL.
Summary/Conclusions: Multiple reports have showed that CD 200 expression is moderate –high in CLL. However, it is dim to negative in mantle cell lymphoma and proposed as another marker to distinguish between CLL and MCL. CLL/ SLL demonstrates low intensity staining for surface immunoglobulin, low expression of CD 22, CD 79B, strong expression of CD 5 and CD 23 but leukemic phase of MCL can be misdiagnosed as CLL. Since MCL is a more aggressive disease and generally treated differently than B-CLL, it is important to differentiate them. In our study, it is showed CD200 expression has a great impact on accurate B-CLDs at diagnosis and it could be added to the routine panels and might be able to show in a single tube by using 8 colour flow cytometer . Also there is high expression of CD200 in B-CLL and it could be a good option for targeted immune therapy in the near future.

PB1779

STRUCTURAL AND FUNCTIONAL ALTERATIONS OF THE COMPLEMENT SYSTEM IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS
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Background: Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the western world, accounting for 30% of all leukemias. The therapeutic approach in CLL includes chemotherapeutic regimens of purine analogues and therapeutic monoclonal antibodies. The immunotherapeutic approach triggers immune responses against the leukemic B-cells that synergize with cytotoxic chemotherapeutic agents. The therapeutic monoclonal antibodies mediate anti-tumor effects through complement-mediated cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and phagocytosis. The efficacy of the CDC thus depends on the expression level of the targeted antigen on B-cells, the integrity of apoptotic cascades within tumor cells, the functional capacity of effector cells, and the availability and activity of the complement system.
Aims: To study structural and functional characteristics of key complement components in untreated CLL patients, in order to develop a marker which will assist in identifying patients who are likely to be less responsive to future immunotherapy treatment.
Methods: Blood samples were collected from 13 untreated CLL patients and 8 healthy controls (HC). Clinical biochemical and haematological parameters, as well as CLL staging were recorded. Three key complement components, C3, C4 and C5 were studied by Western blot analysis. The activity of the complement system before and after activation with Zymosan or aggregated IgG was followed by the levels of C5b-9, the terminal product of complement activation, using ELISA. The link between levels of complement activity and complement isoforms, as revealed by Western analysis, was studied.
Results: The Western analysis results indicate differences in the pattern of C5 and C4 in CLL patients compared to HC subjects. Specifically, the C5 complex, which exists as a single band in HC, appeared in CLL patients as a clear double-band. This alteration was observed in >50% of the patients. Western analysis did not indicate clear differences in C3 pattern in the patients. Activity analysis revealed higher basal levels of C5b-9 in the CLL patients (compared with HC), that were particularly high in the patients presenting altered pattern of C5. Maximal activation, achieved by Zymosan or aggregated IgG, appeared to be inversely correlated with basal activation levels.
Summary/Conclusions: These preliminary data demonstrate a possible link between the activation potential of the complement system in CLL patients and alterations in the complex structure of C5. The exact mechanism by which "modified" C5 distracts the complement activity needs further clarification. Yet, the appearance of "modified" C5 in CLL patients with disturbed complement activity bears the potential to develop a marker which will assist in identifying patients who are likely to be less responsive to future immunotherapy treatment due to compromised CDC. Development of such a marker may assist clinicians in refining and personalizing the immunotherapeutic approach, improving CDC and consequently the therapy results.

PB1780

BACH2 & PRDM1: PREDICTIVE MARKERS OF IMMUNOSENESCENCE IN AGING?
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Background: Aging is characterized by a progressive decline in immune surveillance that favours cancer development. This is illustrated by the high median age of most malignant hemopathies. There is currently a lack of information related to genetic or epigenetic modifications of tumor suppressor genes (TSGs) in ageing immune cells. Our previous studies investigated the 6q deletion in T-cell lymphoproliferative diseases and we reported that BACH2 gene is a candidate TSGs.
Aims: In this study, we examined whether BACH2 and PRDM1 could be predictive markers of immunosenescence in lymphocytes subsets according to age.
Methods: Lymphocytes subsets were analysed using multi-colour flow cytometry to assess expression of CD45, CD3, CD4, CD8, CD45RA, CD25, CD197, CD19, CD27, CD5, CD56 and CD16. Lymphocyte subsets were isolated using the MACS isolation system for subsequent molecular investigations. BACH2 and PRDM1 were quantified by qRT-PCR and western blotting for each purified lymphocytes subset.
Results: Peripheral blood samples were obtained from 35 healthy donors (HDs) of different ages (20y to 90y), including 18 HDs under 50y (median: 36) and 17 HDs above 50y (median: 61). Absolute lymphocyte counts did not vary between these two groups but alterations in lymphocyte subsets distribution were detected. The number of naïve T cells and CD8+cytotoxic T cells was significantly reduced in the older group (p=0.01 and 0.002). Our data confirmed that the CD4:CD8 ratio and effector T cells numbers are significantly increased (p<0.0001 and 0.01) with age. BACH2 and PRDM1 gene expression was studied by using qRT-PCR to evaluate 28 HDs [14 HDs under 50y (median: 38) and 14 HDs above 50y (median: 62)] compared with 23 untreated Chronic Lymphocytic Leukemia (CLL) patients (median age: 69). The purity of each lymphocytes subset was 95%>99%. BACH2 was significantly down-regulated in most lymphocytes subsets in the older group: CD4+, CD4+naïve, CD8+T cells and CD19+B cells (p=0.001; 0.005; 0.004 and 0.03, respectively). Com-

pared with a similar age group of HDs, BACH2 expression was even more severely reduced in CD19+, CD4+, CD8+subsets of CLL patients (p=0.004; 0.001 and <0.001). In contrast, PRDM1 was significantly up-regulated in the CD4+and CD8+T cells (p=0.003; 0.001) of CLL patients but not in leukemic B cells. There was inverse correlation between BACH2 and PRDM1 (Pearson r=0.82; p<0.0001) in T cells of CLL patients. In addition, by using western blot BACH2 and BLIMP1 protein expression in CD4+, CD8+and CD19+lymphocytes correlated significantly with their transcripts expression levels. These observations uncover the roles of BACH2 and PRDM1 in the survival of immune cells in HD and CLL patients.
Summary/Conclusions: Our investigations suggest that BACH2 and PRDM1 expression is correlated with immunosenescence. Moreover, in CLL patients, this down regulation of BACH2 is even more pronounced. Further investigation should be conducted to better understand the role of unbalanced expression of BACH2 and PRDM1 genes in lymphocytes functions and survival.

PB1781

ALTERED SETMAR EXPRESSION ASSOCIATES WITH CLINICAL CHARACTERISTICSIN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The Chronic lymphocytic leukemia (CLL) is the most common chronic lymphoid malignancy in the western population. To date, several studies have been developed to elucidate the genetic factors involved in the biology and progression of CLL. Epigenetic changes have been shown to be increasingly important in the genesis of various tumors and especially in chronic lymphoid neoplasms.Histone methylation is one of the leading and most studied epigenetic events. The gene *SETMAR*(or *METNASE*) was associated with carcinogenesis of acute myeloid leukemia by losing disjuntion checkpoint in cells treated with inhibitors of protein topo II alpha such as etoposide, doxorubicin and mitoxantrone. However, the role of *SETMAR* in CLL leukemogenesis remains unknown.
Aims: Determine relative expression of the gene in SETMAR CLL samples.
Methods: In this study, we evaluated the relative expression of *SETMAR*[FP1] between a group of 59 CLL patients and 10 samples from peripheral blood of healthy blood donors by real-time qPCR.Quantification of *SETMAR* was performed by Real Time PCR (qPCR) and normalized to endogenous *ACTIN* expression. Results were analyzed by the comparative 2 $^{-\Delta\Delta Ct}$ method. The amount of target gene, normalized to the endogenous control gene and relative to a reference sample, was converted into relative quantification. Based on the distribution of *SETMAR* expression in CLL samples, we adopted the median value as the cutoff to dichotomize CLL patients in "low expression group (LEG)" and "high expression group (HEG)". The expression of *SETMAR* was thencorrelated with clinical relevant prognostic factors such ascytogenetic, immunophenotypic and hematologic features in this disease.
Results: We found that *SETMAR* was significantly overexpressed in patients with CLL compared to controls (p <0.0014). After the dichotomy, the LEG showed high leukocyte count (p <0.0050), low platelet count (p <0.0489) and predominance of cytogenetic abnormalities (p <0.030). The clinical staging of BINET and expression of ZAP-70 did not show statistical significance between the LEG and the HEG. Our results shows that SETMAR expression is altered in CLL patients when compared to healthy controls, and revealed that a lower expression of *SETMAR* in patients is clinically associated with chromosomal instability and progression of the tumor mass though increased leukocytosis.
Summary/Conclusions: These findings are immediate in CLL samples, and further studies are necessary for evaluation as a prognostic factor in this disease.

PB1782

P-GLYCOPROTEIN TRANSPORT ACTIVITY UNDER REDOX BALANCE CHANGES IN LYMPHOCYTES OF PATIENTS WITH B-CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: The experimental data about the close relationship between tumor progression and cellular redox balance are published lately. It is shown that the redox balance alterations play a key role in the cell response to the anticancer agents influence, and it is an important regulator of the protein-transporters associated with the phenomenon of multidrug resistance (MDR) expression. However, the impact of the redox imbalance in human tumor cells on the functional activity of MDR proteins has not been studied yet. Such investigations necessary for explanation of the conventional chemotherapy possible failure and their protocols modification, taking into account the features of MDR transporters functioning under the cellular redox balance changing.
Aims: To study the influence of chemotherapeutic agents on the redox status of lymphocytes of patients with B-chronic lymphocytic leukemia (CLL) and to assess the P-glycoprotein (P-gp) transport activity in these cells.
Methods: Lymphocytes were separated from the CLL patients peripheral blood by density gradient centrifugation on histopaque. Monoclonal antibody UIC2-PE (Immunotech) was used to evaluate P-gp expression and functional activity, anti-CD5-FITC (Immunotech) and anti-CD19-PC5 (Beckman Coulter) were used to determinate $CD19^+CD5^+$B-CLL cells. Reactive oxygen species (ROS) level and low-molecular antioxidants content were assessed using CM-H_2DCF-DA (Molecular Probes) and Antioxidant assay kit (Sigma), respectevly. Cells viability was assessed using Calcein-AM test and propidium iodide (Sigma). All investigations were carried out on the FACScan and FACScanto II (BD).
Results: The effect of drugs applied in the treatment of CLL (purine nucleosides - fludarabine and cladribine analogs: fludarabel (Flu), 17,5 μM and leucladine (Leu), 7,0μM (Belmeddrugs, Belarus); anthracyclines – doxorubicin (Dox), 1,72 μM (Sigma); vinca-alkaloids – vincristine (Vincr), 47,2 nM (Lens-Farm, Russia)) on the functional activity of membrane transporter P-gp associated with MDR in lymphocytes of patients with B-CLL is studied. Also the ROS level and low-molecular antioxidants content are evaluated in leukemic cells during the chemotherapeutic drugs metabolism. A degree of low-molecular antioxidants involvement in maintaining of the redox balance in lymphocytes of patients with B-CLL under exposure to drugs was determined. It is found that change of the ROS level in the leukemic B-lymphocytes comparative to values in intact cells, as well as its further recovery leads to increase of P-gp transport activity. The viability of leukemic B-cells under exposure to the antitumor drugs decreases and depends on the redox balance changes but to a lesser degree than in donor's B-cells, as had been established previously. Nevertheless, the statistically significant relationship between the functional activity of the membrane protein P-gp and viability of lymphocytes of patients with B-CLL wasn't detected.
Summary/Conclusions: The received results has allowed to characterize the functioning of the main MDR transporter under redox balance changing caused by the metabolism of the investigated anticancer drugs.

PB1783

IDENTIFICATION AND ROLE OF HUMAN REGULATORY B LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in Europe. There is no curative treatment. CLL is characterized by expansion of CD19+CD5+B cells in blood, lymph nodes and bones marrow. In this pathology, immune responses are disrupted, at least due to dysfunctions of T cells, contributing to the immunodeficiency and the disease progression. Generally speaking, B lymphocytes play an intricate role in the immune system and are able to slowdown the anti-tumor responses favoring tumor escape. They are called regulatory B cells.
Aims: The main focus of this project is to evaluate the regulatory function of CLL B cells, aiming to estimate their influence on the lack of anti-tumor responses mediated by T cells.
Methods: *In vitro* models of co-cultures between T and B cells are used to appraise the regulatory capacity of CLL B cells on T cell proliferation evaluated by flow cytometry. Regulatory activity of CLL B cells, stimulated or not with CpG-ODN, is estimated as their ability to inhibit the T cell proliferation. Concurrently, phenotypic characteristics of regulatory B cells and T cells are evaluated.
Results: Two groups of patients have been identified following CpG-ODN stimulation. The first group presents defective regulatory B cell functions compared with control B cells. In the second group, no inhibitory activity is detected, leading even to T cell proliferation in some cases. Compared with unstimulated cells, stimulation with CpG-ODN do not induce any regulation, suggesting insensitivity of the second group. Surprisingly, the level of TLR9 expression is similar in both groups. Furthermore, no correlation is found between functional and phenotypic signature of T and B cells. However longitudinal study suggests a switch of group with a strong clinical course.
Summary/Conclusions: These results suggest functional TLR9 pathway but alteration of the regulatory-induced function of CLL B cells in the first group, while the TLR9 pathway seems to be totally defective in the second group of patients, explaining the lack of regulation. To go further, it will be of interest to identify the molecular mechanisms damaging the TLR9 pathway. These results would contribute to clarify the lack of anti-tumor immune response found in the CLL patients.

PB1784

PRAME PROTEIN IS LOCATED IN EXTRACELLULAR SIDE OF MEMBRANE AND MIGHT BE A GOOD TARGET FOR ANTIBODY THERAPY OF HEMATOLOGICAL MALIGNANCIES

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Background: PRAME protein is not expressed in normal cells, but is active in large number of tumor cells. Thus PRAME protein might be a promising target for cancer immunotherapy. It is known that PRAME located in nucleus and cytoplasm of tumor cells. Tajeddine *et al.,* (2005) and Wadelin *et al.,* (2010) reported that this protein was located in nucleus and cytoplasm of tumor cells. On the other hand, Proto-Siqueira *et al.,* (2006) and Quintarelli *et al.,* (2008) found this protein on cell membrane.
Aims: To prove that PRAME protein is located on tumor cell surface and can bind with a specific monoclonal antibody.
Methods: We used 11 samples of B-CLL patients blood and cell lines K562, THP-1 and NOMO-1. 5 samples of healthy blood donors were used as negative controls. PRAME expression level was determined by RQ-PCR in blood of 8 B-CLL patients and in cell lines. PRAME epitops in surface of cell from patients and cell lines was determined by flow cytometry and immunocytochemistry experiments. Cell lines were incubated with anty-PRAME monoclonal antibodies (mAbs) 5D3F2 and 6H8F12, together and separately, at concentrations 111 and 112 ug/ml, 11 and 12 ug/ml, respectively. Cell count was carried out after incubation with mAbs. The number of dead cells was evaluated during MTT assay.
Results: PRAME expression level in K562 THP-1 and NOMO-1 cells lines was 104%, 1,6% and 0,5%, respectively. 6 patients were PRAME-negative and 2/8 had PRAME expression median lewel 4,43%. According to flow cytometric data K562, THP-1 and NOMO-1 cell lines had PRAME-positive cells 14%, 3% and 0,9%, respectively. One patient had 0% PRAME-positive cells (flow cytometric data) and none mRNA of PRAME gene expression (RQ-PCR data), one another patient had 100% PRAME-positive cells whith mRNA expression level 8,84%). Immunocytochemistry data showed, that protein PRAME was located in cell's surface of 11,5% cells in blood of 2/4 patients with B-CLL (we hadn't RQ-PCR data in this cases). K562 and THP-1 cells decreased growth rate by 15-20% and about 15-20% of died cells was observed after incubation with mAbs 5D3F2 and 6H8F12, together and separately, at concentrations 11 and 12 ug/ml, respectively. K562 and THP-1 cells decreased growth rate by 50% and about 4% of cells died after incubation with mAbs 5D3F2 and 6H8F12, together and separately, at concentrations 111 and 112 ug/ml, respectively. NOMO-1 cells decreased growth rate by 30% and around 10% of cells died after incubation with mAbs 5D3F2 and 6H8F12 together at concentrations 11 and 12 ug/ml. NOMO-1 cells decreased growth rate by 30% during incubation with mAb 6H8F12 at concentration 112 ug/ml, but this cells didn't die. NOMO-1 cell did not demonstrate slowing of cell growth or dying after incubation with mAb 5D3F2 at concentration 111 ug/ml.
Summary/Conclusions: We confirmed evidence that PRAME protein is located on surface of cancer cells. The protein is expressed only in cases when mRNA of PRAME is expressed. Cytostatic and cytotoxic effect of mAbs 5D3F2 and 6H8F12 were detected. This mAbs are effective against cell lines K562, THP-1 and NOMO-1. Interestingly, at a lower concentration mAbs have a more pronounced effect. In cases with higher expression level of PRAME gene in cells, we obserwed a greater number of cells with PRAME epitopes on cell surface and more powerful MAbs-depended cytostatic and cytotoxic effect. Our data suggest that PRAME might be a promising target for immunotherapy of PRAME-positive patients with B-CLL.

PB1785

CD49D AND CD26 ARE INDEPENDENT PROGNOSTIC MARKERS FOR DISEASE PROGRESSION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: CLL is characterized by extremely variable clinical course. Several prognostic factors can predict disease progression and therapeutic outcomes in those patients.
Aims: The aim was to evaluate the use of CD49d and CD26 as independent prognostic markers in CLL patients. The present study measured surface expression of CD49d and CD26 by three-color flow cytometry in a series of 103 untreated CLL patients.
Methods: The present study measured surface expression of CD49d and CD26 by three-color flow cytometry in a series of 103 untreated CLL patients. We evaluated the prognostic role of CD49d and CD26 to predict the risk of lymphocyte doubling, disease progression and overall survival.
Results: We evaluated the prognostic role of CD49d and CD26 to predict the risk of lymphocyte doubling, disease progression and overall survival.We confirmed that CD49d and CD26 were significant predictors of lymphocyte doubling(P b 0.001 for both markers) and disease progression (P b 0.001 for both markers) but insignificant for overall survival(P=0.303 and 0.519 respectively. Multivariate analysis between clinical parameters and flow cytometry markers revealed that CD49d and CD26 are independent prognostic markers for lymphocyte doubling (HR=1.487 P=007 and HR=2.248, P=0.014 respectively) and progression to a more advanced stage (HR=3.191, P=0.049 and HR=7.887, P=0.003) (Figure 1).
Summary/Conclusions: Also, concordant expression of both markers was found to improve their predictive power. Many studies reported that CD49d and CD26 combined analysis was found to improve their power to predict the risk of lymphocyte doubling and disease progression. CD49d and CD26 have independent prognostic value and we suggest its use as a part of routine panel for prognostic stratification of CLL.

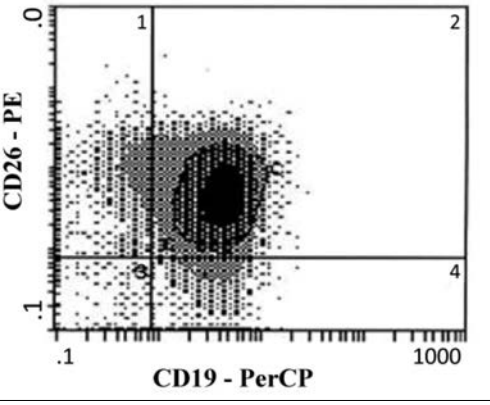

Figure 1.

PB1786

IMPORTANCE OF CD38 MARKER IN PROGNOSIS OF B CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) IN THE NEW ERA

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Background: Several published studies suggested that transmembrane glycoprotein- CD38 on the surface of leukemic cells. CD38 is accepted as a dependable marker of unfavorable prognosis and as an indicator of activation and proliferation of CLL cells.
Aims: The aims of the present study were to establish the predictive value of the CD38 expression and to examine the corelation between CD38 positivity and other established prognostic markers in our CLL patients in the new era.
Methods: Peripheral blood samples from 180 consecutive treatment naïve CLL patients were analyzed by flow cytometry for CD38 expression on CD5/CD19 leukemic cells. Various patients established prognostic characteristics and molecular markers were studied in correlation to time to treatment (TTT). The Kaplan-Meier method was used to construct survival curves, and the log-rank statistic was used to compare these curves.
Results: CD38 was expressed in 63% of the patients. Patients with high CD38 expression (30% or more) with high value of B2M and advance disease according to Binet had significantly shorter survival times (p=0 .00001) and (p=0.00033) respectively.
Multivariate analyses showed that CD38 expression is an important prognostic factor for shorter TTT associated high B2M level (P .000002), age(P.00000), gender(P.00000), lower hemoglobin level (P.00008),hepatomegaly (P.00086)
Summary/Conclusions: CD38 expression identified a group of patients with aggressive disease that was considered by traditional staging to be early-stage disease (Rai stages 0-II or Binet A). Patients with CD38 samples have significantly aggressive disease regardless of their clinical stage. But today in era of molecular and genetics markers when CD38 is loosing it prognostic value in CLL patients prognosis, we propose serial analyses of the percentage of CD38+cells to be done, resembling indicators of leukemiccell proliferation and may signal clone evolution to a more aggressive state.

Chronic lymphocytic leukemia and related disorders - Clinical

PB1787

IMPACT OF IGVH MUTATIONAL STATUS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA WITH ISOLATED GOOD AND INTERMEDIATE RISK GENETIC ABERRATIONS

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Background: Genetic abnormalities in chronic lymphocytic leukemia (CLL) have prognostic impact. The presence of trisomy 12 (T12) and normal karyotype (NK), and 13q deletion (del13q) are associated with an intermediate and good risk profile in CLL, respectively (Dohner *et al.* NEJM, 2000). The unmutated status of the immunoglobulin variable heavy chain gene (IgVH) correlates with survival in sole del13q CLL (Gladstone *et al.,* Leukemia 2011). The prognostic relevance of IgVH mutational status in isolated intermediate risk genetic variants (T12 and NK) has not been described. We investigated the impact of the IgVH mutational status in CLL patients with isolated del13q, T12 or NK in a large single institution cohort.
Aims: To assess the prognostic impact of the IGVH mutational status in CLL patients with isolated del13q, T12 and NK.
Methods: From January 2000 to January 2013 we identified 1267 CLL patients using the Moffitt Cancer Center and Total Cancer Care databases. Descriptive data was reported in patients with both unmutated (<2% from the germline sequence) and mutated IgVH (>2% from the germline sequence). Time to first treatment (TFT) and overall survival (OS) were estimated using the Kaplan-Meier method and the log-rank test. A significant difference was considered at p≤0.05. All analyses were done using SPSS version 19.0.
Results: We identified 145 CLL patients (median age: 59 years) with isolated del13q (mutated IgHV=96, 66.2%; unmutated IgVH=49, 33.8%), 49 patients (median age: 64 years) with isolated T12 (mutated IgVH=17, 34.7%; unmutated IgVH=32, 65.3%), and 79 patients (median age: 57 years) with NK (mutated IgVH=41, 51.9%; unmutated IgVH=38, 48.1%), respectively. Unmutated IgVH was associated with shorter TFT in patients with sole del13q (unmutated 2.1 years *vs* mutated 6.0 years, p <0.001) and isolated NK (unmutated 1.9 years *vs* mutated 3.3 year, p=0.05). IgVH mutational status had no impact in the TFT of patients with sole T12. Patients with isolated del13q and mutated IgVH had a longer median OS compared to unmutated patients (not reached *vs* 10.5 years, p=0.03). Nonetheless, the IgVH mutational status did not significantly impact the OS of sole T12 patients (p=0.29) and NK (p=0.14) (Figure 1).

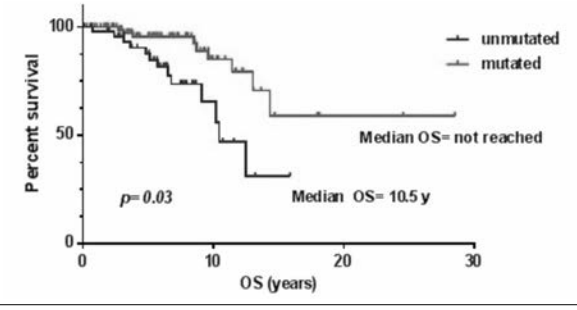

Figure 1. Overal survival per IGVH mutational status.

Summary/Conclusions: Our results confirm the negative impact of the unmutated IgVH status in CLL patients with isolated del13q. The mutational IgVH status did not impact the OS of T12 and NK CLL patients.

PB1788

REDISTRIBUTION PATTERN AND ASSESSMENT OF EARLY RESPONSE TO IBRUTINIB IN CLL BY TOTAL TUMOR MASS SCORE (TTM) - KROHEM CLL2 STUDY

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Background: Ibrutinib is Bruton's tyrosine kinase inhibitor with significant efficacy in CLL. Ibrutinib monotherapy usually leads to redistribution of tumor mass from lymphoid organs to peripheral blood before eventual response in all compartments. This leads to certain problems in response assessment by iwCLL criteria requiring several modifications. Total Tumor Mass score (TTM) (Jaksic, BJH 1980) is a simple quantitative clinical parameter for evaluation of tumor mass in all relevant lymphoid compartments in CLL (PB+BM, LN and spleen) that was successfully used in CLL trials by several international cooperative groups (EORTC, IGCI) in alkylator and purine analog era. It is helpful in routine clinical practice for monitoring of total tumor load, progression and/or response to treatment. Due to its characteristics TTM may overcome problems caused by significant redistribution during TKI treatment (Jaksic, BJH 2014).
Aims: To evaluate usefulness of TTM in evaluation of response and redistribution during ibrutinib treatment in CLL patients in a real life setting.
Methods: This is an observational study from Croatian cooperative group for hematological malignancies (KROHEM) were data from patients included in ibrutinib NPP (420 mg daily) were collected on national level. Thirty patients with relapsed/refractory B-CLL were included, 24 males and 6 females, median age 65.5 years (50-83) and median 2 prior lines of therapy (1-6). There were 4 pts with del 17p and 6 with del 11q (out of 24 with data). Baseline routine evaluation was done before ibrutinib treatment. Clinical and laboratory assessments of tumor mass were collected at start and on months 1, 3, 6, 9 and 12. Bone marrow assessment, US and MSCT scans were performed when clinically indicated. Response was assessed by modified iwCLL criteria (Hallek, Blood 2008, 2012, 2013) and by TTM criteria (Jaksic, BJH 2014). TTM scoring system: TM_1=√Lymphocytes ($\times 10^9$/l), TM_2=largest palpable lymph node (cm), TM_3=spleen below left costal margin (cm). TTM=TM_1+TM_2+TM_3, TTM-D=TM_1/TTM (percentage of tumor mass in leukemic compartment). ΔTTM – response/progression, ΔTTM-D - redistribution
Results: At 6th month 81% of patients responded with tumor mass (TTM) reduction >25%; 67% of patients responded with tumor mass reduction >50% and 43% of patients responded with tumor mass reduction >75%. Modified iwCLL response correlated well with TTM response criteria on months 3, 6 and 9 (Spearman correlation 0.538, p<0.001; 0.762, p<0.001; 0.739, p<0,009 respectively), but not in month 1 (Spearman correlation 0.175, p=0.356). At month 6, there were 13/21 patients in PR and PR+PR-Ly 19/21 (iwCLL) compared to 14/21 (TTM reduced >50%) and 17/21 (TTM reduced >25%) respectively. Lymphocytosis changed from baseline=1.0 to months 1, 3, 6 and 9 (median 2.60; 1.95; 0.67 and 0.51 respectively) taking into account all cases. However, while this describes a typical increase, actually no increase of lymphocytosis at month 1 was observed in 30% of patients. Neither multiple stepwise regression found any statistically significant correlation with baseline predictors, nor were patients with increase of lymphocytosis at month 1 significantly different from typical responders in distribution of baseline predictors according to Mann Whitney U test. Tumor distribution (TTM-D, median) rose from 0.47 (baseline) to 0.84 in month 1, and to 1.0 from months 3 on. Multiple stepwise regression with response at month 6 as dependent variable and 10 predictor variables at baseline: age, disease duration, previous lines of therapy, lymphocytosis, lymph node size, spleen size, TTM, TTM-D, hemoglobin and platelets identified in the model only TTM at baseline as significant negative predictor of response (p=0.001) (Figure 1).

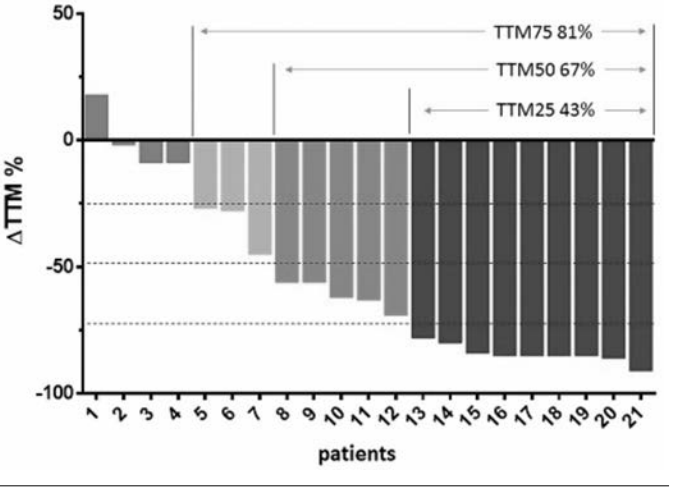

Figure 1. TTM response at 6 months.

Summary/Conclusions: TTM is a simple and useful parameter successfully applied in ibrutinib treated patients in routine clinical practice for response assessment and follow-up. It is highly correlated with modified iwCLL criteria after 3rd month. It is more robust then iwCLL criteria because it incorporates and quantify tumor redistribution and therefore can be applied regardless of therapy used without specific modifications.

PB1789

A NEW PROGNOSTIC SCORING SYSTEM FOR TTFT FOR PATIENTS WITH CLL IN CHINA

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Background: The established clinical staging systems (Rai/Binet) can not accurately discriminate among prognostic groups, especially for patients in early stages. Several prognostic factors have been identified to predict the outcome of patients with chronic lymphocytic leukemia (CLL), but only a few studies analyzed more markers together.
Aims: In this study, we identified the biologic prognostic markers and combined them in a new prognostic scoring system, the CLL prognostic index (CLL-PI) for predicting time to first treatment (TTFT) in patients with CLL in China.
Methods: Taking advantage of a population of 402 untreated Chinese patients with CLL at early and advanced stage of disease, we identified the strongest prognostic markers of TTFT and, subsequently, in a cohort of 173 patients we integrated data of traditional staging system, cytogenetic lesions and immunoglobulin heavy chain variable region (*IGHV*) mutational status in CLL-PI. The median follow-up time was 45 months (ms). Methods of multivariable statistics were applied, and the end point was TTFT.
Results: Based on multivariate Cox regression analysis, three independent factors for TTFT were identified: clinical stage (Rai risk group), del17p and *IGHV* mutation status. Applying weighted grading of these three independent factors based on regression parameters, a CLL-PI was constructed, which could categorize four different risk groups [low (score 1), intermediate (score 2), high (score 3) and extremely high risk (score 4-6)] with significantly different TTFT (median TTFT of NR, 65.0 ms, 24.0 ms and 12.5 ms, respectively, p<0.001). Further, this index provided accurate estimation regarding overall survival (OS) (median OS of NR, 210.0 ms, 97.0 ms and 43.0 ms, respectively, p<0.001] (Tables 1 and 2, Figure 1).

Table 1. Scores assignment to three independent factors of PI

Rai risk group	Scores	*IGHV* status	Scores	17p-	Scores
Low (Rai 0)	1	M-*IGHV*	0	Negative	0
Intermediate (Rai I-II)	2	U-*IGHV*	1	Positive	2
High (Rai III-IV)	3				

Abbreviation: IGHV*, mutated immunoglobulin heavy chain variable region; M, mutated; U, unmutated.*

Table 2. Survival data in each subgroup according to the scoring system

Groups (PI scores)	N	Median TTFT (months)	95% CI (months)	p-value
Low risk (=1)	21	NR	NE-NE	0.000
Intermediate risk (=2)	56	65.0	40.9-89.1	
High risk (=3)	67	24.0	6.2-41.8	
Extremely high risk (4-6)	27	12.5	7.6-17.4	

Abbreviation: CI, confidence interval; TTFT, time to first therapy; NR, not reached; NE, not evaluated.

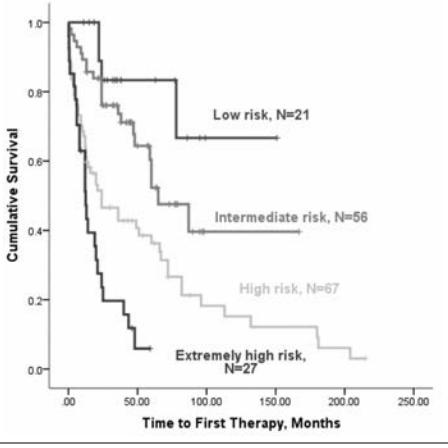

Figure 1.

Summary/Conclusions: This study developed a weighted, integrated CLL-PI which combines the most important genetic prognostic markers (*IGHV* mutation status, 17p deletion) with traditional clinical stage. This newly modified PI could be used to discriminate among groups and may help predict the TTFT and prognosis of patients with CLL.

PB1790

AN ASSESSMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WHO ARE ELIGIBLE FOR FIRST-LINE THERAPY BUT UNSUITABLE FOR FULL-DOSE FLUDARABINE

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Background: Chronic Lymphocytic Leukemia (CLL) is a rare disease which has been granted orphan designation by the EMA. Treatment of CLL is only initiated in patients with symptomatic, active disease and treatment options are dependent on patient characteristics. The European Society for Medical Oncology (ESMO) identifies three subgroups of symptomatic CLL patients who are eligible for front-line treatment; physically fit patients, elderly patients or those with major comorbidities, and patients with del17p/TP53 mutation. Older patients and those with severe comorbidities are less able to tolerate the full-dose fludarabine treatments prescribed to physically fit patients. Fludarabine-based therapies are also considered unsuitable for patients with the del17p/TP53 mutation. These patient subgroups may thus benefit from the introduction of newer, less aggressive therapies.
Aims: The aim of this analysis is to estimate the number of CLL patients who require treatment initiation but who are unsuitable for treatment with full-dose fludarabine, namely those who are elderly (≥70 years), or those who are younger but have comorbidities (aged between 65 and 69 with comorbidities). It also identifies the number of patients with the del17p/TP53 mutation. To our knowledge this is the first study to stratify the total symptomatic, treatment naïve CLL patient population by these patient characteristics which influence treatment decisions.
Methods: A targeted literature review was conducted in Medline and Embase, as well as in cancer-related statistical sources in nine European countries (Belgium, France, Germany, Italy, Spain, UK, Netherlands, Poland and Sweden), applying all relevant keywords related to the disease and to the parameters to be assessed (incidence, prevalence, mortality, survival, asymptomatic, symptomatic, age, fitness, comorbidities). Based on relevant articles extracted and reviewed, the annual number of patients unsuitable for full-dose fludarabine is estimated.
Results: Patients eligible for first-line therapy comprise all newly diagnosed symptomatic CLL patients as well as patients who have transitioned from an asymptomatic disease state to an active disease state. The crude incidence rate of CLL was found to range from 3.88 per 100,000 in Germany to 6.9 per 100,000 in the UK. Of these newly diagnosed patients, between 51.9% and 75.6% are asymptomatic. Approximately 5.0% to 7.8% of previously diagnosed patients transition from an asymptomatic state to a symptomatic state every year. Assuming a mean CLL incidence rate of 4.94 per 100,000 and a mean yearly transition rate of 6.4%, the total estimated number of patients eligible for front-line treatment in 2015, across the nine European countries considered, was 12,370. Of these symptomatic patients, 986 (8.0%) had the del17p/TP53 mutation. Among symptomatic patients without the del17p/TP53 mutation, 56.5% were aged ≥70, and 6.7% were aged between 65 and 69 with major comorbidities. Thus in 2015, across the nine European countries considered, there were an estimated 7,191 symptomatic CLL patients aged ≥70 or aged between 65 and 69 with major comorbidities, who were eligible for first-line treatment but not suitable for full-dose fludarabine.
Summary/Conclusions: This study provides an in-depth overview of the treatment-naïve CLL patient population across nine European countries and provides an estimate of the number of patients unsuitable for full-dose fludarabine and fludarabine-based therapies, and who could potentially benefit from newer, more tolerable therapies.

PB1791

INSULIN-LIKE GROWTH FACTOR SYSTEM AS A PROGNOSTIC BIOMARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA: A CROSS-S ECTIONAL STUDY

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Background: B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the accumulation of long-survived B lymphocytes arrested in $G_{0/1}$ phase by impaired apoptosis. Growth factors may contribute to paracrine or autocrine loops affecting CLL cell survival via the induction of apoptosis-related genes. Insulin-like growth factor I (IGF-I), which is mainly produced by hepatocytes, constitutes an important anti-apoptotic factor for several malignant cells. Furthermore, IGF-I and its main binding protein IGFBP3 are related to the development and pathogenesis of insulin resistance, diabetes as well as in obesity-associated malignancies.
Aims: The goal of the present study was to evaluate circulating levels of IGF-I and IGFBP3 in patients suffering from B-CLL and control participants. We also

explored the ratio of IGF-I/IGFBP3, which gives a mechanistic insight into IGF-I bioavailability, as well as IGF-I and IGFBP3 as prognostic parameters of B-CLL.
Methods: Blood samples were collected from ninety five cases with incident B-CLL and an equal number of hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2001 and 2007. Informed consent was obtained from all study participants. Serum IGF-I and IGFBP3 were determined using commercially available ELISA assays (DIAsource ImmunoAssays S.A, Louvain-la-Neuve, Belgium). Moreover, serum lactate dehydrogenase (LDH), β2-microglobulin (BMG), lymphocyte morphology and the surface expression of CD38 in >30% of B-CLL lymphocytes were assessed. Statistical analysis of the data was performed using univariate and multivariate analyses with IBM-SPSS® version 23 for Windows.
Results: Overall, B-CLL patients had a significantly elevated body mass index (BMI) as compared to control participants (27.8 *vs* 26.7 kg/m^2; p<0.01). In univariate analysis, circulating levels of IGF-I were similar in cases compared to controls (126.75±21.41 *versus* 125.10±25.50 ng/mL, respectively, p=0.63). However, serum IGFBP3 was significantly lower in cases than controls (3.16±0.96 *versus* 4.22±1.03 µg/mL, respectively, p<0.001) while the IGF-I/IGFBP3 ratio was significantly elevated in cases compared to controls (0.049±0.07 *versus* 0.030±0.05, respectively, p=0.01). Lower serum IGFBP3 levels were associated with B-CLL risk in multivariable analyses adjusting for age, gender, date of diagnosis, family history of lymphohemopoietic cancer, BMI, serum IGF-I and insulin (<0.001). In B-CLL patients, serum IGF-I was significantly and positively associated with Binet stage, IGFBP3, LDH and BMG (p<0.001), and negatively associated with age (p<0.001).
Summary/Conclusions: The observed results highlight the potential involvement of IGF-I bioavailability in the B-CLL pathogenetic process. Circulating IGF-I might be a potential biomarker for B-CLL prognosis. Further prospective and longitudinal studies are needed in order to confirm these observations as well as to elucidate the contribution of the IGF-I system in B-CLL progression.

PB1792

SECONDARY MALIGNANCIES IN CHRONIC LYMPHOCYTIC LEUKEMIA: A SINGLE CENTRE RETROSPECTIVE ANALYSIS

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Background: Chronic lymphocytic leukemia (CLL) is characterized by progressive immunodeficiency with high prevalence of infections, autoimmune phenomena and secondary malignancies. The immune deregulation may be due to the disease itself or it may be a consequence of the treatment performed.
Aims: To evaluate the occurrence of second cancers in CLL patients and their relationship with clinical and laboratory features as well as with therapy lines.
Methods: Clinical history of 514 CLL patients diagnosed and followed from 1983 until 2014 at our Institution were retrospectively evaluated and the diagnosis of a second cancer was collected. History of neoplasia preceding CLL diagnosis was also registered. Student t test for continuous variables, chi-square test for categorical ones and Log rank test for survival analysis were performed.
Results: Clinical, hematological and biological characteristics are listed in Table 1. Secondary cancers were categorized according originating organ/tissue; skin cancers were divided into melanoma and non-melanoma. During the follow up 88 patients (17%) developed secondary cancers, with a mean time from diagnosis to secondary neoplasia of 9 years. Considering tumor histology, we observed 9 blood, 9 lung, 5 breast, 19 uro-genital tract (5 kidney, 10 prostate, 4 bladder, 2 uterus, and 2 ovarian), 15 gastro-enteric tract (12 colon, 2 gastric and 1 tongue), 4 pancreas, 3 melanoma and 15 skin cancers other than melanoma. No significant differences were observed according to age, gender, Rai/Binet stage and hematologic parameters in patients with or without secondary tumors (Table 1). Considering CLL prognostic features, the development of second cancers was associated with higher age (p<0.001) at diagnosis, increased beta2microglobuline levels (p=0.03) and un-mutated VHIG status (p<0.005; Figure 1). At variance, no association was found with 13q, 11q or 17p deletion, chromosome 12 trisomy, nor with ZAP-70 and CD-38 positivity (Table1). Past history was positive for malignancies in 70 patients (13%): 2 blood, 3 airways (2 lower and 1 upper), 3 breast, 6 uro-genital tract (3 bladder, 3 prostate, 2 uterus and 1 ovarian), 3 gastro-enteric tract, 3 skin cancers other than melanoma, and 3 melanoma. On the whole, 46/88 (52.3%) and 219/426 (51.4%) patients with or without secondary cancers, underwent at least one therapy line. More specifically, 86 patients were treated with fludarabine containing regimens, of whom 11 developed a secondary cancer; 180 with chlorambucil, of whom 34 developed a secondary tumor and 65 with alemtuzumab, of whom 10 were later diagnosed with a second cancer. During the follow up, 121 patients died, of whom 8 directly from secondary malignancies, 41 from CLL progression, 2 from thrombotic events, and 11 from infections.

Table 1.

Clinical and laboratory features of 514 CLL patients with or without secondary malignancies. Data are expressed as median (range) or absolute number (%).

	Secondary malignancies	
	Yes (N=88)	No (N=426)
Age years	64 (39-80)	63 (31-90)
Gender M/F	57/31	250/176
Follow-up years	12 (0-30)	12 (1-25)
Rai/Binet		
<C/III	85 (97)	401 (94)
≥ C/III	3 (3)	25 (6)
WBC x10e3/mmc	18.74 (3.6-138)	17.3 (3-384)
ALC x10e3/mmc	12.63 (1.8-91.8)	11.9 (1.4-381)
Hb g/dL	14 (9-17)	14 (8-18)
PLT x10e3/mmc	198 (77-608)	184 (58-472)
LDH U/L	325 (144-838)	334 (142-795)
Beta2microglobulin mg/L	2 (1-23)	2 (0-10)
FISH*		
Del11q	6 (13)	23 (9)
Del13q	19 (40)	101 (40)
Del17p	4 (8)	15 (6)
+12	6 (13)	38 (15)
VHIG unmutated**	15 (42)	80 (39)
ZAP-70 positive***	14 (39)	73 (36)
CD-38 positive****	15 (42)	76 (37)

*Tested in 48 and 252 patients respectively. **Tested in 36 and 205 patients respectively. ***Tested in 33 and 182 patients respectively. ****Tested in 56 and 291 patients respectively.

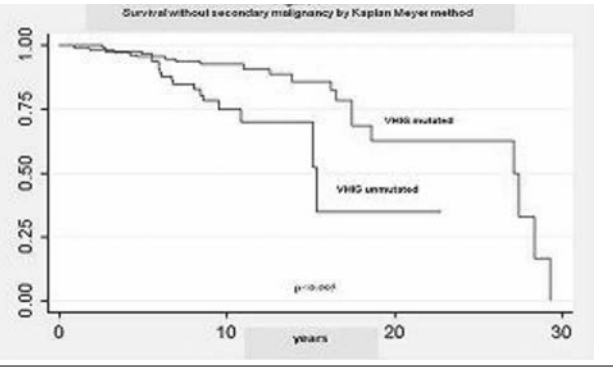

Figure 1.

Summary/Conclusions: Secondary malignancies are not infrequent in patients with CLL and patients with un-mutated VHIG status are at higher risk of developing a second cancer. As secondary neoplasia are not clearly related to biologic markers or to the treatment performed, a careful clinical follow up, encompassing sex and age adjusted tumors screening is advised.

PB1793

COMPLEX ACTIVATION OF ANGIOGENIC SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA: EVIDENCE FROM CIRCULATING ANGIOGENIC CYTOKINES

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Background: Angiogenesis is an important and well-known player in the biology of chronic lymphocytic leukemia. Elevated plasma/serum concentrations of various angiogenic cytokines have been reported in CLL; however, their exact prognostic role, association with modern prognostic factors and impact on clinical course (especially overall survival) is largely unknown.
Aims: To evaluate prognostic significance of vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), soluble endoglin (sCD105), angiopoietin-2 (Ang-2), transforming growth factor-1 (TGF-β1) and inhibitors endostatin and thrombospondin-1 (TSP-1) in patients with untreated CLL followed at a single tertiary center.
Methods: Plasma levels of VEGF, FGF-2, sCD105, Ang-2, TGF-β1, endostatin and TSP-1 were quantified using enzyme-linked immunosorbent assay (ELISA Quantikine kits, RD Systems) in 206 patients with CLL (median age, 64 year; males, 69%; Rai modified risk: low/intermediate/high in 38/46/16%; unmutated IGVH genes, 58%; del 11q/del 17p by FISH, 19 and 4%) and 80 healthy controls. Plasma concentrations ≥median of the CLL cohort were considered elevated.
Results: VEGF, FGF-2, sCD105 and Ang-2 were significatly elevated in comparison to the control group; differences in TGF-β1, endostatin and TSP-1 were on the border of statistical significance (Table 1). There was a positive association of high Ang-2 with unmutated IGHV genes (p=0.027); patients with high sCD105 and lowTGF-β1 had more advanced Rai stages (p=0.0004 and p=0.052). High levels of Ang-2, endostatin, sCD105 and low TGF-β1 correlated

with shorter time to first-line therapy (p=0.0001, p=0.0001, p=0.0021 and p=0.027); in addition, patients with high Ang-2 and endostatin had significantly shorter overall survival (p=0.0006 and p=0.015).

Table 1.

Group	n	Median	95% CI	Units	p
VEGF CLL	206	70	60-90	ng/l	0.0092
VEGF controls	80	46	26-56		
FGF-2 CLL	206	115	78-144	ng/l	< 0.001
FGF-2 controls	80	9	5-10		
sCD105 CLL	200	6,2	5.6-6.5	µg/l	< 0.0001
sCD105 controls	69	4,2	4.1-4.5		
Ang-2 CLL	158	2030	1903-2457	ng/l	0.0016
Ang-2 controls	30	1631	1453-1856		
TGF-β1 CLL	97	3700	3098-4471	ng/l	0.066
TGF-β1 controls	13	4960	4550-6140		
TSP-1 CLL	97	704	391-1097	µg/l	0.088
TSP-1 controls	13	324	288-372		
Endostatin CLL	165	165	152-182	µg/l	0.073
Endostatin controls	34	150	135-170		

Summary/Conclusions: Our results indicate that a complex network of angiogenic signaling is active in CLL and has an impact on clinical course; Ang-2 and endostatin appear to possess the best prognostic value and deserve further evaluation.

Supported by DRO (Univ Hospital Hradec Králové, 00179906).

PB1794

FINAL RESULTS OF THE NCRI CLL210 TRIAL OF ALEMTUZUMAB, DEXAMETHASONE AND LENALIDOMIDE IN PATIENTS WITH HIGH-RISK CLL (ORIGINAL PROTOCOL)

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Background: 17p- chronic lymphocytic leukaemia (CLL) is notorious for its resistance to chemotherapy. The Phase II NCRI CLL206 trial showed that alemtuzumab plus high-dose methylprednisolone was safe and effective in this setting, although the median progression-free survival (PFS) was only 11.8 months (J Clin Oncol 2012;30:1647-55).

Aims: In an attempt to improve on these results, the NCRI CLL210 trial was developed to examine the safety and efficacy of alemtuzumab, dexamethasone and lenalidomide which has activity in 17p- CLL.

Methods: Patients with previously untreated 17p- CLL or CLL progressing within 12 months of FCR received dexamethasone (40 mg po day 1-4 of weeks 1,3,5,7,9,11,13,15), lenalidomide (5 mg od weeks 3-4 and 10 mg od weeks 5-24) and alemtuzumab (30 mg sc days 1,3,5 of weeks 7-22). Patients who achieved a complete response (CR) or partial response (PR) were allowed to proceed to allogeneic stem-cell transplantation if considered appropriate, or were randomised to lenalidomide maintenance (10 mg od until disease progression) *versus* no further treatment. Supportive medication consisted of allopurinol, G-CSF, co-trimoxazole, aciclovir, itraconazole, lansoprazole, alendronic acid, aspirin, plus immunoglobulin replacement therapy where appropriate. Written informed consent was obtained from all patients prior to entering the study. The primary endpoints were post-induction CR/CRi rate and progression-free rate after 2 years of maintenance therapy. Response data were assessed by an independent endpoint review committee using the 2008 NCI/IWCLL criteria.

Results: Sixteen patients out of the planned 85 were recruited from 7 UK sites during the first 7 months of recruitment before accrual was halted in September 2012 due to withdrawal of marketing authorisation for alemtuzumab. The protocol was subsequently amended to replace alemtuzumab with ofatumumab. This report describes the outcome of the initial cohort of alemtuzumab-treated patients (8 untreated and 8 FCR failures; 17p- in 13). Ten patients (62%) completed induction, whereas 6 (38%) stopped induction prematurely due to toxicity (3), disease progression (1), change in diagnosis (1) or death (1). Three patients (19%) proceeded to allogeneic stem-cell transplantation, 5 (31%) were randomised to lenalidomide maintenance (3) or no further treatment (2) and 2 (12%) withdrew from the randomised part of the trial despite completing induction successfully. The post-induction OR and CR/CRi rate among evaluable patients (11) was 91% and 18% respectively. The progression-free rate after 2 years of maintenance phase among evaluable patients (4) was 50% in both treatment arms. With a maximum follow-up period of 46 months, the median PFS for all 15 eligible patients was 29.3 months. The median OS was not reached since there were only 4 deaths. Grade ≥3 toxicity occurred in 93% of patients and the treatment-related mortality was 6%.

Summary/Conclusions: Although the small sample size prevents definitive conclusions from being drawn, the results of this prematurely terminated study suggest that alemtuzumab, dexamethasone and lenalidomide is feasible to deliver in most patients with high-risk CLL and has an acceptable safety profile. The regimen also appears to be effective, with a high overall response rate and a median PFS somewhat longer than the 11.8 months that was observed with alemtuzumab plus methylprednisolone. We conclude that the efficacy of glucocorticoid/alemtuzumab in this setting may be enhanced by the addition of lenalidomide without incurring significant additional toxicity.

PB1795

SERUM RESISTIN IN RELATION TO B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA RISK AND ITS CORRELATIONS WITH PROGNOSTIC BIOMARKERS

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Background: Excess weight and insulin resistance are now considered risk factors for many types of malignancies, including leukemia and lymphoma. Potential mechanisms that may link obesity and insulin resistance to B-cell chronic lymphocytic leukemia (B-CLL) include also the abnormal secretion of adipocytokines. Resistin, an adipocytokine belonging to the family of resistin-like molecules, was originally discovered as a molecule enhancing insulin resistance or impaired hepatic sensitivity to insulin and provoking hyperglycemia without affecting peripheral insulin sensitivity. However, data in humans are controversial.

Aims: In this cross-sectional study, we attempted to investigate the contribution of resistinemia to B-CLL risk taking into account potential important confounders including the family history of lymphohematopoietic cancer (LHC), body mass index (BMI), serum insulin and other adipocytokines. We also attempted to ascertain whether a relationship between serum resistin and prognostic markers exists amongst patients with B-CLL diagnosis.

Methods: Blood samples were collected from 95 cases with incident B-CLL and 95 hospital controls, admitted for non-neoplastic and non-infectious conditions, matched on gender, age and year/month of diagnosis (±1 month) between 2001 and 2007. Informed consent was obtained from all study participants. Serum resistin was determined using a commercially available ELISA assay (Phoenix, CA, USA). Furthermore, serum lactate dehydrogenase (LDH), β_2-microglobulin (BMG), lymphocyte morphology and the surface expression of CD38 in >30% of B-CLL lymphocytes were assessed. The statistical analysis of the data was performed using IBM-SPSS® version 23 for Windows.

Results: Patients with B-CLL had on average a higher BMI as compared to control participants (27.8 *vs* 26.7 kg/m^2; p<0.01). Significantly, more patients than controls presented a positive family history of LHC (13 *vs* 3 controls; p<0.01). In univariate analysis, circulating levels of resistin were statistically significantly elevated in cases as compared with controls (10.43±5.42 *versus* 8.12±4.88 ng/mL respectively, p=0.002). However, although serum higher resistin was associated with B-CLL risk in unadjusted analyses, in multivariable analyses controlling for age, gender, date of diagnosis, family history of LHC, BMI, serum insulin, leptin and adiponectin levels, serum resistin presented a borderline statistical significance with B-CLL occurrence (OR: 1.08, 95% CI: 0.99-1.16, p=0.06). Amid patients, serum resistin was not associated with Binet stage (p=0.85) and absolute lymphocyte count (p=0.55). Nevertheless, circulating resistin presented a borderline negative correlation with BMG (p=0.07) and CD38 (p=0.07).

Summary/Conclusions: Circulating resistin was found to be elevated among cases as compared to controls in univariate analysis. It has been recently shown that hyperesistinemia is linked to the risk for many malignancies. These results need to be confirmed in larger study populations using a prospective study design; but if reproduced in adjusted analyses they may suggest that resistin may be upregulated and/or upregulate the synthesis of other inflammatory factors etiologically linked to B-CLL etiopathogenetic process.

PB1796

CALRETICULIN GENE MUTATION IN HAIRY CELL LEUKEMIA AND RELATIONSHIP WITH PROGNOSIS

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Background: Hairy cell leukemia (HCL) is a malignant hematologic neoplasm characterized by peripheral blood and bone marrow hairy cells. Etiology of disease is not clear. Calreticulin (CRT) is a protein located on endoplasmic reticulum which plays role on protein folding, calcium homeostasis, regulation and loading antigenic peptides into major histocompatibility class-I in the cells. As of today, CRT gene mutations were determined in JAK2 and MPL negative myeloproliferative neoplasms. However, the effect of this gene mutation on hairy cell leukemia which is a lymphoproliferative disease is unknown.
Aims: To study the presence of CRT mutations in hairy cell leukemia and effects.
Methods: A retrospective single institution study chart review of cases with HCL at Gaziantep University between 2005- 2015 was performed. Patients with precise hairy cell leukemia were included. Bone marrow tissue biopsy samples were used. Following the isolation of genetic material and gene sequence analysis by PCR, mutations were investigated for CRT. Complete blood count, peripheral blood smear, bone marrow aspiration and biopsy, flow cytometry were used to diagnose HCL patients. Laboratory counts were determined as anemia <12g/dL, thrombocytopenia <100x10^9/L, neutropenia <1 x10^9/L and monocytopenia <0.2 x10^9/L. We used French Society of Hematology remission criteria for treated hairy cell leukemia. Appropriate ethics approval approval was obtained in accordance with the Helsinki declaration. Comparison between group medians was done using Mann Whitney U, while survival estimates were calculated using Kaplan-Meier curves SPSS V22.
Results: Out of 33 HCL samples, 21 (63.6%) CRT exon-9 sequencing analyses could be performed and CRT gene mutation was not detected in any. In the clinical analysis of these patients median age was 54 years, with 26 (78.8%) patients were males. Labs at diagnose included median white blood cell was 2.7 x10^9, Hemoglobin 9.5 g/dL, platelet 58 x10^9, monocytes 0.28x10^9, lymphocyte 1.3 x10^9, neutrophil 1 x10^9. Laboratory count were found anemia in 22 (%73.3), trombocytopenia in 21 (70%), neutropenia in 14 (48.3%) and monocytopenia in 12 (41.4%) of patients. Out of 33 patients, 27 (90%) had splenomegaly documented at the time of diagnosis. When evaluated pretreatment splenic size by ultrasound, median spleen size was 20.3 centimeter (median%25-75, 16.5-24). Cladribine was used in the treatment of 23 (69.7%) patients. Among these patients' complete response was 13 (56,5%), partial response was 3 (13%) and overall response was 69.5%. Splenomegaly was significantly high in patients without monocytopenia (p=0.05). There were survival differences between treated (n=23) and untreated (n=10) patient groups (85.6±3 and 17.7±7.8 median months, respectively p=0.001). The patients who treated had significantly longer overall survival compared to those untreated. Furthermore, pretreatment median hemoglobin level was higher in remission group compared to no remission group as 10.1 g/dL *vs* 8.5 g/dL respectively and there was a statistically significant difference between them (p=0.047).
Summary/Conclusions: This is the first study carried out regarding CRT gene mutation in HCL in the literature. We consider that CRT gene mutations are not effective on disease pathogenesis. As a result, spleen size was larger in patients with who had no monocytopenia and initial hemoglobin levels were higher in remission group. This result may be an important finding in terms of timing of treatment in patients with HCL. In this respect, it may be appropriate approach to start to the treatment before anemia develops. Additional larger studies are needed to confirm our results.

PB1797

FACTORS ASSOCIATED WITH TREATMENT CHOICE AND RESPONSE IN RELAPSED AND REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS RE-TREATED WITH RITUXIMAB: THE OBSERVATIONAL PERLE STUDY

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Background: The treatment of patients with relapsed and refractory chronic lymphocytic leukemia (r/rCLL) previously treated with rituximab-based regimen (R) and re-treated with rituximab is relatively complex.
Aims: Exploratory analyses were conducted to identify factors leading to the choice of a specific combined chemoimmunotherapy: R-Bendamustine (RB), R-purine analogs (RP), R-alkylating agents (RA) or others regimens (Oth) and factors associated with the quality of response.
Methods: PERLE is a prospective non-interventional cohort study (main results presented at EHA 2014 - P876) conducted in r/rCLL patients. Two sets of exploratory analyses using data-mining methods were performed on baseline characteristics: decision trees to find patterns of factors defining subgroups of patients and random forests to provide a robust predictor model with a ranking between all available information. To confirm these outputs, standard logistic models, univariate and multivariate were implemented. Among baseline characteristics (clinical, demographics, disease and patients' management) analysed with data mining, some were identified as candidate factors. The most impacting factors associated either with choice of chemotherapy or with response were confirmed by multivariate analyses.
Results: Overall, 310 patients were included. Their median age was 72 years [35 - 93] and 68% were men. The majority (187, 60%) was in first relapse. Rituximab was administered during first (69%), second (94%), or both (16%) previous treatment lines. More than half of the patients received RB, 16% received RP and 18% received RA (including 40% with chlorambucil). RB was favored in patients with a previous purine based-treatment (OR=2.36 [1.4; 3.9]) which is the opposite of RP (OR=0.39 [0.2; 0.8]). RA was preferentially prescribed in elderly patients (≥70 y.o., OR=4.29 [2.0; 8.9]). Overall response rate (ORR) at the end of induction was 84%. A significantly better response was observed with the use of 500mg/m^2 rituximab dose *versus* 375mg/m^2 (84.5% *versus* 53.4%), when neutrophils count was ≥1.10^9/L *versus* <1.10^9/L, (76.3% *versus* 47.4%) and when response duration was ≥12 months *versus* <12 months (80.1% *versus* 62.5%). The number of previous rituximab-based treatment lines also impacts the quality of the response: ORR 76.2% with 1 treatment line *versus* ORR 45.0% with 2 previous treatment lines. Imbalance between responders and non-responders according to 17p deletion was also observed (patients with deletions: 49% responders; without deletion: 80%). Furthermore, ORR differences were observed between treatment groups: 85% with RB, 89% with RP, and 78% with RA. In this observational study, adverse events (all grades) were hematological (26.9%, including neutropenia (11.9%)) and infectious (14.4%). Grade ≥3AEs occurred in 28.4% of patients and were mainly hematological (19.7%); 22 patients (6.9%) died during induction treatment (15 deaths related to progression; 7 for AEs).
Summary/Conclusions: In real-life setting, treatment choice for r/r CLL is multifactorial. Overall, whatever the patient's profile and the treatment strategy, the dose of rituximab (500mg/m²), as well as a single previous rituximab-based treatment line, are associated with a significantly better quality of response.

PB1798

PLATELET DYSFUNCTION CAUSED BY IBRUTINIB TREATMENT IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. MONOCENTRIC EXPERIENCE: CLINICAL AND LABORATORY CHARACTERIZATION

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Background: Ibrutinib (IBR) is a potent and irreversible inhibitor of Bruton's tyrosine kinase (Btk), approved for treatment naïve of chronic lymphocytic leukemia (CLL) with del 17p or TP 53 mutation or for patients (pts) with relapsed/refractory (R/R) disease. IBR treatment is associated with bleeding events, mostly mild to moderate (I-II grade of severity), rarely severe (III-IV grade of severity). The mechanism causing these bleeding events remains unknown. A defect of platelet function has been hypothesized and inhibition of signaling by glycoprotein VI (GP-VI) has been previously described. IBR associated bleedings and platelet dysfunction may be relevant in CLL pts who are elderly and with comorbidities.
Aims: To investigate and characterize the effect of IBR on platelet function *in vitro* and *in vivo* in pts with CLL.
Methods: Nine pts with CLL were treated with oral IBR at dose of 420 mg/day; 7 received IBR in monotherapy for relapsed/refractory CLL and 2 in association with monoclonal antibody anti-CD20 for treatment-naïve CLL. Median age was 68 years (57-75); 5 pts had unmutated IgVH and 2 had 17p deletion on FISH. The median number of prior therapies in R/R CLL pts was 3 (range 2-7). After a median follow up of 14 months (range 6-20) all pts achieved a partial response. Thereafter 2 pts discontinued IBR therapy: 1 for Richter's transformation, 1 underwent allogeneic HSCT. All pts before and after initiation of treatment with IBR were studied with light transmission aggregometry (LTA) using platelet-rich-plasma and the following agonists : ADP 2-4 uM, PAR1-AP 25 uM, Collagen 10 ug /mL, arachidonic acid 1 mM, ristocetin 0.6-1.2 mg/mL; measurement of von Willebrand factor(vWF) antigen and ristocetin cofactor activities by chemiluminescent immunoassay.
Results: We recorded only grade I or II bleeding events (bruising, petechiae, conjunctival hemorrhage, rectal bleeding) in 7 pts at a median time of 3 months after IBR treatment (range 1-9); no patient needed treatment interruption or dose reduction. Eight pts displayed abnormalities of the aggregation induced by 10 ug/ml collagen after initiation of IBR treatment. At these collagen concentration, only significant prolongation of the lag phase was measured (74.6+/- 23.7 sec *vs* basal 40.4+/- 17.2 sec), whereas the maximal aggregation was not impaired (67.9+/- 21.4% *vs* basal 85.5+/-5.8%). Interestingly, in 5 pts a significant improvement of the aggregation by 2 uM ADP (91.2+/- 5.1% *vs* basal 39.3+/- 24.6%) and 4 uM ADP (91.6+/- 2.9% *vs* basal 65.4+/- 19.4%) during IBR treatment was reported. On the contrary the aggregation by PAR1-AP, ristocetin and arachidonic acid was not affected under IBR. Finally, in 3/3 pts the vWF antigen and ristocetin cofactor activity were higher at the onset of the disease (169+/- 38%) and returned to normal values under IBR treatment (111.4+/- 47%).

Summary/Conclusions: Our study showed that collagen induced platelet aggregation resulted impaired while ADP induced aggregation improved upon IBR treatment. Finally the levels of vWF were significantly higher in CLL pts before treatment and normalized during IBR. In conclusion, IBR treatment in CLL pts causes a mild bleeding phenotype most probably due to platelet dysfunction. The bleeding risk was unrelated to platelets count. The assessment of platelet aggregation in IBR treated CLL pts could help to predict and monitor bleeding risk, and to guide pts through invasive procedures.

PB1799

EFFICACY OF IBRUTINIB-BASED THERAPY IN THE TREATMENT OF RECURRENT AND REFRACTORY FORMS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Several studies in chronic lymphocytic leukemia (CLL) have determined the achievement of minimal residual disease (MRD) negativity as an independent favorable prognostic factor, leading to prolonged disease-free and overall survival, regardless of the treatment protocol or the presence of other pre-existing prognostic indicators. Novel therapies that incorporate purine analogs, monoclonal antibodies and new agents targeting the B-cell receptor signalling pathway recently improved response rates in relapsed/refractory CLL. The number of patients achieved MRD negative remission increased.
Aims: To estimate of response rate and MRD after ibrutinib therapy in the treatment patients with relapsed/refractory CLL.
Methods: 47 pts were included in the analysis. Stratification of patients into prognostic groups based on identified chromosomal abnormalities by standard GTG-method and interphase FISH analyses with use of DNA probes: LSI *RB1* (13q14), LSI *ATM* (11q22), CEP12, LSI *TP53* (17p13.1) (Abbott). Group 1 (n=26): 1st line rituximab-based chemotherapy (RB-12, FCR-14); Group 2 (n=17): 2nd and subsequent lines of rituximab-based chemotherapy (RB-12, FCR-4, R-CHOP-1) and Group 3 (n=9): ibrutinib-based therapy (420 mg daily oral Ibrutinib±Rituximab). Median age in Group 1 was 57 years (35-67), in Group 2-62 years (49-83), in Group 3-65 years (51-82). Patients received, regardless of the programs of therapy. We have used NCI-IWCLL revised guidelines for treatment initiation and assessment of response. MRD was detected by multicolor flow cytometry of bone marrow in patients achieved a complete or partial remission: Group 1-26 pts, Group 2-7 pts, Group 3-4 pts.
Results: Patients with unfavorable chromosomal abnormalities were detected in each group: Group 1-4 pts (del(11q)-3, complex karyotype-1); Group 2-2 pts (combination del(11q) with del(13q)); Group 3-1 pts (del(17p)). Overall response rate (ORR) in Group 1 was 100%: complete remission (CR)-10 pts (unfavorable prognosis (UP)-2), partial remission (PR)-16 pts. Group 2: ORR 82% (CR-1 pts, PR-13 pts (UP-2)); stable disease (SD)-1 pts, progression disease-2 pts. Group 3: ORR 89% (CR-3 pts, PR-5 pts (UP-1); SD-1 pts). MRD-negative remission was achieved in 46% pts in Group 1 (12/26: CR-6 pts (UP-1), PR-6 pts (UP-2)), in Group 2-14% (1/7, CR), in Group 3-25% (1/4, CR).
Summary/Conclusions: Evaluation of response and MRD after ibrutinib-containing programs therapy in the treatment patients with relapsed/refractory chronic lymphocytic leukemia require further research. The influence of genetic abnormalities on the treatment efficacy and MRD status has yet to be defined.

PB1800

USE OF IBRUTINIB IN PATIENTS WITH B CELL LYMPHOID MALIGNANCIES: ADVERSE EVENTS AND MANAGEMENT IN COMMUNITY SETTING

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Background: Bruton's tyrosine kinase (BTK) is expressed in B-cell malignancies, playing an important role in B-cell receptor (BCR) signaling and offering a promising new strategy for the development of targeted drugs. Malignant B cells in mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) rely on BCR signaling pathways for cell survival, proliferation, adhesion, and migration. Ibrutinib is a BTK inhibitor which has been found to be an effective therapy option in patients with relapsed/refractory mantle cell lymphoma, relapsed/refractory chronic lymphocytic leukemia/small cell lymphoma (SLL) or newly diagnosed patients with CLL and a chromosome 17 deletion (del 17p)[1]. Most responses tend to be partial, but nearly two thirds of MCL and CLL patients treated have had a durable response, despite many being refractory, having high risk cytogenetic abnormality and/or heavily pre-treated[2]. The medication is tolerable with <10% of trial patients discontinuing use due to side effects[3]. Published trial data highlight side effects including nausea, diarrhoea, fatigue, bleeding, decrease in blood cell counts, renal impairment[2]. Our analysis of data on side effects from this drug in our cohort differs from those side effects highlighted in drug trials.
Aims: To better understand the side effect profile of ibrutinib and management of these patients in real life.
Methods: A cohort of 36 patients all refractory to at least two lines of therapy or with del 17p were entered into this study; with a mix of 25 patients with CLL, 7 with SLL, 4 with MCL. All were treated with ibrutinib at either 560mg/day (MCL) or 420mg/day (all others) until disease progression or death with a median treatment time of 9 months.
Response and adverse event data has been recorded for these patients and subsequently analysed.
Results: In our cohort the most common side effects were dermatologic complications, affecting nearly one third of patients to some degree. As well, one sixth of patients in our cohort experienced symptomatic gastro-oesophageal reflux requiring treatment with proton pump inhibitors. We had one patient who experienced transaminitis related to ibrutinib requiring cessation of therapy. No patient in our cohort experienced cytopenias or renal impairment or significant diarrhoea or nausea related to therapy. Bruising and platelet dysfunction was noted in 20% of patients, especially if already on antiplatelet therapy (APA) or anticoagulants (OAC). None of these patients required cessation of either therapies, but dose or frequency reduction of APA or OAC.
Most of the patients in our cohort have achieved at least very good partial response, with only six patients with progressive disease (all with CLL or SLL).
Summary/Conclusions: Our real life experience of using ibrutinib in patients with refractory CLL or MCL has shown very good response with most patients tolerating the therapy very well. In our cohort the side effects were different from those recorded in clinical trials. Cutaneous reactions and acne like lesions as well as staphylococcal infections appear to be more common than appreciated and GIT side effects were less common in our experience. We have found topical treatment of acne lesions with antibiotics to be mostly effective and oral or intravenous antibiotics required for extreme cases. Ibrutinib therapy is a well tolerated and effective therapy option for patients with high-risk CLL or MCL. Therapy can be managed in the community without requiring hospitalisation.

PB1801

APPLYING PROBED CAPILLARY ISOELECTRIC FOCUSING (PROBED CIEF) FOR RESPONSE MONITORING OF SIGNAL INHIBITOR THERAPY DIRECTED AGAINST BRUTONS TYROSINE KINASE (BTK) IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Background: Signals mediated through the B cell receptor (BCR) of chronic lymphocytic leukemia (CLL) are central to disease pathobiology. Bruton's Tyrosine Kinase (BTK) is a key regulator of BCR signalling. Small molecule inhibitors of BTK (BTKi) have therefore emerged as effective therapeutic agents. BTKi treatment is particularly successful in CLL; however responses may not be complete or durable. It is therefore important that we have tools to monitor disease response and direct therapy. Probed capillary isoelectric focussing (probed cIEF) uses fine capillaries that contain an ampholyte pH-gradient to separate protein within cell-lysates according to their charge. Many post-translational modifications induce significant changes to charge, and modified proteins are readily separated. Antibodies that recognise specific signal-molecules are then used to probe the samples and to generate profiles that demonstrate the presence and relative abundance of different charge-forms of the target molecule. These charge-forms reflect structural alteration, lipid modification or different phosphorylation states, modifications that reflect differential activation of the signalling molecule. The technique offers the capability to detect and evaluate the full range of changes to BTK, and therefore can be used to support clinical monitoring of drug effectiveness.
Aims: To evaluate the use of probed cIEF in signal response monitoring for patients treated with BTK inhibitors.
Methods: CLL cells were cultured *in-vitro* in the presence or absence of BCR activation (anti-IgM cross-linkage) with and without BTKi (ibrutinib). Cells lysates were separated according to charge using an ampholyte pH-gradient (nested 5-8). To assess BTK response, separated protein was probed with total BTK antibody to generate profiles of the presence and relative abundance (area under curve for peak volumes (Compass software)) of different charge-forms of the molecule. *Ex-vivo* cells of CLL pre and post *in-vivo* BTK signal inhibitor therapy were analysed to compare with *in-vitro* profiles.
Results: Phosphoprotein traces were reliably obtained yielding reproducible dose-response profiles. A significant complexity of charge-forms was identified for BTK, with a range of peaks differing substantially between resting and activated cell states. Ibrutinib greatly changed the profile of BTK, both through new peak formation and peak shifts that were not attributable to recognised effects of the drug against standard regulatory motifs. The peak changes we observe are therefore complex, they combine the expected prevention of BTK activation, but also new and unexpected changes not simply a return to the "resting state profile" of the molecule. We suggest these form a molecular signature representing a biomarker of drug response. Studies of inhibitor action in clinically treated and clinically responsive CLL cells mirrored that of our profile

of BTKi treated cells *in-vitro* indicating our profile to represent biochemical modification of BTK signalling after clinically relevant therapy.
Summary/Conclusions: Probed cIEF offers the capability to monitor complex signal-interactions, and to identify important signalling changes in a simple, objective and reproducible manner. Future work will aim to precisely identify the biochemical changes that underlie the specific peaks of the BTK profile that may represent clinically useful biomarkers of treatment response.

PB1802

IS C-REACTIVE PROTEIN A RELIABLE SURROGATE MARKER FOR INFECTION TO HELP FACILITATE DISCHARGE IN PATIENTS ON IBRUTINIB? (A SINGLE CENTRE EXPERIENCE

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Background: C-Reactive Protein (CRP) is an acute phase protein made by the Liver in response to interleukin-6 (IL-6). It is produced from cells including macrophages, T and B Cells in response to inflammation. It is a poor marker of sepsis at day zero, rising at 4-6 hours and peaking at 36 hours. Ibrutinib is a covalent Bruton's tyrosine kinase (BTK) inhibitor. BTK is an essential part of the B-cell receptor signalling pathway. It mediates both the microenvironment system and promotes survival and proliferation of B-cells. Anecdotally, since Ibrutinib's introduction as a treatment for Chronic lymphocytic Leukaemia (CLL), Mantle Cell Lymphoma (MCL) and Waldenstrom's Macroglobulinaemia (WM) we have noticed some patients admitted with confirmed infections whose CRP response was lower than expected.
Aims: To analyse the correlation between CRP and confirmed infections in all patients on Ibrutinib at a small single centre in the UK.
Methods: We retrospectively reviewed the notes of all 46 patients who had been started on Ibrutinib since its approval, and collected data including sequential CRP's (significant if >60 mg/L) for all episodes requiring admission with confirmed infection, defined as a positive culture result (blood, sputum, skin swab, mid stream urine), or imaging changes consistent with an infection (as reported by Radiology).
Results: Of the 46 patients (29 CLL, 17 MCL) to have received treatment, only 16 (10 CLL, 6 MCL) were admitted to hospital with infection. 5 of these 16 patients had multiple admissions resulting in 25 admission episodes. Of these 25 episodes only 15 met the criteria for confirmed infection. On analysis of the data, the mean length of stay was 10.1 days with a mean admission CRP of 73 mg/L which rose to 85 mg/L at 48 hours. The majority of the episodes (10/15, 67%) appear to have had an appropriate CRP response in the first 48 hours. Of interest one of these 10 was diagnosed with *Listeria monocytogenes* on multiple blood cultures, the admission CRP (58 mg/L) did not rise significantly at 24 hours (68 mg/L) and fell thereafter. The patient never mounted an appropriate response for the clinical picture and by the time she died at day 15 her CRP was 3 mg/L. Of the 5 remaining episodes who's CRP remained low in the first 48 hours (mean CRP 25 mg/L) 4 had imaging and symptoms consistent with a chest infection and 1 was blood culture positive for *Pseudomonas aeruginosa* sepsis (mean length of admission in this group 10.2 days). The data shows 33% of admission episodes with confirmed infection did not have a significant rise in CRP in the first 48 hours.
Summary/Conclusions: In clinical trials Ibrutinib has shown excellent results and the number of patients taking it stand to rise considerably over the coming years. Our single centre study is relatively small and did not obtain enough data to say whether CRP is a reliable surrogate marker for infection in this group. However the 5 cases with low CRP and to a certain extent the 6th (*Listeria monocytogenes* case) mentioned above raise the possibility that Ibrutinib could have an effect on CRP that has yet to be identified. The proposed possible mechanism would be IL-6 reduction. For this reason we have changed departmental policy, stopping Ibrutinib on admission, relying on clinical picture in the first 48 hours before re-checking CRP to assess response to treatment and therefore likelihood for discharge. Possible areas for further investigation include a multicentre study looking for correlation, comparing alternative inflammatory markers and evaluating IL-6 release in patients with infections on Ibrutinib.

Chronic myeloid leukemia - Biology

PB1803

DYNAMICS OF BCR-ABL1 MULTIPLE MUTATION DETECTED BY SUBCLONING AND SEQUENCING IN TYROSINE KINASE INHIBITOR RESISTANT CML

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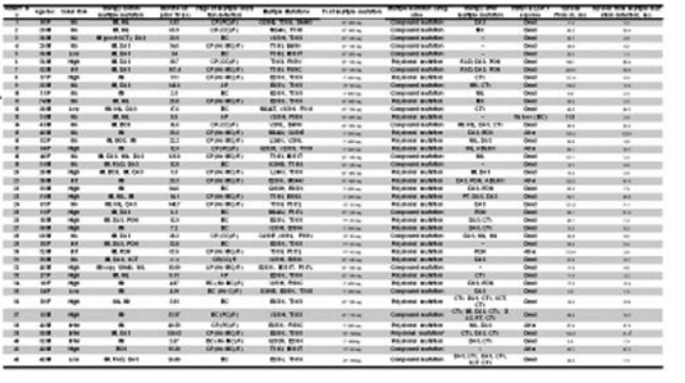
Background: BCR-ABL1 kinase domain (KD) point mutation causes resistance to tyrosine kinase inhibitors (TKI) in CML patients through impaired binding of TKI to the target site. One of the characteristics of patients with BCR-ABL1 kinase domain point mutations is the fact that some patients have multiple mutations. However there have not been many studies showing that data about clinical relevance or dynamics of multiple mutation during CML treatment.
Aims: The aim of this study to was to evaluate dynamics and characteristics of multiple mutations in the serial samples from the patients carrying multiple mutations using subcloning and sequencing.
Methods: Since 2002, 735 CML patients were screened for mutation analysis due to sign of resistance to TKI including imatinib, nilotinib, dasatinib, bosutinib, radotinib or ponatinib at Seoul St Mary's Hospital using direct sequencing and ASO-PCR. Among them, 42 patients showed multiple mutations. We analyzed serial samples from the 42 patients using subcloning and sequencing to investigate whether the multiple mutations are on same clone (defined as compound mutation), separated clones (defined as polyclonal mutation) and characterize its clinical relevance and dynamics.
Results: Details on response and survival outcome of 42 patients with multiple mutations is shown in Table 1. In order to investigate whether the multiple mutations are on same clone or on separated clone, we cloned serial samples from the 42 patients. Cloning of cDNA region corresponding to BCR-ABL1 KD into plasmid was performed and followed by transformation into competent cells, colony formation, plasmid preparation of 20 colonies from each sample, and then direct sequencing. Distribution of disease phase at first multiple mutation detection, advanced phase or blast phase was 48% and chronic phase was 52%. With a median follow-up of 35.6 months (range, 4.8-224), 20 different mutations were detected by direct sequencing in 42 patients. 36 patients harbored 2 mutations and 6 patients harbored 3 mutations. Among 42 patients with Multiple mutation, 19 (45%) and 23 (55%) patients had compound mutation and polyclonal mutation, respectively. of 19 patients with compound mutation, 18 patients (95%) were dead. 26 of 42 patients (62%) developed multiple mutations including T315I. Among them, 16 patients (62%) also had P-loop mutation.

Table 1.



Summary/Conclusions: Analysis of serial samples from a same patient provided evidence of dynamic change of portion of compound mutation. In most case, portion of the containing compound mutation was increased as treatment went on, indicating the clone harboring compound mutation can take survival advantage over TKI treatment in comparison of the clone containing individual type of mutation. In addition, some patients showed change in individual mutation type comprising multiple mutations as treatment went on. Patients with compound mutation showed poor outcomes compared with polyclonal mutation in our cohort, further investigation on a large patient cohort will be needed. Updated data about dynamics and characteristic of multiple mutations with longer follow-up duration will be presented in the meeting.
**Even though E255k detected by direct sequencing, it was not detected by subcloning and sequencing.*

PB1804

PPARΓ IS A NOVEL THERAPEUTIC TARGETS IN PH-POSITIVE LEUKEMIA CELLS

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Background: ABL tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib and dasatinib have demonstrated the potency against Philadelphia chromosome (Ph)-positive leukemia patients. However, resistance to ABL TKI can develop in chronic myeloid leukemia (CML) patients due to BCR-ABL point mutations and leukemia stem cells, because ABL TKIs cannot eradicate leukemia stem cells. Therefore, new approach against BCR-ABL mutant cells and LSCs may improve the outcome of Ph-positive leukemia patients. Peroxisome proliferator-activated receptors (PPARs) compose a superfamily of nuclear hormone receptors and regulate the transcription of target genes. Three subtypes of PPARs (α, γ and δ/β) have been identified. PPARγ is expressed not only adipose tissue but also cancer cells. The PPARγ agonist, pioglitazone was used for the treatment of diabetes patients. Recently, it has been reported that PPARγ agonist eradicate leukemia stem cells of CML patients (Prost S *et al.* Nature. 2015;525:380-3).
Aims: We hypothesized that targeting PPARγ, in combination with ABL TKI, would result in enhanced therapeutic activity in Ph-positive leukemia cells including T315I mutation and primary samples.
Methods: In this study, we investigated the effect of PPARγ agonist, pioglitazone on Ph-positive leukemia cell lines (K562, NCO2, Ba/F3 BCR-ABL, Ba/F3 T315I) and primary samples.
Results: 72 h treatment of pioglitazone did not exhibit cell growth inhibition against K562 cells.
We found that mRNA of PPAR subunit, PPARα and PPARγ was increased after pioglitazone treatment. We examined the intracellular signaling after treatment of pioglitazone. We found phosphorylation of BCR-ABL and Crk-L was not reduced after pioglitazone treatment. In contrast, phospohorylation of AMP-activated protein kinase (AMPK) was increased and S6 ribosomal protein was reduced. We investigated the pioglitazone activity against T315I positive cells. Pioglitazone did not induce cell growth inhibition of Ba/F3 T315I cells. However, combined treatment of Ba/F3 T315I cells with ponatinib and high concentration of pioglitazone caused more cytotoxicity than each drug alone. Phosphorylation of BCR-ABL and Crk-L was not reduced. However, phosphorylation of S6 ribosomal protein was reduced and AMPK phosphorylation and cleaved poly (ADP-ribose) polymerase (PARP) was increased after ponatinib and pioglitazone treatment. We also found that the treatment of ponatinib and pioglitazone exhibits cell growth inhibition against Ph-positive primary samples with T315I mutation. Phosphorylation of S6 ribosomal protein was reduced and AMPK phosphorylation and cleaved poly (ADP-ribose) polymerase (PARP) was increased after ponatinib and pioglitazone treatment. We next investigated the anti-angiogenic effects of PPARγ agonist on human umbilical vein endothelial cells (HUVEC). Pioglitazone inhibited tube formation of HUVEC (size, length and junction) in matrigel assay. Pioglitazone also inhibited chemotaxis of HUVEC.
Summary/Conclusions: Our study indicated that administration of the PPARγ agonist, pioglitazone enhances the effects of ABL TKI and is effective in suppression of angiogenesis, suggests that PPARγ agonist may possess promising clinical relevance as a candidate for treatment of Ph-positive leukemia patients.

PB1805

PLASMA PROTEOMICS IN CHRONIC MYELOID LEUKEMIA PATIENTS BEFORE AND AFTER INITIATION OF TYROSINE KINASE INHIBITOR THERAPY REVEALS INDUCED TH1 IMMUNITY AND LOSS OF ANGIOGENIC STIMULI

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Background: Chronic myeloid leukemia (CML) treated with tyrosine kinase inhibitors (TKIs) in most cases have an excellent long-term prognosis. However, there are still problematic issues in a smaller proportion of CML patients related to TKI resistance and long-term treatment side effects and costs. Much is still unknown about why patients respond differently to TKI treatment and why some patients are even able to stop TKI treatment without disease relapse while others relapse quickly despite seemingly good, durable treatment responses. Proteomics is an area of growing interest and the simultaneous measurement of many proteins is now possible using multiplex assays. Multiplexing is used for several purposes, such as surveys of changes in protein abundance, biomarker validation and clinical diagnostics and to our knowledge, the usefulness of plasma proteomics has not been evaluated in CML patients.
Aims: In this pilot study we investigated a total of 124 proteins in plasma from CML patients with the purpose of identifying proteins that are differently expressed at diagnosis and after TKI treatment initiation, either as a result of the decreased disease burden or as an effect of the TKI treatment itself.
Methods: Samples were taken from 14 CML patients at diagnosis and after three months of TKI treatment (imatinib or dasatinib). Samples were analyzed by three different multiplex platforms: Human proinflammatory 9-plex Ultra-Sensitive kit by MesoScale Discovery, Multi-analyte profiling (MAP) technology by Myriad RBM and Proseek Oncology 1 by Olink. Results were correlated to disease activity (Sokal score, presence of Ph+stem and progenitor cells at diagnosis) and treatment response.
Results: Many protein markers were significantly altered after three months of TKI treatment. Some proteins were analyzed on more than one platform and results for markers present in low concentration in plasma were sometimes contradicting between platforms, which may reflect the specificity and sensitivity variation of the platforms used. It is also highlighting possible difficulties analyzing multiple markers of different concentration ranges in a single sample at one dilution. The protein patterns demonstrated a decrease of pro-tumorigenic analytes (VEGF, TGFβ, IL10, CD31, MICA) while some analytes known to be of importance to T-helper 1 (Th1) immune responses and anti-cancer immunity were increased after TKI initiation (IL12, CXCL9/MIG, IFNγ) (Figure 1), likely reflecting a restoration of normal immune functions after treatment initiation. Interestingly, the level of TGFβ, which has been connected to the maintenance of leukemic stem cells, was correlated to the leukemic stem cell burden ($Ph^+CD34^+CD38^-$ cells) at baseline. We also found reduced angiogenic stimuli which could reflect normalization of bone marrow angiogenesis after treatment initiation since increased bone marrow vascularity and elevated angiogenic factors have been described in untreated CML patients. Further, some single plasma proteins were identified that can be of potential interest to study further for biologic, prognostic or therapeutic significance such as E-selectin, uPAR, growth hormone and carbonic anhydrase IX.

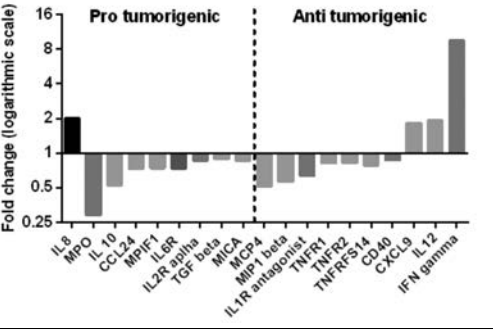

Figure 1.

Summary/Conclusions: Plasma proteomics seems feasible and useful in CML patients, both for studying patterns of protein expression and for identifying single proteins differentially expressed before and after treatment. Hence, plasma proteomics can be used to gain better understanding of drug mechanisms and treatment responses in CML. Some of the significantly altered proteins indicate novel disease or treatment mechanisms and further studies may give novel insights in CML and TKI therapy.

PB1806

LEUKEMIC STEM CELLS DEFINED BY DIPEPTIDIL-PEPTIDASE IV (CD26) EXPRESSION IN CD34+CD38- STEM CELLS AND TYROSINE KINASE INHIBITOR THERAPY IN CHRONIC MYELOID LEUKEMIA

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Background: Recently, dipeptidil-peptidase IV (DPP IV; CD26) has been described as a specific marker of leukemic stem cells (LSC) within the $CD34^+CD38^-Lin^-$ compartment present in chronic myeloid leukemia (CML) patients. These cells seem to be difficult to eradicate by tyrosine kinase inhibitors (TKI), although the efficacy of different generations of TKI remains unknown. In addition, the negative influence of these LSC on optimal therapy responses (time to achieve major molecular response) is under investigation in CML.
Aims: In this study, we aimed at the evaluation of the presence of LSC in chronic phase CML patients to establish the additional parameters to better define the efficacy of different therapeutic strategies.
Methods: Peripheral blood samples from chronic phase CML patients (n=45) under Interferon-alpha 2b (IFN-α 2b), imatinib, dasatinb, nilotinib, bosutinib and ponatinib therapy were analyzed by multi-parametric flow cytometry for the characterization of LSC. Buffy coats (n=3) from healthy blood donors were

used as control. Cytokines and chemokines were evaluated in a 34-plex panel by xMAP technology (Luminex®).
Results: Circulating CD34 positive cells were increased in chronic phase CML patients when compared to controls (0.15±0.05% *vs* 0.06±0.05%). CD26 was found expressed in CML $CD34^+CD38^-Lin^-$ stem cells (12.07±4.14%) and absent in controls. CD26 expression was higher in LSC compared to progenitor cells (MFI 704.6±200.8 *vs* 270.4±34.8). We also found SDF-1 significantly overexpressed in CML patients undergoing TKI treatment when compared to IFN-α 2b. An impairment of LSC to respond to SDF-1, associated with less migration to stem cells niche, has been observed, although they express high levels of CXCR4. All of our chronic phase CML patients with detectable LSC in peripheral blood samples needed to change therapy and/or increase TKI dose and the majority of these patients present high or intermediate clinical prognostic risk score (Sokal) on initial evaluation.
Summary/Conclusions: Detection of LSC defined by CD26 in CD34+CD38-Lin- stem cells might be an important tool to improve CML treatment strategy in the near future.

PB1807

MODULATION OF LEUKOTRIENE SIGNALING INHIBITING CELL GROWTH IN CHRONIC MYELOID LEUKEMIA
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Background: The introduction of tyrosine kinase inhibitors (TKI) has dramatically improved the outcome in chronic myeloid leukemia (CML). However, for most patients cure remains unlikely due to residual, detectable leukemic stem cells. This may be due to aberrant regulation of BCR-ABL-independent signaling pathways. One such suggested pathway leads to the formation of leukotrienes (LT), inflammatory mediators indicated to play a role also in the tumorigenicity of several malignancies (*e.g.* in the colon, prostate, breast, lung). We have previously shown that CML patient-derived myeloid cells have an elevated capacity to synthesize LT, paired by an increased expression of the enzyme LTC_4 synthase (LTC4S). Recently an up-regulation of the arachidonate 5-lipoxygenase (5-LO) gene was observed in BCR-ABL-positive CML mice, together with an improved animal survival when this enzyme was pharmacologically inhibited.
Aims: To examine the effect of leukotriene-signaling-modulating compounds on the growth of human CML cells.
Methods: Human CML cells were grown in 3-day *in vitro* cultures (MTT), with and without addition of different specific blockers of LT-signaling and tyrosine kinase inhibitors (TKI). Protein expression was determined by Western blot.
Results: The cysteinyl-LT1-receptor (CysLT1rec) antagonist montelukast significantly reduced the growth of K562, KCL22 and KU812 CML cells in culture in a dose-dependent manner (IC50 1.1-1.7 μM, *i.e.* clinically achievable concentrations). Montelukast also inhibited the growth of the CysLT1rec-expressing colon cancer cell line HCT116, but not of normal fibroblasts. As expected, several TKIs, including imatinib, also clearly suppressed the growth of the CML cells. When combining montelukast with imatinib an additive inhibition occurred. Similarly, inhibition of CML cell growth was also evident after addition of the 5-LO-inhibitor BWA4C, the 5-LO-activating protein (FLAP) inhibitor licofelone and the LTB4(BLT)1-receptor antagonist LY293111. All three CML cell lines where shown to express 5-lipoxygenase, LTA4hydrolase and LTC4S, key enzymes in the formation of bioactive leukotrienes.
Summary/Conclusions: Our data indicate that blocking of LT-signaling selectively suppresses expansion of human CML cells and may thus provide an additional therapeutic possibility targeting residual disease in this leukemia. Some of the tested pharmaceuticals are already in clinical practice for non-hematological disorders, facilitating the initiation of prospective CML intervention trials.

PB1808

ISODERIVATIVE PHILADELPHIA CHROMOSOME IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA
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Background: The key point in pathogenesis of chronic myeloid leukemia (CML) is occurrence of Philadelphia chromosome as a result of reciprocal translocation of chromosomes 9 and 22 leading to formation of the *BCR-ABL* fusion gene. Increasing number of copies of this oncogene may be one of the reasons for disease progression and lack of response to treatment with tyrosine kinase inhibitors. Usually, this process develops due to occurrence of additional copies of the Ph-chromosome without alteration of its structure. This report however describes 5 cases of changed morphology of derivative chromosome 22 (particularly isochromosome 22), leading to increased number of *BCR-ABL* gene copies. Despite the fact that occurrence of an isochromosome can be quite frequent in various malignancies reports concerning isoderivative chromosome 22 are quite rare.
Aims: The aim of this study was an attempt to establish the mechanisms leading to modifications of derivative 22 structure; and to investigate the number of *BCR-ABL* gene copies and its localization.
Methods: Methods of conventional cytogenetics (karyotyping of bone marrow cells) and FISH using BCR-ABL DC DF probe (Vysis) were applied.
Results: Cytogenetic aberrations involving increased numbers of copies of Ph-chromosome and *BCR-ABL* gene were detected in 30 patients overall. Occurrence of 1 or more additional Ph-chromosomes without structural changes was found in 25 of the cases. Structural modifications of derivative chromosome 22 were revealed in 5 patients. Evidences of isoderivative chromosome 22 were found in 4 of them in amount of 1-2 copies per metaphase plate, whereas in 1 case dicentric isoderivative chromosome 22 was detected in amount of 1-5 copies per metaphase plate (Figure 1). Presence of two copies of *BCR-ABL* on both arms of isoderivative 22 in all cases was confirmed by FISH on metaphase plates. It was hence proved that the detected isoderivative 22 in these 4 patients was composed of the long arms of Ph-chromosome with fragments of chromosome 22 and 9 linked by centromere of chromosome 22. Presence of two copies of *BCR-ABL* was also confirmed by FISH for the patient with dicentric isoderivative chromosome 22. However, in this case the isoderivative comprised two cetromeres of chromosome 22 and the long arms were connected by telomeric areas of the translocated fragments of chromosome 9. Total number of *BCR-ABL* copies in cells with isoderivative was 2-10. The described abnormalities were detected in 4 patients treated with imatinib. Therapy was subsequently changed to nilotinib in 3 of them and to dasatinib in 1 patient. After switching to nilotinib cytogenetic response was not achieved in 2 patients who subsequently died of disease progression. In case of dasatinib the patient also failed to reach cytogenetic response. Only in one patient after 2 years of nilotinib complete cytogenetic response (CCyR) was obtained. In remaining 5th patient who failed after 1 year of imatinib without any clonal evolution treatment was changed to nilotinib. After 6 months of this treatment CCyR was achieved, however after 12 months occurrence of isoderivative 22 was for the first time detected in 50% of the cells. The patient currently continues being followed.

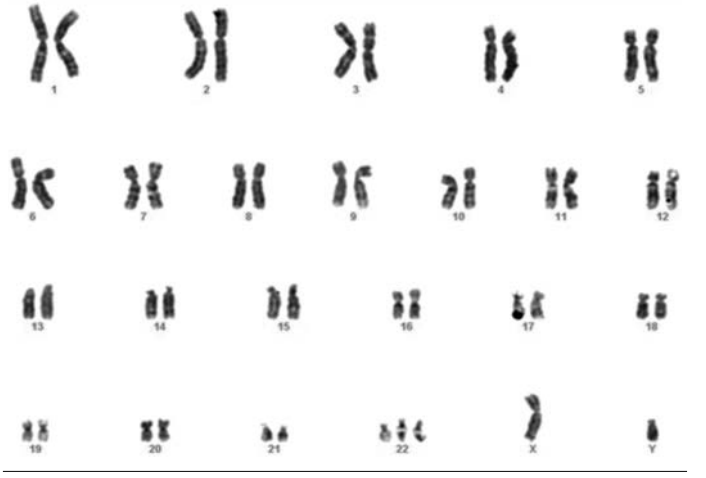

Figure 1.

Summary/Conclusions: The paper describes 5 cases of rare structural modifications of derivative chromosome 22, leading to increased numbers of copies of *BCR-ABL* gene. In majority of these cases patients were resistant to treatment with tyrosine kinase inhibitors. Detected aberrations most probably represent one of the mechanisms of the secondary resistance and disease progression in CML.

PB1809

MOLECULAR AND FUNCTIONAL INTEGRITY OF CRYOPRESERVED CHRONIC MYELOID LEUKAEMIA CD34-POSITIVE HAEMATOPOIETIC STEM CELLS
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Background: Biopreservation is a billion dollar industry, despite this, access to large numbers of high quality biospecimens remains a challenge for many researchers. Chronic myeloid leukaemia (CML) is a rare condition of approx-

imately 1 new case per 100,000 in the UK per year. CML is a clonal disorder originating from a CD34-positive haematopoietic stem cell. Studies of molecular targets and functional processes in tumour-initiating stem cells have greatly enhanced the understanding of oncogenesis and disease progression, but the impact of freezing, thawing and long-term storage on tumour-initiating cells is under reported. Knowledge of these bioprocesses is critical to the development of high-quality biobank collections.
Aims: To investigate the effect of cryopreservation on CML CD34-positive cells and the utility of such material for downstream molecular and functional analyses.
Methods: Mononuclear cells (MNCs) and CD34-positive cells were isolated from excess cryopreserved autologous peripheral blood stem cell (PBSC) harvests, collected between 1990 and 2006, fresh whole blood from untreated CML patients, and healthy NHS blood donors. Cells were cryopreserved by controlled-rate freezing, stored in gas-phase liquid nitrogen and underwent three cycles of freeze-thaw (FT). Molecular and functional assays were performed at each cycle. RNA integrity number (RIN), as a measure of total RNA quality, were assessed on an Agilent Bioanalyzer, and semi-quantitative PCR, using GUSB as a reference gene, was performed to evaluate BCR-ABL gene expression. CD34-positive cell frequency was determined by two-colour flow cytometry using CD34 PE-Cy5.5 and the vital dye Calcein. Analysis of CD34-positive cell clonogenic potential was conducted using a colony-forming unit (CFU) assay, whereby cells were incubated in complete CFU media, supplemented with 3U/ml erythropoietin, for 16 days.
Results: Mean RIN values for normal and CML cell subsets were ≥7. RIN values for normal MNCs and CD34-positive cells were significantly higher ($p<.01$) compared to CML cellular subsets; there was no significant effect of repeat FT on total RNA derived from either normal or diseased cell subsets. GUSB and BCR-ABL expression were significantly higher ($p<.05$) in freshly isolated CML cell subsets compared to FT cycles 2 and 3, but not cycle 1. A downward trend in the ratio of BCR-ABL:GUSB expression was observed with repeat FT cycles but this did not reach statistical significance. The frequency of live CD34-positive cells remained stable with successive FT cycles. No difference in CFU potential was observed for CD34-positive cells in healthy donors between fresh and FT cycle 1, but significantly fewer ($p<.05$) colonies were observed for successive FT cycles. Cryopreservation of CML CD34-positive cells resulted in a significant reduction ($p<0.5$) in CFU potential compared to fresh material.
Summary/Conclusions: Long-term storage and cryopreservation has minimal impact on the quality of genetic material for use in downstream molecular analysis. However, gene expression data indicates a potential global down-regulation of gene regulation post freeze-thaw and functional studies suggest that while CD34-positive cells are sufficiently robust to withstand cryopreservation they experience a loss in proliferative potential, to which CML derived cells are more sensitive. This implies that there are different handling and storage requirements for cell subsets derived from healthy and diseased individuals, highlighting the need to standardise biospecimen preparation and storage for meaningful analyses where access to fresh material is limited.

PB1810

COMBINED ANTICANCER ACTIVITY AND SIRNA DELIVERY BY DENGUE VIRUS CAPSID PROTEIN PEPTIDES PEPR AND PEPM AGAINST CHRONIC MYELOID LEUKEMIA

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Background: Cancer remains a major cause of morbidity and mortality worldwide. Although progress has been made regarding chemotherapeutic agents, new therapies that combine increased selectivity and efficacy with low resistance are still needed. In the search for new anticancer agents, therapies based on biologically active peptides, in particular, antimicrobial peptides (AMPs), have attracted attention for their decreased resistance development and low cytotoxicity. AMPs, which have been essentially studied and developed as potential alternatives for fighting infectious diseases, have been tested as anticancer peptides (ACPs) in cancer therapy either alone or in combination with other conventional drugs. Moreover, the usage of cell-penetrating peptides (CPP) to deliver anticancer drugs, such as siRNA targeting molecular causes of cancer are currently being developed.
Aims: Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder caused by a single genetic mutation, a reciprocal translocation that originates the BCR-ABL gene with constitutive tyrosine kinase activity. Because there is a specific gene associated to this pathology it is an optimum therapeutic target for RNA silencing therapy (siRNA). Thus we developed a siRNA-based therapeutic approach in which the siRNA is delivered by Dengue Virus Capsid Protein-derived peptides, pepM and pepR. These peptides were used with the intention of having have dual role, to deliver siRNA into cells and act as Anticancer molecules by targeting intracellular cancer signaling events.
Methods: Dengue virus-peptides ability to transfect the positive BCR-ABL$^+$Cell Line (BV-173) was evaluated by confocal microscopy following GFP fluorescence emission after plasmid expression. Anti-BCR-ABL siRNA design was performed using a siRNA design web-tool and the BCR-ABL downregulation kinetics (48h to 168h) after transfection by Dengue virus-peptides was evaluated by RT-PCR. The Anticancer action of either pepR or pepM was assessed by genome-wide analysis microarray and further validated by testing BV 173 cell cycle and cell proliferation analysis by RT-PCR.
Results: siRNA design for BCR-ABL retrieved 148 potential siRNA sequences, which were reduced to 5 BCR-ABL siRNA for *in vitro* analysis after thorough screening. Positive efficacy of siRNA targeting BCR-ABL was tested using a commercial transfection agent. Significant BCR-ABL gene knockdown were observed for siRNA #3 when delivered by pepM with maximum decrease at 120h. Both pepM and pepR showed downregulation effects on proliferative CML cancer-related signaling pathways having direct impact on BV 173 cell cycle and proliferation at the G2/M phase.
Summary/Conclusions: With this work we showed the potential therapeutic technology of combining a drug delivery system (CPP) with anticancer properties to deliver functional siRNA into CML cell model. Acting together, these conjugates significantly decreased BCR-ABL gene expression levels, and perturbed leukemogenic cells homeostasis, revealing a potential scaffold to develop an alternative CML therapy. The development of a selective ACP is still a current challenge. It is not straightforward the prediction and design peptides with antitumor activity and we believe that these results will enrich ACP-structure based prediction.

PB1811

ASSOCIATION OF 3'BCR GENE DELETION WITH LACK OF MOLECULAR RESPONSE IN CML PATIENTS WITH PH-VARIANTS TREATED WITH TYROSINE KINASE INHIBITORS

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Background: Chronic myeloid leukemia (CML) is a disease of the clonal hematopoietic stem cells caused by a balanced translocation t(9,22)(q34;q11) forming derivate 22 or Philadelphia chromosome (Ph), and molecularly BCR/ABL gene fusion. The standard for treatment of CML patients (pts) is therapy with tyrosine-kinase inhibitors (TKIs). About 8-10% of Ph+CML pts have more composite rearrangements involving additional chromosomes (Ph-variants) or cryptic translocations. Ph-variants are accompanied more frequently by microdeletions in the regions of chromosome breakpoints than by standard t(9,22)(q34;q11). Although the proportion of CML pts with Ph-variants resistant to TKI therapy is the same as in the group with standard translocation, the specific mechanisms involving Ph-variants in TKI resistance are still unclear.
Aims: Evaluating the molecular-cytogenetic impact to resistance of TKIs treated CML pts with Ph-variants.
Methods: We have analyzed 13 CML cases, 12 of which were with Ph-variants and 1 of the patients was with cryptic translocation detected via G-banding cytogenetic analysis (CyG) and metaphase fluorescence *in situ* hybridization (met-FISH). The pts are aged 16 to 78 years and were treated with TKIs and followed for at least 18 months in hematology clinics in Sofia, Bulgaria. No less than 20 metaphases with CyG and no less than 10 with met-FISH were examined in each patient. Hematologic, cytogenetic and molecular responses were evaluated regularly.
Results: Our data showed that the following breakpoints were included in three- or four-way translocations: 1q42, 2q37, 3p25, 4p14, 4q33, 6q21, 7q34, 8q24, 11q11, 17q21, 19p13 (in 2 cases), 22q13 and Xq26. At diagnosis, two of the 13 pts had additional aberrations in the karyotype besides Ph-variants. The met-FISH technique allowed us to detect that the rearrangements in 10 of the cases are more complex than registered by CyG: additional chromosomes or additional regions in the visibly rearranged chromosomes were involved in 2 of the cases; loss of the 5' portion of *ABL* gene (*5' ABL*) was observed in 3 of the cases, deletions in *3'BCR* and *3'ABL* genes were detected in 4 and 1 cases, respectively. Complete cytogenetic (CCgR) and major molecular response (MMR) were achieved in 61.5% (8/13) of the pts. The *3'BCR* gene deletions were observed in 80% (4/5) of the pts without MMR. Two out of 5 resistant pts have the same t(9;19;22)(q34;p13;q11), but only one of them has a *3'BCR* deletion with no achievement of CCgR and MMR. The the latter patient (without the *3'BCR* deletion) has achieved CCgR and suboptimal molecular response on the 18 month after the start of the therapy.
Summary/Conclusions: We suggest that the lost of material in the chromosome 22 region including *3'BCR* gene in CML pts with Ph-variants may be associated with TKIs resistance. Further research is necessary to confirm these results.

PB1812

PROTEOMIC PROFILE CORRELATES TO MOLECULAR RESPONSE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Recent advances in the proteomic field have allowed us to better understand the biology of several cancer types and/or discover new candidate biomarkers. Very few data are available about the serum protein expression of patients with Chronic Myeloid Leukemia (CML).
Aims: The aim of our pilot study was to evaluate a correlation between depth of molecular response (MR) and proteomic profile of CML patients, and if possible to find novel potential biomarkers complementary to currently existing proven tools to monitor therapy response.
Methods: Thirty sera from peripheral blood (PB) were sampled from 8 patients in MR1 response, 6 in MR2, 11 in MR3, and 5 in MR4. For 11 patients serum from bone marrow (BM) was also available. In particular 3 were sampled from patients in MR1, 3 in MR2, 4 in MR3, 1 in MR4. The one patient at diagnosis was included in MR1 group. Samples were divided into 2 groups according to the achievement of MR3: Group A, which comprised 14 samples from patients with a MR lower than MR3, and Group B, including 16 samples from patients who experienced a MR greater than or equal to MR3.The association of proteomic profile with MR was investigated using the SELDI ToF Mass Spectrometry platform. Each specimen was analyzed in duplicate. Expression Differences Mapping analysis was applied in order to generate a cluster peaks list, which describes how a singular peak is expressed in the specimen spectrum. Finally, with the aim to give a preliminary identity to the most differentially expressed peaks, an in silico identification was attempted using the Mascot database search available at www.matrixscience.com.
Results: Comparing PB sera from group A and group B, only a peak at 5075 Da had a p-value=0.05. Comparing sera from patients with different MR (MR1 *vs* MR2 *vs* MR3 *vs* MR4), statistical analysis found two features at 11092 Da and once again that at 5075 Da as differentially expressed and statistically significant (P-value=0.0034 and 0.0084 respectively) between MR1 and MR4. In particular the peptide at 11092 Da was overexpressed in MR4 patients. Also the peak at 5075 Da was highlighted as statistically significant, but its significance was less important than that of 11092 Da due to a slightly worse sensitivity (80%). Sera from PB were compared with those extracted from BM. Interestingly, no difference was highlighted. Regarding the two peaks (5075 Da and 11072 Da) we hypothesized that they were closely related, probably as a fragmentation product. With the aim to quickly give a preliminary identity to the peptide, we attempted an "in silico" mass peptide finger print using the open-source tool available at www.matrixscience.com. The bioinformatic tool identified the peptide with a high probability score, as a truncated part of a larger nuclear protein ZMIZ2. It would interact with the Wnt/β-catenin pathway participating in its activation. As suggested by data from literature, we can try to hypothesize that, unlike non responders, in the serum of responsive patients a part of ZMIZ2 is present, as a consequence of the degradation of the entire protein; this finding results in an under-regulation of the Wnt/β-catenin pathway with consequently a better response.
Summary/Conclusions: These preliminary data suggest that the peptide at 11092 Da is very closely related to a good response. Our efforts are now oriented to definitively confirming the identity of the peptide and to clarify its role.

PB1813

EVALUATION OF BECLIN-1 PROTEIN EXPRESSION IN CHRONIC MYELOID LEUKEMIA PATIENTS ON IMATINIB THERAPY

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Background: Imatinib is a tyrosine kinase inhibitors that used for the treatment of chronic myeloid leukemia (CML). Recent studies showed imatinib-induced cell death in many types of cancers. Autophagy is the physiological process in which cellular components are broken down by the lysosomal activation. Beclin-1 is a protein that encoded by the BECLN1 gene which sits at the core of autophagy regulation.
Aims: the aim of this study is to evaluate the role of Beclin-1 in regulation of BCR-ABL1 fusion gene in patients with chronic myeloid leukemia.
Methods: Peripheral blood samples collected from 30 patients (16 males and 14 females) diagnosed with CML at Oncology Center Mansoura University. Ages ranged from 18 to 50 (44.7±10.2) years. Diagnosis was based on typical finding in the peripheral blood smear and bone marrow aspiration then confirmed by detection of Philadelphia chromosome by cytogenetics or BCR/ABL1 fusion gene using Quantitative RT-PCR. Further samples collected form 30 apparently healthy individuals (16 males and 14 females) subjected as matched controls. Beclin-1 protein was measured by ELISA technique in serum of controls and patients before starting treatment (Time point A) and after 6 months of Imatinib therapy (Time point B).
Results: there was a significant increase in mean Beclin-1 protein level in CML patients either at initial diagnosis (32.6 ng/dl, p ≤0.001) or after 6 month of imatinib therapy (44.3, p ≤0.001) compared to matched control (9.5 ng/dl). Although its mean level increased after 6 month of imatinib therapy, this increase was not statistically significant. There was a strong negative correlation between mean Beclin-1 protein and hemoglobin level (r=-0.56, p=0.001), white blood cells count (r=-0.62, p=0.008), BCR/ABL1 fusion gene level (r=-0.43, p=0.03).
Summary/Conclusions: there was a statistically significant negative correlation between Beclin-1 protein expression and BCR/ABL1 fusion gene level. Beclin-1 protein expression may be used as a dynamic biomarker to predict response to imatinib therapy. However, larger studies are needed to validate these results.

PB1814

REVERSINE TRIGGERS MITOTIC CATASTROPHE AND APOPTOSIS IN BCR-ABL POSITIVE CELLS

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Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm of the hematopoietic stem cell characterized by the presence of the oncoprotein BCR-ABL1, which have a constitutive tyrosine kinase activity. The tyrosine kinase inhibitors for BCR-ABL1 (*e.g.* imatinib, dasatinib and nilotinib) can lead towards the elimination of the BCR-ABL1 clone and increase survival of CML patients. However, acquisition of resistance to tyrosine kinase inhibitors has emerged as a challenge for CML patients and the identification of novel targets is necessary. BCR-ABL1 activation induces aurora kinase A (AURKA) and aurora kinase B (AURKB) expression, which are serine-threonine kinases that play an important function in chromosome alignment, segregation and cytokinesis during mitosis. Aurora kinase inhibition has proven to be an attractive anticancer approach. In the present study, we explored the cellular effects of reversine, an AURKA and AURKB inhibitor, in the BCR-ABL1positive K562 cell line.
Aims: Based on the evidence of participation of aurora kinases in the BCR-ABL1 signaling pathway and CML malignant phenotype, we explored the cellular effects of reversine in BCR-ABL1 positive cells.
Methods: K562 cell line was treated or not with the AURKA and AURKB pharmacological inhibitor, reversine, at 1, 10, 20 and 50μM for 24, 48 and 72 hours. After drug exposure, cells were evaluated for cell viability (MTT assay) and apoptosis (Annexin V/PI and cleavage caspase 3). For DNA content distribution analysis (Cycletest™ Plus DNA Reagent Kit) and Confocal immunofluorescence microscopy, K562 cells were treated or not with reversine at 1 and 10μM for 48 hours. Comparisons between the control and treated groups were performed by the ANOVA test and Bonferroni post-test. A p value <0.05 was considered statistically significant. At least three independent experiments for each method were tested.
Results: In K562 cells, reversine treatment significantly reduced cell viability in a dose- and time-dependent manner (all p<0.05). Using a nonlinear regression analysis, IC50 citotoxicity for K562 was 44.6 μM for 24 hours, 12.9 μM for 48 hours and 9.4 μM for 72 hours. We evaluate the DNA content distribution of K562 cells, since aurora kinases are known to be an important protein family for cell cycle progression and success of mitosis. We observed an increased percentage of K562 cells with tetraploidization (4N) or endopolyploidization (>4N) upon reversine treatment. These findings were confirmed by confocal microscopy analysis, which reveals large cells with several nucleus. Taken together, these data indicate that reversine is a potent inductor of mitotic catastrophe. Reversine significantly induced apoptosis in dose- and time-dependent manner (all p<0.05). In order to confirm our results regarding reversine-induced apoptosis, we also evaluated the caspase 3 cleavage in K562 cells and increased levels of cleaved-caspase 3 were observed in a dose-dependent manner.
Summary/Conclusions: Our preclinical study establishes that reversine presents an effective antileukemia activity against K562 cells and provide new insights on anti-cancer opportunities for CML.

PB1815

COMPARISON BETWEEN AUTOMATED AND MANUAL INTERPHASE FLUORESCENT *IN SITU* HYBRIDIZATION (FISH) ANALYSIS OF T(9;22) (Q43;Q11.2)

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Background: Metafer 4 or Metacyte is an automatic interphase FISH scoring system offered by MetaSystems. The scanning system contains a motorized microscope and a computer that achieves microscope control and real time image analysis.
Aims: Around the world, many laboratories have adopted Automated FISH

scoring for interphase FISH analysis, however few have reported their experience. In our laboratory, we have validated automated FISH analysis for a number of probes, including BCR/ABL ES Dual Color Translocation (Abbott). Detection of t(9;22)(q34;q22) generating the *BCR-ABL* gene fusion by FISH is a tool available for clinical diagnosis and treatment monitoring of Chronic Myeloid Leukemia (CML).
Methods: Six different pools of leukemic blood cell suspensions were used as negative controls and 55 CML patient samples were analyzed by automatic FISH and manual analysis.
Results: A Cut off value of 0.8µm was set as maximum distance between signals for fusion count and 1.2µm as minimum spot distance for red and green spot count. Compared to manual read, the scored nuclei are categorized in a higher number of signal patterns with automated FISH analysis. In addition, the number of nuclei per category is different between the two methods. However, there was a strong correlation between automated and manual interpretation results. Only one case out of the 55 cases analyzed was discordant (1.8%), with results above normal reference range by Automated analysis, but within the normal range by Manual analysis.
Summary/Conclusions: Our scoring and interpretation criteria, as well as the advantages of the automated approach will be discussed.

Chronic myeloid leukemia - Clinical

PB1816

HALVING TIME AND LOG REDUCTION ARE GOOD PREDICTORS OF OPTIMAL MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH SUB-OPTIMAL RESPONSE AT 3 MONTHS

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Background: In Chronic Myeloid Leukemia (CML) the 3-month molecular evaluation defines suboptimal responders with BCR-ABL1>10%. This group shows worse prognosis and is thought to benefit from treatment change to 2nd-line TKIs in an early onset. Nonetheless, some patients respond favorably long-term without treatment change. The decline rate of the BCR-ABL1, measured at 3M of treatment, may distinguish patients with suboptimal response that will achieve good responses from those who will need therapy switch.
Aims: In a group of CML patients, analyze clinical and laboratorial differences between patients with optimal/sub-optimal response, after 3M of Imatinib. To determine the predictive value of halving time and log reduction for optimal molecular response at 6 and 12M in patients with suboptimal response.
Methods: Single center retrospective analysis of 24 CP-CML patients treated with Imatinib 1st-line. Halving time at 3M of treatment calculated according to the formula proposed by Branford *et al.,* (2014), $c=-ln(2)/k$, c is the halving time, k ($k=[ln(b)-ln(a)]/d$) the reduction in BCR-ABL1 transcript between initial (a) and 3M quantification (b), divided by the number of days (d) between the two evaluations. Log reduction ($log(a/b)$) calculated at 3M of treatment, a is the 3M QPCR value and b the baseline value. Descriptive statistic analysis performed using the SPSS (v.21). The predictive value was calculated through the area under the curve (AUC), using *MedCal* statistical program. The optimal cut-offs were calculated using the Younden (J) Index.
Results: Between January 2006 and August 2014, 24 patients were diagnosed with CP-CML and started on Imatinib 400mg id; 16 male (66,7%) and 8 female (33,3%), median age=57,71 years(±15,75). Clinical-laboratory findings at diagnosis: mean Hb=12,42g/dL (±1,81;), Plts=452,92x10^3/uL (±299,51), median Leuc=75,1x10^3/uL (141), mean peripheral basophil percentage=2,43%(±1,8) and peripheral blasts=2,3% (±2,07); mean LDH=1302,79U/L (±852,41); mean palpable spleen size=13cm (±5,97). Sokal and EUTOS scores were obtained for 21/24 patients (Sokal score: 3 (14,3%) low-risk; 9 (42,9%) intermediate-risk; 9 (42,9%) high-risk. EUTOS score: 19 (90,5%) low-risk; 2 (9,5%) high-risk). The mean time between initial and 3M QPCR evaluation was 93,25 days (±18,43). At 3M, 14 patients (48,3%) had optimal and 10 (41,7%) suboptimal response. Transcript level at baseline was significantly higher in the suboptimal group (127,3±70,62 *vs* 73,53±25,5;p=0,04). In the suboptimal group, the halving time was highly predictive of optimal molecular response at 6M (AUC=0,93;p<0,0001), as well as response failure (AUC=0,83;p=0,02). The log reduction was highly predictive of optimal molecular response at 6M (AUC=0,96; p<0,0001), at 12M (AUC=0,83;p=0,03) and for response failure at 12M (AUC=0,85;p=0,01). The most predictive cut-off values (J) for halving time and log reduction were obtained for the 6M optimal response (J=0,87). A halving time of 22,59 days and a -1,1 log reduction were predictors of optimal molecular response at 6M.
Summary/Conclusions: In this group, patients with suboptimal response at 3M had a significantly higher BCR-ABL1 transcript at baseline. A halving time of 22,59 days and a -1,1 log reduction predicted optimal molecular response at 6M. In an early time-point of molecular evaluation, the use of parameters evaluating kinetics of the transcript decline will contribute to discriminate patients outcome and guide treatment options.

PB1817

EVALUATION OF CLINICAL EFFICACY AND SAFETY OF GENERIC IMATINIB IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA

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Background: Application of generic imatinib (GI) should allow to lower price of cancer drug making it available to a larger number of patients, that way improving health care of the population. Serbia was one of the first countries in Europe to adopt generic imatinib which has been used since 2012. Thereafter, all patients with CML have been switched from the original imatinib (OI) to GI, while newly diagnosed CML patients have been frontline treated with GI.
Aims: The aim of this study was to evaluate the clinical efficacy and safety of GI in the treatment of patients with CML both as frontline therapy and continued therapy after switching from the OI.
Methods: Our study included 70 adult patients with chronic phase CML that were treated with OI and/or GI in the period from August 2006 until February 2016. Patients were divided into two study groups: the first one had 34 patients

switched from the OI to GI, second had 36 newly diagnosed patients who were treated with GI as frontline therapy. All patients underwent hematological, cytogenetic and molecular monitoring and had been treated in accordance with the applicable national guidelines and ELN recommendations.
Results: The median follow-up was 74.7 months (range 46-115) for the patients who started treatment with OI and 20.3 months (range 6-43) for those receiving frontline GI. In the both analysed groups a slight predominance of female subjects was found. In the first group, at the time of the switch from OI to GI, all patients achieved complete cytogenetic response (CCgR) while major molecular response (MMoR) achieved 25/34 (73.5%) of analysed patients. During 41 months of follow-up, 6 patients lost CCgR, but after switching to the 2nd line therapy nilotinib, all have achieved secondary CCgR. The overall rate of MMoR for the group of patients that switched from OI to GI was 22/28 (78.6%). Among newly diagnosed patients receiving frontline GI, the overall rate of CCgR was 29/36 (80.6%), while overall rate of MMoR was 17/36 (47.2%). During the study follow-up, 10 patients were switched to 2nd line therapy nilotinib due to cytogenetic refractoriness or relapse and 7 of them achieved secondary CCgR. When the two study groups were compared regarding the most common non-hematologic adverse events (AEs) of any grade, in the group that started with OI therapy, there were peripheral edema (n=8), skin reactions (n=4) and myalgia (n=3), while in group with frontline GI there were nausea (n=10), peripheral edema (n=6) and fatigue (n=4). In both groups most common hematological AEs were neutropenia (n=5 *vs* n=4) and thrombocytopenia (n=2 *vs* n=3). There were no dose reductions due to toxicities in both groups.
Summary/Conclusions: The results of our study have shown that generic imatinib is not inferior in efficacy and safety compared to the original imatinib. Despite these encouraging results, the need remains for the large prospective randomized studies that would allow for the definitive confirmation of the generic imatinib efficiency in the treatment of patients with CP-CML.

PB1818

PREDICTIVE FACTORS OF STABLE DEEP MOLECULAR RESPONSE IN CHRONIC PHASE CML PATIENTS TREATED WITH DASATINIB OR NILOTINIB AFTER IMATINIB FAILURE

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Background: Front-line treatment of newly diagnosed Chronic Myeloid Leukemia (CML) patients with 2nd generation tyrosine kinase inhibitors (2G-TKIs) dasatinib (DAS) or nilotinib (NIL) resulted in higher rates of deep molecular response (MR) as compared to imatinib. Very little is known about rates and stability of deep MR when 2G-TKIs are used after imatinib failure.
Aims: To assess the probability and predictors of stable deep MR in ≥2nd line of treatment.
Methods: We restrospectively analyzed 127 chronic phase CML patients treated with imatinib 400 mg daily as first-line therapy and then switched to 2G-TKIs for resistance or intolerance. Patients progressing to advanced phases before switch were excluded. Deep molecular response (MR^4) was defined as $BCL\text{-}ABR^{IS}$ ratio ≤0.01% or undetectable disease with ≥10,000 ABL copies. Patients with MR^4 lasting ≥2 years, ongoing at the last contact, and with at least a Q-PCR test every 6 months were defined as stable MR^4. Patients with any sample >0.01% $BCR\text{-}ABL^{IS}$ after the achievement of MR^4 were defined as unstable MR^4. Age, sex, Sokal and EUTOS risk score, type of BCR-ABL transcript, duration of imatinib, reason for switch to 2G-TKIs and early molecular response to 2G-TKIs have been examined for the association with stable MR^4. Frequencies were compared by Fisher's exact test. Univariate and multivariate regression analysis were performed using the competing risk model of Fine and Gray.
Results: Median age at diagnosis was 55 years (range 20-88) and median duration of imatinib treatment was 19 months (range 1-115). Patients resistant (n=89; 70%) or intolerant (n=38; 30%) to imatinib were switched to DAS (n=82; 64.5%) or NIL (n=45; 35.5%). Thirty-six patients were resistant or intolerant to their first 2G-TKI and were switched to 3rd-line NIL (n=20), DAS (n=12), bosutinib (n=2), or ponatinib (n=2). At a median follow-up of 52 months after switch to 2G-TKI (range 6-126), best deep MR to 2G-TKI was: no MR^4 in 57 patients (45%), unstable MR^4 in 28 patients (22%; 24 with 2nd line and 4 with 3rd line treatment), and stable MR^4 in 42 patients (33%; 37 with 2nd line and 5 with 3rd line treatment). Five-year cumulative incidence of stable MR^4 was 28.7% (95%CI: 18.9-37.3%). Age, sex, risk scores at diagnosis, type of BCR-ABL transcript, duration of imatinib and type of 2G-TKI were similar between patients with or without stable MR^4 (Table 1). Predictors of stable MR^4 were reason for switch to 2G-TKI (intolerance *vs* resistance HR 0.41, 95%CI: 0.22-0.79; p=0.007) and 3-month BCR-ABL level after 2G-TKI start (≤10%^{IS} *vs* >10%^{IS} HR 0.08, 95%CI: 0.01-0.59; p=0.01). Three stable MR^4 patients attempted discontinuation and are presently in treatment-free remission phase at 2, 22 and 27 months after 2G-TKI stop, respectively.

Table 1. Characteristics of patients and frequency or MR^4 (unstable or stable).

Characteristics	No MR^4 (n=57)	Unstable MR^4 (n=28)	Stable (≥2 yrs) MR^4 (n=42)	p
Sex (male / female)	34 / 23	19 / 9	22 / 20	0.43
Age, median (range)	59 (22-88)	55 (20-80)	55 (23-78)	0.77
Sokal risk				
- low	21 (37)	12 (44)	16 (38)	0.56
- intermediate	22 (39)	14 (50)	18 (43)	
- high	11 (19)	1 (3)	7 (17)	
- not evaluable	3 (5)	1 (3)	1 (2)	
EUTOS risk				
- low	50 (88)	22 (79)	39 (93)	0.33
- high	2 (3)	2 (7)	2 (5)	
- not evaluable	5 (9)	4 (14)	1 (2)	
BCR-ABL transcript				
- b2a2	26 (46)	15 (54)	19 (45)	0.74
- b3a2	18 (31)	10 (36)	18 (43)	
- b2a2/b3a2	7 (12)	1 (3)	3 (7)	
- other	6 (11)	2 (7)	2 (5)	
Duration of imatinib				
- <12 months	24 (42)	7 (25)	13 (31)	0.27
- ≥12 months	33 (58)	21 (75)	29 (69)	
Reason for switch				
- intolerance	10 (17)	11 (39)	17 (41)	**0.019**
- resistance	47 (83)	17 (61)	25 (59)	
Type of 2G-TKI				
- dasatinib	39 (68)	14 (50)	29 (69)	0.20
- nilotinib	18 (32)	14 (50)	13 (31)	

Summary/Conclusions: In this retrospective, real-life experience, long-term use of 2G-TKIs in ≥2nd line of treatment after imatinib failure resulted in more than half of patients achieving the "safe haven" of deep MR, with around 60% of them in stable MR^4, a prerequisite for discontinuing treatment.

PB1819

MTOR INHIBITION IN CML: A NEW THERAPEUTIC OPTION

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Background: *BCR-ABL1* fusion gene, which encodes an oncoprotein with a deregulated tyrosine kinase activity, is the hallmark of chronic myeloid leukemia (CML) – a myeloproliferative disorder. Despite the high rate of response to treatment with Imatinib and other tyrosine kinase inhibitors (TKI), the number of patients with suboptimal response or resistance to TKI has been increased. The BCR-ABL oncoprotein activates multiples signaling pathways responsible for tumor cells characteristic, namely the high cellular proliferation rate and resistance to apoptosis. One of these pathways is the PI3K/AKT/mTOR pathway, which is correlated with the increase in cell survival and resistance to apoptosis. Leukemic stem cells use these pathways as a mechanism of defense against treatment and as tumor maintenance.
Aims: The aim of this study was to evaluate the therapeutic potential of the mTOR inhibitor, Everolimus, in CML cell lines sensitive and resistant to TKI and in primary cultures of CML patients.
Methods: For this purpose, we used a CML cell line sensitive to Imatinib, the K562 cells, and established two sub-cell lines resistant to Imatinib, the K562-RC and K562-RD cells. Cell lines were treated in the absence and presence of different concentrations of Everolimus and the effect in cell viability was analyzed by the resazurin assay. Cell death was determined by flow cytometry (FC) using the Annexin V/Propidium Iodide staining. The cell cycle analysis was accessed using Propidium Iodide incorporation by FC. *Ex-vivo* studies were performed in peripheral blood samples of 56 patients under TKI treatment. In our cohort, 52 (93%) patients presented molecular response and 4 (7%) cytogenetic response. Moreover, 39 (74%) patients were treated with Imatinib, and 14 (26%) with 2nd or 3rd generation TKI. Patients' samples were culture with increasing concentrations of Everolimus during 48h. Cytotoxic effect was evaluated by FC in different cell populations using Annexin V staining.

Results: Our results show that Everolimus induced a reduction in cell lines viability, with an IC50 of 20μM for sensitive cells and 25μM for Imatinib resistant cell lines. The cell death was induced by apoptosis and this drug has also an antiproliferative effect through an arrest in cell cycle progression in G_0/G_1.
In *ex-vivo* studies, Everolimus reduced cell viability by increasing apoptosis of hematopoietic stem cells ($CD34^+$cells) without cytotoxicity to lymphocytes. In the the dose of 25 μM this mTOR inhibitor induced in CD34 cells an increase of 19% of these cells positive to annexin V compared with control, and only 8% of lymphocytes are in apoptosis. Comparing the response to TKI treatment with sensibility to Everolimus, we observed a tendency to higher efficacy in patients with cytogenetic response (CR) comparing with patients under molecular response (MR) (24,5% *vs* 18,7% of CD34 cells positive to annexin V). When compared patients under Imatinib treatment *versus* patients treated with 2nd or 3rd generation TKI, we observed a better response to Everolimus in the second group, also associated with lower toxicity to lymphocytes.
Summary/Conclusions: Our results reveal the efficacy of Everolimus in inducing cell death in CML cells, without cytotoxicity to normal cells, suggesting that Everolimus could be an alternative targeted therapeutic approach in CML patients. However, it is important to increase the number of patients in the study to confirm our results.
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PB1820

EFFICACY AND SAFETY EVALUATION OF NILOTINIB AND DASATINIB (2G-TKI) ON FIRST LINE TREATMENT IN 73 PATIENTS WITH CML-CP OUTSIDE OF CLINICAL TRIALS. ANDALUSIAN CML REGISTRY (RALMC)

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Background: Even though they were approved last June 2011 to be used on first line, it is not a common procedure to begin treatment of CML-CP with 2G-TKI, despite it has been demonstrated its efficacy and safety against imatinib on ENESTnd and Dasision clinical trials.
Aims: To describe the RALMC experience in terms of efficacy and safety of 2G-TKI on first line of treatment in CML-CP in real life evidence.
Methods: Descriptive analysis of 73 RALMC patients, treated from the outset in 18 hospitals of Andalusia (Spain) with nilotinib and dasatinib from June 2011 out of clinical trials. Results of BCR ABL are expressed in IS by means of GenXpert system. Responses are cataloged according to the 2013 ELN group guidelines. Toxicity of each treatment, overall survival (OS), failure free survival (FFS), event free survival (EFS) and progression free survival (PFS) were evaluated. Event was defined as death from any cause, progression to accelerated phase (AP) or blast crisis (BC), loss of CCgR or MMR and change of treatment for any reason.
Results: Median of 49 years of age (18-78) and median of follow-up of 38 months (3-56). 59% male, 41% female. 54% low, 26% intermediate, 20% high Sokal index. 79% low, 21% high Eutos score (n 63). Treatment of first line: nilotinib 46 (63%), dasatinib 27 (37%). Probability of achieving CCgR at 6 months was 100% (54 of 54 evaluated patients). Probability of achieving MMR at 12 months was 85,7% (54 of 63 evaluated patients) and MMR at 18 months 93,4% (57 of 61 evaluated patients). Depth of the responses are detailed in the Table 1. No statistically significant differences are found between both treatments as they achieve MMR in months 12 and 18. In our patients treated with 2G-TKI in first line, probability to obtain BCR ABL ≤10% in month 3 (65 evaluated patients) was 100% and ≤1% of 77% (50 of 65 evaluated patients). In both branches of treatment, 77% of patients obtained rates of BCR ABL ≤1% (30 patients of 39 evaluated with nilotinib and 20 of 26 with dasatinib). Overall median value of BCR-ABL at 3 months was 0.16% (0.22% nilotinib, 0.15% dasatinib). Probability to achieve MMR in month 12 if BCR ABL in month 3 was <1.5% is of 94% as opposed to BCR ABL ≥1.5% which is 55% (p-value 0.001). Only one death is reported on the dasatinib branch (death related with CML). EFS with nilotinib was 85.5%, and 74.4% with dasatinib. FFS with nilotinib was 89.9% and 86.1% with dasatinib; there is no statistically significant differences between both treatments in terms of EFS and FFS (p-value 0.28 and 0.73 respectively). No patient of the series progresses to AP or BC. 12 treatment changes are carried out; 5 due to toxicity (7%): 2 with nilotinib (1 neutropenia, 1 dermatological toxicity) and 3 with dasatinib (1 pleural effusion, 1 thrombocytopenia, 1 ocular thrombosis). 7 treatment changes due to lack of efficacy (9.5%): 4 failures due to CCgR loss, 2 dasatinib and 2 nilotinib (mutational study was positive with nilotinib: 1 E308V and 1 T315I mutation), 2 fails due to MMR loss (1 dasatinib, 1 nilotinib) and 1 change from nilotinib to dasatinib in month 9 due to warning.

Table 1.

Evaluated responses at 12 month (n 63)					
TKI	**CCgR**	**MMR**	**MR 4.0**	**MR 4.5**	**MR 5.0**
Nilotinib	7 (18.4%)	8 (21.1%)	7 (18.4%)	11 (28.9%)	5 (13.2%)
Dasatinib	2 (8%)	6 (24%)	6 (24%)	7 (28%)	4 (16%)
MR at 12 month according BCR ABL at month 3, n 59 (p-value 0.001)					
BCR ABL ratio		**No MR**		**MMR**	
< 1.5%		3 (6.25%)		45 (93.75%)	
≥ 1.5%		5 (45.455)		6 (54.54%)	

Summary/Conclusions: The use of nilotinib and dasatinib as first-line treatment is consolidated as an excellent therapeutic alternative to CML-CP. Showing with our series the efficacy and safety of 2G-TKI, with high rates of cytogenetic and molecular responses, deep and early, and low rates of toxicity carrying over treatment changes. The cutoff point of BCR ABL in month 3 of 1.5% could determines the optimal response in month 12 as soon as they achieve MMR.

PB1821

OPTIMIZATION OF RADOTINIB DOSES BASED ON DOSE-EFFICACY AS WELL AS DOSE-SAFETY RELATIONSHIP ANALYSES FOR NEWLY DIAGNOSED PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA

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Background: Radotinib is a selective second generation BCR-ABL1 tyrosine kinase inhibitor approved for the treatment of chronic myeloid leukemia (CML). In the previous dose-safety relationship analyses (Leuk Lymphoma. DOI 10.3109/10428194.2015.1113278), a positive association was found between the radotinib dose adjusted for the patient's body weight (Dose/BW) and the risk of dose-limiting toxicity (DLT). Hence, a weight-based dosing method was suggested to improve the safety outcomes of radotinib.
Aims: To explore an optimal radotinib dosing regimen for the treatment of chronic phase (CP) CML based on the dose-efficacy as well as dose-safety relationship analyses of Phase 3 study data
Methods: The Phase 3 data were derived from a total of 160 newly diagnosed patients with CP CML treated with radotinib 300 mg BID or 400 mg BID (fixed dose regardless of BW). A logistic regression analysis was conducted to assess the impact of Dose/BW of radotinib on the achievement of major molecular response (MMR) (dose-efficacy relationship) or occurrence of DLT (dose-safety relationship) within 48 weeks of treatment. Subsequently, an optimal Dose/BW cut-off was selected based on chi-square tests and Kaplan-Meier analyses with log-rank test.
Results: *Efficacy.* A statistically significant inverse relationship was found between Dose/BW and the probability of achieving MMR when gender was controlled for (p=0.033). A significantly higher rate of MMR was achieved in patients who received <6.5 mg/kg than ≥6.5 mg/kg (56% *vs* 34%; chi-square test, p=0.045). *Safety.* A statistically significant positive relationship was found between Dose/BW and the probability of DLT occurrence (p=0.003). Among various Dose/BW cut-offs, the greatest difference in the rate of DLT occurrence was observed between patients who received <6.5 mg/kg and ≥6.5 mg/kg (57% *vs* 91%; chi-square test, p <0.001) with the median time to first DLT being 194 days and 83 days, respectively (log-rank test, p <0.001). Therefore, Dose/BW of 6.5 mg/kg BID appears to be a threshold dose of radotinib below which the efficacy is improved as well as the risk of toxicity is reduced.
Summary/Conclusions: The results indicate the need for dose adjustment of radotinib according to the patients' individual BW. Based on the proposed cut-off of Dose/BW 6.5 mg/kg BID, the radotinib doses for patients weighing ≤60 kg and >60 kg are suggested to be 300 mg BID and 400 mg BID, respectively. A randomized well-controlled clinical trial would be needed to confirm the efficacy and safety of this weight-based dosing strategy.

PB1822

REAL-WORLD DATA FROM 54 BELGIAN PATIENTS FROM THE PONATINIB NAMED PATIENT PROGRAMME (NPP)

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Background: Ponatinib is a tyrosine kinase inhibitor (TKI) indicated for adult patients with refractory chronic- (CP), accelerated- (AP), or blast-phase (BP) chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL), or those with the T315I mutation. Fifty-four patients from 17 Belgian centres entered the ponatinib Named Patient Programme (NPP) from August 2012 through September 2015.
Aims: To provide evidence of the efficacy and safety of ponatinib in real-world Belgian patients.
Methods: Limited patient data were collected prospectively for patients enrolled in the NPP, based on a questionnaire approved by the Ethics Committee of the Cliniques Universitaires Saint-Luc. Demographic data are presented for all patients, and efficacy and safety data for patients recruited from August 2012 through June 2015 with at least 3 months of follow-up. All patients provided informed consent.
Results: Patients with CP-CML (N=35), AP/BP CML (N=5) or Ph+ALL (N=14) had a median age of 62 years; 89% were in ≥3rd-line of TKI therapy. Mutation analysis revealed baseline mutations in 43% of CP-CML and 83% of Ph+ALL patients; 42% and 40% of mutations were T315I. Of CP-CML patients 63% received a starting dose of 45mg/day, 29% 30mg/day and 8% 15mg/day. Dose reductions occurred in 42% of CP-CML patients: 29% due to adverse events and 13% to prevent cardiovascular complications after achieving response. For Ph+ALL patients, starting doses were 45 mg/day (69%), 30 mg/day (23%), and 15 mg/day (8%). Among 27 CP-CML patients with outcome data, 10 patients stopped ponatinib within 3 months due to: disease progression (n=2), thrombocytopenia (n=2), gastrointestinal complaints (n=2), cutaneous lesions, uncontrolled hypertension, ischemic CVA and planned allotransplant (n=1 each). Best responses in CP-CML patients treated beyond 3 months were: major molecular response (MMR; n=10), complete cytogenetic response (CCyR; n=3), partial cytogenetic response (PCyR; n=2), and complete hematologic response (CHR; n=2). Four of 17 CP-CML patients discontinued due to disease progression (n=2) and vascular occlusive events (toe necrosis after 19 months [n=1]; cardiac death after 14 months [n=1]). Thirteen CP-CML patients remain on treatment (median: 17 months; Figure 1). Among 10 evaluable Ph+ALL patients, 4 achieved MMR, 3 of whom remain in remission after 15, 24 and 33 months.

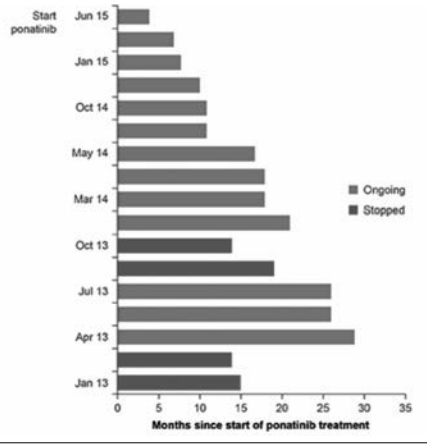

Figure 1. Treatment duration for 17 patients with CP-CML that continued ponatinib treatment beyond 3 months.

Summary/Conclusions: Despite limited data, ponatinib appears efficacious in the Belgian NPP; results appear comparable to the PACE clinical trial, which included resistant or intolerant CML and Ph+ALL patients.

PB1823

PREDICTORS FOR 12-MONTH MAJOR MOLECULAR RESPONSE IN CP CML PATIENTS WHO FAILED 3-MONTH EARLY MOLECULAR RESPONSE WITH IMATINIB THERAPY

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Background: Recent studies have demonstrated that measurements of BCR-ABL1 transcript levels at 3 months were able to identify high-risk patients treated with imatinib (IM). However, the value of early molecular response (EMR) has not been fully defined. Until now, whether no achievement of BCR-ABL1 transcripts ≤10% after 3 months of treatment is sufficient to define failure necessitating a change of treatment remains obscure.
Aims: The aim of this study was to identify predictive factors for an achievement of 12-month major molecular response (MMR) in the patients who failed 3-month EMR, as additional information to guide clinical decisions on selecting high-risk patients.
Methods: Among 413 newly diagnosed CP CML patients who received IM with no prior treatment and had available molecular data at 3 months, 120 (29.1%) patients failed to achieve 3-month EMR. Finally, to identify predictive factors for an achievement of 12-month MMR, 94 patients with available decision for 12-month MMR were included in this study. All qRT-PCR were tested with at least 4.5-log sensitivity in a single laboratory (Leukemia Research Institute, The Catholic University of Korea, Seoul, Korea).
Results: Ninety-four newly diagnosed CP CML patients (including 69 men and 25 women) were analyzed. With a median age of 36 years (range, 14-73 years), the distribution of low, intermediate, and high Sokal risk scores were 30%, 42% and 25%, respectively, with 4% unknown risk. 16 (17%) patients of the patients who did not achieve a 3-month EMR had MMR at 12 months, while 78 (83%) patients failed to achieve MMR at 12 months. Univariate analyses revealed that female sex, transcript type of b3a2, >0.65 of log reduction of BCR-ABL1 from individual baselines to 3 months, and smaller spleen size were potential predictive factors for an achievement of 12-month MMR. After adjusting for factors affecting achievement of 12-month MMR on univariate analyses, multivariate analyses showed that transcript type of b3a2, compared to b2a2 (RR of 0.09, *P*=0.022) and >0.65 of log reduction of BCR-ABL1 from individual baselines to 3 months (RR of 5.75, *P*=0.031) were independent factors for the achievement of 12-month MMR. The patients with larger spleen size showed a trend for no 12-month MMR (RR of 0.82, *P*=0.057).
Summary/Conclusions: This study analyzed various predictive factors for an achievement of 12-month MMR in the patients group who failed 3-month EMR. It provides additional information on selecting high-risk patients necessitating a change of treatment. Base on our findings, the patients with transcript type of b2a2, larger spleen size, and ≤0.65 of log reduction of BCR-ABL1 from individual baselines to 3 months need a clinical decision of changing therapy according to the results of qRT-PCR at 3 months. Further clinical investigations in a larger patient population with longer follow-up are needed.

PB1824

REAL-WORLD TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) IN PATIENTS WHO FAILED ≥2 PRIOR TYROSINE KINASE INHIBITORS (TKIS) AMONG US COMMUNITY ONCOLOGISTS

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Background: In the era of tyrosine kinase inhibitors (TKIs) most (chronic phase) CP-CML patients can expect to achieve excellent response with 1st-line (1L) therapy. However, in those who relapse, as many as one-third will not achieve a major molecular response in 2nd line (2L), and over time, up to one-half relapse. Lipton *et al.* (*Leuk Res, 2015)* reported that for CP-CML patients receiving a 3rd line (3L) TKI, sequential treatment with a 2nd-generation (2G) TKI after a prior 2G TKI failure is effective in only 22-26% of patients. Understanding real-world patterns of care and outcomes in this poor prognosis subset of CML patients is critical to developing new treatment strategies.
Aims: To document treatment patterns and measure real-world duration of treatment (DOT) across therapy lines in CML patients who were treated with ≥2 prior TKI lines.
Methods: In a retrospective study, the Navigating Cancer® electronic medical record (EMR) database was used to examine treatment patterns and estimate the benefit of TKI therapy in real-world practice. This database is comprised of community oncologists across the US. Patients with an ICD-9 code for diagnosis of CML, between August 2001-August 2015, who were treated with ≥2 prior lines of TKIs from pharmacy records were included. Patients included were ≥18 years of age at index date (initiation of 3L treatment), with no prior history of concomitant treatment for secondary cancers. Disease phase was reported in clinical progress notes. Line of therapy (LOT) was assigned for each specific regimen. Treatment discontinuation was defined as a gap in therapy of >90 days. As a surrogate for treatment benefit, DOT was calculated from prescription dates and days of therapy dispensed.
Results: One-hundred and fifty two CML patients met all inclusion criteria: 51% were female, and 67% Caucasian (16% unknown), with a mean age at index date of 58.7 years (23-88 years). Disease phase was available for 85 patients, among whom 64 were in CP-CML at most recent follow-up. For all 152 patients, the median DOT was 2.98 months in 1L, 1.01 months in 2L, and 5.45 months in 3L. For the 64 CP-CML, 3L DOT was 7.89 months. The most common treat-

ment sequence for the first 3 lines was imatinib/dasatinib/nilotinib (observed in 20% of patients). 61% of 3L therapy received 2G agents (24% dasatinib, 28% nilotinib, 9% bosutinib), 30% received imatinib, and 9% received ponatinib. Among 3L patients, 35% discontinued treatment and 38% progressed to 4-6 total lines. Most common 4L treatment was dasatinib (33%) followed by imatinib (24%), ponatinib (16%) and nilotinib and bosutinib (both 14%). 52% of all 4L TKI use was in patients who had received the same drug in an earlier LOT.
Summary/Conclusions: Our analysis affirms the limited effectiveness of current therapies in patients who relapse after 2LTKI for CP-CML. Use of 2G TKI and imatinib in 3L remains common among community oncologists. Our analysis identifies a subset of CML patients who not only fail quickly after TKI initiation, but subsequent 2G TKI treatment has short DOT. A variety of treatment sequences were observed across patients in ≥3L, and early switching of therapy appears common, suggesting an absent standard of care. In addition, the observed 52% repeat use of TKI in 4L suggests significant unmet need. More research into understanding the optimal treatment of these poor prognosis patients is needed to address the unmet clinical need.

PB1825

STEPWISE DOSE ESCALATION METHOD FOR NILOTINIB STOP TRIAL IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (STEP STOP TRIAL): A MULTICENTER PHASE 2 TRIAL

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Background: The STIM trial, stood in innovative point of view, indicate that imatinib may be successfully stopped in patients with deep molecular responses. Subsequently, the feasibility of various TKIs discontinuation trials have been investigated. The most important factor in TKIs discontinuation trials is the molecular response of BCR-ABL. The molecular response affected by many factors, such as Sokal score, percentage of basophil, additional chromosome anomaly and so on. Another important factor for the success of TKIs stop study is adverse events of TKIs itself. Several patients are not able to continue the TKIs by the adverse events.
Aims: Stepwise dose escalation method of NIL improved the proportion of patients who had treatment free Remission or not. A prospective, multicenter, single-arm phase 2 trial was conducted.
Methods: The Stepwise Dose Escalation Method for Nilotinib Stop trial in Patients with Chronic Myeloid Leukemia (Step Stop trial) was a prospective, multicenter, single-arm phase 2 trial. Adult CML-CP patients with newly diagnosed or experienced TKIs (imatinib, dasatinib or nilotinib) was enrolled. The first half of this study was "stepwised induction (achieve CMR) and consolidation (for 2 years) phase", stepwise dose escalation of NIL was conducted to avoid discontinuation of NIL by the adverse events. And the second half was "stop and monitoring phase".Eligible patients were administered nilotinib with stepwise dose escalation method, *i.e.* 150 mg per day for 2 weeks, 300mg per day for 2 weeks, 450mg per day for 2 weeks, and a final dose, 600 mg per day was continued. After achieve MR4.5, patients was received nilotinib consolidation therapy for 2 years. The primary end point was the proportion of patients who had treatment free remission at 12 months from the final day of the administration. The trough levels of NIL were examined and relapse was defined as a loss of MMR.
Results: Between November 2013 and August 2015, 26 patients was enrolled. 22 patients were previous treated and 4 patients were newly diagnosed. 16 patients were male and 10 patients were female. A median age was 55.2 years old (range 30-79). 21 patients were PS0, and 5 patients were PS 1. No patients were dropped out of stepwise Dose Escalation phase and consolidation phase. A total of 21 pts were found adverse events (81%), and 14 pts were found cutis such as itching and rash (54%). No severe adverse event was found and tolerability for NIL administration was 100%.No significant changes were found in the peripheral Lymphocytes count (CD3+, CD19+, and CD16+56+), but NK activities were significantly increased between before and after treatment by NIL (n=25, Before:6.184(%), After 9.48(%),. %: +53%, p-value 0.004).
Summary/Conclusions: The sustainable rate of the chemotherapy by NIL for CML-CP was 100% by the stepwise dose escalation method. These method may improve CML treatment. NK activities might be induced by NIL. Primary endpoint of this study,the proportion of patients who had treatment free remission at 12 months, would be presentated at EHA after day.

PB1826

IMMUNOMODULATORY EFFECT OF DASATINIB THERAPY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Dasatinib is a second generation tyrosine kinase inhibitor (TKI), widely used in patients (pts) with chronic myeloid leukemia (CML), especially in failure after imatinib. The quite often side effect of dastinib is lymphocytosis and pleural effusion (PE). These phenomenons are probably connected with immunological mechanisms.
Aims: The aim of our study was analysis of the influence of three presently used TKIs: imatinib, nilotinib and dasatinib on different populations of lymphocytes: B cells (Bc), T helper (Th), T suppressor (Ts), T regulatory (Treg), NK cells (NKc) and NKT cells (NKTc).
Methods: A total 37 pts with CML from Department of Haematology and Bone Marrow Transplantation in Lublin were recruited to this study. There were 17 pts on imatinib therapy (imatinib group; IG), 10 pts on nilotinib therapy (nilotinib group; NG) and 10 pts on dasatinib therapy (dasatinib group; DG). As control group there were 23 healthy volunteers. Material for the analysis was vein blood collected from CML pts after minimum 3 months of TKIs therapy. Blood samples were used for the evaluation of lymphocyte count and its immunophenotype to distinguish such subpopulataions as: Bc, Th, Ts, Treg, NKc and NKTc.
Results: Patients treated with dasatinib had significantly higher median number of lymphocytes in blood (3.59 G/L) compared with pts treated with nilotinib (2.31 G/L; p<0.01) and imatinib (1.93 G/L; p<0.001). The median CD19+expression (Bc) in comparison with control group was significantly lower in DG than in IG and NG (p=0.00039). There was no significant difference in Th, Ts and NKTc antigens expression. The analysis showed that in DG there was significantly higher expression of NKc antigens (CD3-CD16+CD56+) compared with control group than in IG and NG (p<0.05). The expression of Treg antigens (Foxp3+CD4+CD25high) was significantly lower in DG compared with control group than in IG and NG (p<0.001).
Summary/Conclusions: The results of our study showed immunomodulatory effect of dasatinib therapy in pts with CML manifested by increase of lymphocyte count and NK cells and decrease of Treg and B cells. This action of dasatinib is associated with such immunological phenomenon as PE and may be considered as additional factor influencing on better clinical response.

PB1827

2013 EUROPEAN LEUKEMIANET RESPONSE CRITERIA VALIDATION IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED FRONTLINE WITH 3 AVAILABLE TKIS OUTSIDE CLINICAL TRIALS

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Background: 2013 ELN recommendations defined endpoints in the treatment of chronic myeloid leukemia patients, introducing the concept of early molecular and warning response, but did not provide precise indications on timing of possible early switch to overcome negative prognosis.
Aims: Aim of our study is to evaluate how Italian physicians are adherent to ELN recommendations and how the different use of available TKIs would have changed therapeutic strategies.
Methods: We collected 218 patients treated consecutively, outside clinical trials, in 13 different Italian centers.
Results: There were 124 males and 94 females, median age 58 years (range 18-90), 20 patients with a previous neoplasia. Ten patients had at baseline ACA (6 major routes). According to Sokal risk, 50% of patients were low, 35% were intermediate and 16% were high risk. According to Eutos risk, 87% were low and 13% were high risk. As frontline treatment 55 patients received dasatinib (52 patients started with 100 mg, 3 at reduced dose), 82 patients received nilotinib 300 mg BID and 81 patients started imatinib (67 patients at 400 mg, 14 at <400 mg). Median age and comorbidities at baseline strongly influenced choice of TKI at baseline. At 3 months, in the dasatinib-treated cohort, 81% of patients were classified as optimal responders and 7% were warning but none of them changed therapy, due to concomitant comorbidities or toxicity experienced that allowed modification of dose. At 6 months, 84% of patients were in the optimal response category, 6% in the warning response (none of them changed) and 4% in failure category (1 patient increased the dose to 140 mg/day). At 12 months, 72% were in the optimal category, 25% in the warning category (none of them changed due to BCR-ABL/ABL ratio slightly above 0.1%) and 6% were in failure category (1 patient switched to nilotinib). At 3 months, in the nilotinib-treated cohort, 90% of patients were classified as opti-

mal responders and 4.8% as failure (1 patient switched to dasatinib). At 6 months, 87% of patients were optimal, 6% warning (none of them changed) and 3% were failure (only 1 patient switched to dasatinib). At 12 months, 80% were in the optimal category, 16% in the warning category (none of them changed due to a ratio BCR-ABL/ABL slightly above 0.1%) and 9% in the failure category (1 patient switched to dasatinib and 1 patient switched to ponatinib). In the imatinib-treated cohort, at 3 months, 74% of patients were classified as optimal responders, 16% patients were warning (5 switched to second-generation TKIs), 3.7% were failure (2 switched to other TKIs). At 6 months, 72% of patients were in the optimal category, 27% were in the warning category (1 patient only changed therapy) and 3% were failure (only 1 patient switched to dasatinib). At 12 months, 52% were in the optimal category, 32% were warning category (none of them changed due to comorbidities) and 15% were in failure category (3 patients switched to nilotinib or dasatinib, 1 patient to high dose imatinib). Cytogenetic analysis at 6 and 12 months was not performed in 40% of patients already in MR3.

Summary/Conclusions: The results of this retrospective analysis indicated that with frontline second-generation TKIs, warning and failure category were reduced at 3 and 6 months and that with this approach, Italian physicians were less likely to perform an early switch.

PB1828

COMBINATION OF HEMOGLOBIN LEVEL AT PRESENTATION WITH MOLECULAR RESPONSE AT 3 MONTHS BETTER PREDICTS SURVIVALS AND DEEP MOLECULAR RESPONSE TO IMATINIB IN CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA PATIENTS

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Background: Anemia at presentation is not uncommon in newly diagnosed chronic-phase chronic myeloid leukemia(CP-CML). However, hemoglobin level at diagnosis of CP-CML is not included in the formulas for calculating three scoring systems, namely Sokal, Hasford and EUTOS scores, for predicting survival outcomes. To our best knowledge, studies on the prognostic values of hemogobin levels at diagnosis of CP-CML were scarce, especially in association with survial outcomes and treatment responses.

Aims: We retrospetively collected data from consecutively newly diagnosed CP-CML patients treated with Imatinib to explore the value of hemoglobin level at presentaiton in predicting survival outcomes and treamtment responses as compared with current scoring systems.

Methods: A cohort of 154 newly diagnosed CML-CP patients treated with frontline Imatinib was surveyed retrospectively from January 2001 to April 2014. The cut-off value of baseline hemoglobin (Hb) level at diagnosis was defined as 10.0 g/dl arbitrarily. The influence of severe anemia (Hb<10.0 g/dl) and three CML scoring systems (Sokal, Hasford, and EUTOS) at diagnosis on treatment response landmarks, progression-free survival (PFS), event-free survival (EFS) and overall survival (OS), were examined by univariate and multivariate analyses. The discriminatory abilities of three prognostic models and baseline characteristics were examined by using the Cox proportional hazards model, and the consequences of the Cox model were expressed with the Akaike information criterion (AIC), which revealed how the EFS, PFS and OS were affected. The model with lower AIC is more explanatory and informative.

Results: Severe anemia with Hb<10.0 g/dl was identified in 43 (27.9%) of 154 newly diagnosed CP-CML patients treated with Imatinib. Patients with Hb<10.0 g/dl was associated with splenomegaly (76.7% *vs* 52.3%, *p*=0.006), high EUTOS risk (37.2% *vs* 16.2%, *p*=0.005), high Sokal score (48.8% *vs* 22.5%, *p*=0.001), high Hasford score (27.9% *vs* 7.2%, *p*=0.001), additional chromosomal abnormalities (25.6% *vs* 12.6%, *p*=0.049) but not associated with gender. Hb<10.0 g/dl was associated with higher white blood cell count (median=177.4 *vs* 88.6, *p*<0.001), blast percentage in peripheral blood (PB)(median=2.0% *vs* 1.0%, *p*=0.002), and eosinophil percentage in PB (3.0% *vs* 2.0%, *p*=0.038), but not assciated with MCV, basophil percentage in PB or platelet counts. Patients with Hb<10.0 g/dl had less favorable treatment responses including *BCR-ABL*≦10% at 3 months (51.2% *vs* 68.5% in Hb≧10.0 g/dl, *p*=0.045), complete cytogenetic response at 6 months (19.0% *vs* 50.5% in Hb≧10.0 g/dl, *p*<0.001), and major molecular response at 12 months (22.5% *vs* 45.2%in Hb≧10.0 g/dl, *p*=0.009). Furthermore, Hb at 10.0 g/dl could significantly distinguish EFS, PFS and OS with lower AIC value as compared with EUTOS, Hasford and Sokal scores. Moreover, combination of *BCR-ABL*≦10% at 3 months with Hb≧10.0 g/dl at presentation, it could predict the patients with better EFS, PFS, and OS and deeper molercular responses (MR4.5) (Figure 1).

Summary/Conclusions: Severe anemia (Hb<10.0 g/dl) at presentation was associated with less favorable treatment responses, poor EFS, PFS and OS in newly diagnosed CP-CML patients treated with frontline Imatinib. Combination of Hb≧ 10.0 g/dl at presentation with early molecular resposne (BCR-ABL≦10%) a 3 months, it could better predict survival outcomes and cumulative incidence of deep molecular response (MR4.5) as compared with current three scoring systems. Our study highlights the need for revisiting traditional CML scoring systems in the TKI era and suggests that CP-CML patients with Hb level≧ 10.0 g/dl at presentation and with early molecular response at 3 months might respond to Imatinib well thereafter.

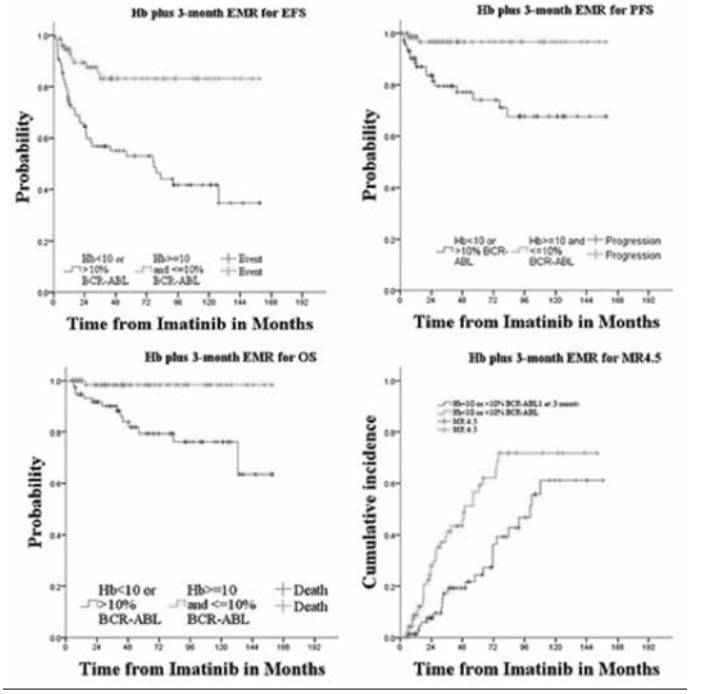

Figure 1.

PB1829

TREATMENT, RESPONSE, AND OUTCOME IN UNSELECTED ELDERLY PATIENTS WITH NEWLY DIAGNOSED, CHRONIC PHASE (CP), PHILADELPHIA CHROMOSOME-POSITIVE (PH+) CHRONIC MYELOID LEUKEMIA

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Background: Elderly patients are a substantial proportion of chronic myeloid leukemia (CML) patients (22% in a population-based registry study performed in Europe) (Hoffman V *et al.,* Leukemia 2015;29:1336-43) but are under-represented and selected in academic and commercial studies. Their treatment and their outcome are poorly known.

Aims: To investigate the treatment, response and outcome in an unselected cohort of elderly patients with newly diagnosed chronic phase (CP) CML.

Methods: In a cohort of 337 newly diagnosed, adult, CP, Ph+,BCR-ABL1+, CML patients who were registered according to the population-based criteria in two Italian Regions (Emilia-Romagna and Sicily) between 2008 and 2012, we identified 85 patients (25%) who were ≥70 years old. Five of them were treated only with hydroxyurea (HU); 17 of them were treated in first-line with nilotinib. We report here on the 63 patients who were treated in first-line with imatinib, 400 mg once daily.

Results: Males were 54%. Median age at diagnosis was 77 years (range 70-95). Median follow-up was 48 months (range 30-65), so that median patients age at data analysis was 81 years. Sokal risk distribution was 3% low, 56% intermediate, and 41% high. 41 patients (65%) received only imatinib. 14 patients (22%) were switched to a 2nd generation TKI for failure. 8 patients

were switched to a 2nd generaion TKI (n=5) or to HU (n=3) for toxicity. Responses and outcomes are shown in the Table 1. Molecular responses (early molecular response, major molecular response, and deeper molecular response) were in the range reported for younger patients in prospective studies of treatment. Overall survival was poorer, because 19% of patients died in MMR or in CP without any evidence of progression, due to age-related complications, mainly cardiovascular and cerebrovascular.

Table 1.

No. of patients	63
Early molecular response (BCR-ABL1 ≤ 10%) at 3 months	76%
MMR or better (BCR-ABL1 ≤ 0.1%) by 1 year	53%
MMR or better (BCR-ABL1 ≤ 0.1%) by 4 years	74%
MR 4.0 or better (BCR-ABL1 ≤ 0.01%) by 4 years	53%
Failure@ (including progression to blast phase@)	27%
Progression to blast phase/death in blast phase	9%
Death in remission or in chronic phase@	19%
Deaths, total	28%
@As defined by European LeukemiaNet, 2009	

Summary/Conclusions: These data reinforce the concept that in CML age is not a barrier to the success of a targeted therapy.

PB1830

THYROID DYSFUNCTION CAUSED BY SECOND-GENERATION TYROSINE KINASE INHIBITORS IN PHILADELPHIA CHROMOSOME-POSITIVE CHRONIC MYELOID LEUKEMIA

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Background: Protein tyrosine kinase inhibitors (TKI) are currently an important drug class in the treatment of leukemia. They represent targeted cancer therapy and have become the treatment of choice in chronic myeloid leukemia (CML). Many studies clearly have demonstrated that they were able to induce thyroid abnormalities including hypothyroidism and less often hyperthyroidism. Increased awareness and monitoring of thyroid function tests are important in patients maintained on TKIs. Thyroid dysfunction is a well-known adverse effect of first-generation tyrosine kinase inhibitors (TKIs), like sunitinib.
Aims: The aim of this study was to investigate the effect of second-generation TKIs on thyroid function in chronic myeloid leukemia (CML) patients treated in 2 centres.
Methods: We retrospectively assessed the effect of the first-generation TKI imatinib and the second-generation TKI nilotinib and dasatinib on thyroid function tests in 73 Philadelphia chromosome-positive (Ph-positive) chronic myeloid leukemia patients.
Results: Overall, 11 of 73 (15%) had one or more thyroid function test abnormalities during follow-up. Hypothyroidism or hyperthyroidism were found in 6 of 73 (8,2%) and 3 of 73 (4.1%) cases after a median of 6 and 22 weeks, respectively. In most patients thyroid dysfunction was transient without clinical symptoms. Therapy of hypo-/hyperthyroidism was required in three patients. Thyroid dysfunction never resulted in the discontinuation of TKI therapy. Under treatment with imatinib, nilotinib, and dasatinib, thyroid abnormalities were detected in 25%, 35%, and 40%, respectively. Three of 45 patients treated with nilotinib had evidence for an autoimmune thyroiditis (antibody positive in 2 of 3 patients) with an episode of hyperthyroidism preceding hypothyroidism.
Summary/Conclusions: Thyroid dysfunction is a common adverse event with second-generation TKI therapy in patients with Ph-positive chronic myeloid leukemia. Although the mechanism is still unclear, the higher frequency of thyroid abnormalities, including autoimmune thyroiditis, warrants regular and long-term monitoring of thyroid function in these patients.

PB1831

ASSESSMENT OF THE MOLECULAR DISEASE LEVEL IN PATIENTS WITH BCR-ABL1 POSITIVE CML IN DENMARK AND ICELAND

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Background: Since the introduction of TKI treatment in CML in the beginning of this century, the expected survival of CML patients has increased substantially and is now close to the survival of the background population. Thus, the prevalence of CML has increased dramatically, and the number of patients in therapy, needing molecular monitoring increases accordingly. Whereas a large number of studies analyze the outcome of CML in cohorts, no population-based studies to our knowledge have focused on the distribution among the various TKIs and the molecular response.
Aims: The aim of this multicenter study was to measure the cross sectional molecular *BCR-ABL1* response in CML patients in a demographic area and to analyze the molecular response across the different treatments available along with the distribution of treatment duration.
Methods: All hematological centers in Denmark and Iceland were invited to participate. One blood sample was taken in two Paxgene tubes per CML patient after informed consent as part of a routine visit and shipped to central *BCR-ABL1* analysis in the EUTOS MR4.5 certified laboratory in Vejle along with information on date of birth, gender, current treatment, dose, start date and line of treatment.
Results: Eight out of ten hematological centers I Denmark participated in the study as well as the Icelandic center. Five of the centers included more than 95% of their CML patients and in total 360 samples were included. One blood sample was lost during preparation, and of the remaining 359 samples 354 samples fulfilled the EUTOS quality criteria for Molecular Response (MR) scoring (>24,000 *GUSB* copies per qPCR well). In the MR scoring system the patients were found to be 6.5% >MR2.0, 7.3% in MR2.0, 19% in MR3.0 (=MMR), 17% in MR4.0 (of which 55/59 were *BCR-ABL1* positive) and 50% in MR4.5 (of which 26/176 were *BCR-ABL1* positive). Thus 150 pt (42%) were *BCR-ABL1* negative and in MR4.5, indicating that the potential for TKI interruption was high in this population, predominantly treated with imatinib. The average and median age of the patient group at sampling time was 58.7 years and 61.0 years, respectively with a gender distribution of 57% males and 42% females. Of the 360 patients, 214 pt (59%) were in 1st line treatment, average duration 10.4 years (197/Imatinib, 4/Dasatinib, 12/Nilotinib and 1/NA); 97 pt (27%) were in 2nd line treatment, average duration 3.3 years (16/Imatinib, 47/Dasatinib, 28/Nilotinib, 5/TKI and/or interferon combinations and 1/Pause (*BCR-ABL1* negative); 33 pt (9%) were in 3rd line treatment, average duration 2.6 years (3/Imatinib, 8/Dasatinib, 18/Nilotinib, 3/Bosutinib and 1/Pause (*BCR-ABL1* negative); 10 pt (3%) were >3rd line treatment, average duration 1.1 year (1/Imatinib, 5/Dasatinib, 3/Bosutinib, 1/Ponatinib); 3 pt were transplanted; 1 *BCR-ABL1* positive pt had been in treatment free remission for 12 years; and 2 pt lacked information on treatment line.
Summary/Conclusions: This study describes in the Danish and Icelandic CML patient population the present distribution of treatment, treatment line and duration combined with molecular response.

PB1832

SAFETY AND EFFICACY OF PONATINIB IN REAL WORLD CLINICAL PRACTICE. RESULTS FROM THE SPANISH COMPASSIONATE USE PROGRAM. A GELMC STUDY

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Background: In clinical trials, ponatinib has demonstrated excellent efficacy in heavily pretreated chronic myeloid leukemia (CML) patients. However, a high rate of vascular complications has been reported with the standard dose of 45 mg per day, and seems to be associated with previous cardiovascular (CV) risks. Hence, close safety monitoring and tapering of the dose have been recommended, although there are limited data supporting their benefit. Since patients included in clinical trials are a selected population, there is a need of real world data to properly assess the results of ponatinib treatment.

Aims: The purpose of this study is to provide safety and efficacy information from patients treated with ponatinib in real world clinical practice.
Methods: We have retrospectively collected data from 23 patients treated with ponatinib after resistance to previous treatment with tyrosine kinase inhibitors (TKIs). Molecular biology tests were done according to ELN guidelines and BCR-ABL/ABL is expressed as% IS. Clinical and comorbidities information (including CV risk factors were collected before and during ponatinib treatment.
Results: Median age at diagnosis was 56 years (34-83). Most (70%) had CV risk factors and 20% had suffered a previous CV event. The percentage of low, intermediate and high-risk Sokal groups were 25%, 45% and 15%, respectively. Median time of TKIs exposure before ponatinib was 43 months. One patient was treated in accelerated phase (AP), while the rest were in first or second chronic phase (CP). Thirty seven (%) of the patients had previously received 2 TKIs (imatinib, dasatinib, nilotinib, or bosutinib), and N (62%) had received ≥3 . At ponatinib start, 69% of patients had not achieved complete cytogenetic response (CCyR) while the rest were in CCyR (including 17% with major molecular response (MMR)). Sixty percent of the patients had one or more *BCR-ABL1* kinase domain mutations at the time of ponatinib start (45% had T315I mutation). Median follow-up was 29 months (3-53). Initial daily dose was 45, 30, and 15 mg in 85%, 5%, and 10% of patients, respectively. The initial dose was reduced in half of the patients, either due to side effects or to minimize the CV toxicity. In the last control, ponatinib daily dose was 45 mg, 30 mg and 15 mg in 65%, 10%, and 25% of the patients, respectively. Probabilities to obtain or maintain previous CCyR or MMR were 65% and 25%, respectively. Fifty eight percent of patients without CCyR at baseline achieved CCyR (17% achieving MMR), while 60% of patients with CCyR at baseline improved their molecular response. No differences in the response rates were observed depending on the mutational status or the initial ponatinib dose. By 43 months, event free survival, and progression free survival were 50% and 80%, respectively. At time of analysis, 40% of the patients had discontinued ponatinib due to toxicity (25%), lack of efficacy (25%), hematopoietic stem cell transplantation (37%) or death (13%). Most common non-hematological toxicities were liver toxicity (20%), lipase increase (10%), and hypertension 15%. No CV events were reported during treatment.
Summary/Conclusions: Ponatinib was shown to rescue a significant proportion of patients failing prior TKI treatment. It is worth noting that, with of a median follow-up of more than 2 years, no CV events appeared, suggesting that early dose modification could be protective against the occurrence of vascular complications.

PB1833

EARLY GOOD LATER BETTER. RELATIONSHIP OF BCR-ABL LEVEL AT 12 MONTHS OF TARGET THERAPY WITH INDIVIDUAL CHARACTERISTICS OF MOLECULAR RESPONSE IN EARLY TREATMENT PERIOD IN CHRONIC MYELOID LEUKEMIA

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Background: The search of surrogate prognostic markers for early detection of high risk disease progression still is actual problem in chronic myeloid leukemia (CML) management. The existing prognostic scales consider characteristics only at moment of diagnosis, whereas individual *BCR-ABL* level trend remains out of its vision.
Aims: The aim of this study was to evaluate the relationship between individual characteristics of early response to treatment and *BCR-ABL*^{IS} level at 12 months.

Table 1. The frequency of the optimal response (MMR) achievement at 12 months of TKI therapy in the relationship with type of first line therapy.

Groups of patients	TKI1 (*n* = 41)			Switching from TKI1 to TKI2 (n = 14)			TKI2 (*n* = 13)		
	n	MMR at 12 months:		n	MMR at 12 months:		n	MMR at 12 months:	
		abs.	%		abs.	%		abs.	%
BCR-ABL at 3 months of therapy:									
≤10%	33	23	69,7	6	4	66,7	13	10	76,9
> 10%	8	2	25	8	2	25	0	0	0
Ratio *BCR-ABL* 3 months to baseline:									
< 0,1	23	17	73,9	1	1	100	11	9	81,8
> 0,1	18	8	44,4	13	5	38,5	2	1	50

Methods: Fifty-four patients with chronic phase CML were included in the study. Forty-one patients started treatment with the first generation of tyrosine kinase inhibitors (TKI1) - Imatinib 400 mg QD. Twelve patients were treated with the second generation of tyrosine kinase inhibitors (TKI2) as first-line: Nilotinib 300 mg BID and 1 patient with Dasatinib 100 mg QD. Fourteen patients initially treated with TKI1 were subsequently switched to TKI2 (Table 1). We have conducted multivariate analysis of relationship between *BCR-ABL*^{IS} levels at 12 months and early molecular response (EMR, *BCR-ABL*^{IS} level ≤10% at 3 months) achievement, individual rates of *BCR-ABL* decline, type of first line therapy, fact of switching therapy from TKI1 to TKI2.
Results: The parameters that have statistical significance influence on *BCR-ABL*^{IS} level at 12 months of therapy were as follows: individual rate of *BCR-ABL* decline from 3 months to baseline (<0,1/>0,1) – p=0.044, type of first-line therapy (TKI1/TKI2) – p=0.0002, switching of therapy from TKI1 to TKI2 (yes/no) – p<0.0001. Achievement of EMR did not reach statistical significance – p=0,51. In addition, we have revealed the fact, that timely switch to TKI2 for resistant CML patients led to the rates of MMR achievement at 1 year of therapy similar to the MMR rate in the remaining TKI1 treated patients, eliminating resistance to TKI1.
Summary/Conclusions: Individual characteristics of the early response to treatment, for example individual rate of *BCR-ABL* decline, might be used as predictors of achievement optimal treatment result in CML treatment. The adequate treatment switching from first to second generations can restore treatment efficacy in resistant patients.

PB1834

EFFICACY OF GENERIC IMATINIB IN NEWLY DIAGNOSED CP-CML PATIENTS IN ESTONIAN POPULATION

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Background: Tyrosin kinase inhibiitor (TKI) imatinib is the gold standard 1st line therapy of chronic phase chronic myeloid leukemia (CP-CML). Imatinib was implemented in Estonia for the 1st line therapy of CML in July 1, 2006. However in 2012 generic version of imatinib was approved in European Union and since January 1, 2014 only generic imatinib has been available in Estonia.
Aims: The aim of the study was to evaluate the clinical efficacy of generic imatinib in the treatment of CP-CML in Estonia and to compare results with historical group of patients treated brand name imatinib.
Methods: 25 patients in CP-CML have been diagnosed during 2014-2015 and have started 1st line treatment with generic imatinib. Optimal response was assessed at 3, 6 and 12 months after the start of treatment according to European LeukemiaNet 2013 guidelines: BCR/ABL ≤10%, <1% and ≤0,1% at 3, 6, 12 months respectively. For comparison historical group of 72 patients in CP-CML who received brand name imatinib for 1st line treatment during 2006-2013 were used. In this group pre-treatment with hydroxyurea for maximum 3 months but not interferon-alfa was allowed, OR was assessed at 6, 12 and 18 months after the start of imatinib and defined according to European LeukemiaNet 2010 recommendations.
Results: OR with generic imatinib was achieved in 16/21 (76.2%) evaluable patients at 3 months, 11/14 (78.6%) patients at 6 months and 7/11 (63.6%) at 12 months. At 18 months there were only 3 evaluable patients who all obtained OR. None of the patients not having OR at 3 months and continuing with imatinib, obtained OR later. One patient without OR at 3 and 6 months, progressed to lymphoid blastic crisis in 9 months after diagnosis. Two patients had failure to generic imatinib - one patient having BCR/ABL level at 6 months over 10% and one patient over 1% at 12 months; both patients were switched to the 2nd generation TKIs. Three patients having OR at 3 and 6 months lost it by 12th month, but 2 of them had treatment interruption periods due to poor tolerability. No new safety signal emerged. The most common adverse effects to generic imatinib occurring more than 10% of patients were nausea/vomiting, diarrhea/constipation, muscle pain in legs, periorbital oedema and allergic skin reactions. Due to adverse events 6 patients were switched to different generic imatinib and one patient not tolerating two different generic imatinibs was switched to the 2nd generation TKI. In the historical group OR was achieved in 33/45 (73%) evaluable patients at 6 months and 33/50 (66%) patients at 12 months. At 18 months OR was obtained in 22/51 (43%) evaluable patients. None of 11 patients not in OR at 6m obtained OR at 12m or 18 months.
Summary/Conclusions: Generic imatinib appears to have the same efficacy as brand name imatinib. Despite differences in group size and OR definition by European LeukemiaNet 2010 *vs* 2013 recommendations the treatment results with generic and brand name imatinib were quite comparable with 78.6-66% of OR rate at 6 months and 63.6-66% at 12 months. To improve treatment results, for patients not achieving early OR at 3 months or 6 months should be offered treatment with 2nd generation TKI.

PB1835

ROLE OF CHR AS A SURROGATE FOR MMR AT 12 AND 18 MONTHS IN RESOURCE CONSTRAINT SETTINGS: PATIENTS UNABLE TO AFFORD FREQUENT MOLECULAR MONITORING

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Background: Studies on CML in last decade have correlated early molecular response (EMR) both at 3/ 6 months and velocity of BCR-ABL reduction with MMR at 18 months. But, this requires molecular monitoring at 3 monthly interval to the least till the achievement of MMR as per NCCN/ELN guidelines. For studying velocity of fall, the molecular monitoring is required at further greater frequency. In resource constraint settings, it is very difficult to monitor patient with qPCR for BCR-ABL transcripts at regular interval owing to the poor educational status, financial constraints, logistic issues (non-availability of testing facilities at all places) and lack of standardization at various labs. Considering these limitations, it is very essential to identify surrogate markers which are universally available for predicting MMR at 18 months.
Aims: To study the role of CHR as a surrogate for MMR at 12 and 18 months in resource constraint settings in patients unable to afford frequent molecular monitoring.
Methods: *Study Design:* It's a prospective single center observational study from northern India. This is part of PRICE study (PGIMER Real world Indian CML Experience) registered vide UTN U1111-1177-8918 and under registration in CTRI. *Patients:* As part of this study a total of 230 CML patients were screened prospectively attending Adult Hematology Clinic of the host institution. All patients (age>12 years) who were fulfilling the diagnostic criteria of CML-CP before initiation of imatinib therapy were included in the study. Exclusion criteria were either (a) patients in accelerated phase and blast crisis or (b) patients in whom Imatinib was discontinued due to hypersensitivity/ intolerant. *Protocol:* As part of this study protocol, all newly diagnosed cases of CML-CP were managed with TKI therapy with regular molecular monitoring (3, 6, 12, 18 and 24 months). Patients were initially evaluated with complete hemogram, bone marrow analysis and diagnosis was then confirmed with qualitative molecular test to detect the presence of BCR-ABL translocation. Sokal, Hasford and EUTOS scores were calculated at baseline. Imatinib, was started and Clinical examination and hemogram was done at weekly intervals to assess for complete hematological remission. Molecular responses at 3, 6 months for EMR and 12, 18 months for MMR was monitored in all these patients.
Results: 85.1% patients achieved CHR within 12 weeks with median time to achieve CHR being 40 days. Median time to achieve CHR was longer in female patients (42 days) as compared to male patients(36 days), difference was not statistically significant (p=0.636). Patients who achieved MMR at 12 months had a mean CHR duration of 42 days (SD-437.79, n=73) and the patients who didn't achieve MMR at 12 months the mean duration of CHR was 62.81 days (SD-55.8, n=69). The difference was statistically significant (p=0.01). We also compared the duration of CHR with MMR at 18 months. The patients who achieved MMR at 18 months had a mean CHR duration of 35.45 days (SD-25.41 n=56) and among the patients who didn't achieve MMR at 18 months the mean duration of CHR was 56.71 days (SD-59.6, n=28). The difference was again significant with p value of 0.024 (Table 1).

Table 1.

Categories of MR	Groups	n	Days to achieve CHR	P value
EMR at 3 months	BCR/ABL <10%	159	46.79	
<0.001				
	BCR/ABL >10%	32	81.34	
OMR at 6 months	BCR/ABL <1%	113	46.11	0.02
	BCR/ABL >1%	77	64.16	
MMR at 12 months	BCR/ABL <0.1%	69	39.16	0.001
	BCR/ABL >0.1%	69	62.81	
MMR at 18 months	BCR/ABL <0.1%	56	35.45	0.024
	BCR/ABL >0.1%	28	56.71	

Summary/Conclusions: CHR is a good marker to predict the disease response in patients with CML on therapy.

PB1836

DEVELOPMENT OF INTERNATIONAL SCALE SECONDARY STANDARDS FOR BCR-ABL1 TRANSCRIPT QUANTITATIVE ASSAY

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Background: Presence of fusion gene BCR-ABL1 is indicative of chronic myelogenous leukemia. Quantitative assay of BCR-ABL1 transcript in patient blood can measure response to therapy. Harmonization of BCR-ABL1 transcript assay results among laboratories is needed in order to standardize clinical reporting. In 2009 the World Health Organization (WHO) developed a panel of four BCR-ABL1 primary standards to calibrate secondary standards and to establish an international scale (*IS*) for reporting assay results as a ratio of fusion transcript to control gene transcript (%*IS*). Reliable secondary standards are essential for monitoring performance of BCR-ABL1 quantitative assays.
Aims: This study creates a panel of BCR-ABL1 secondary standards, based on the WHO primary standards, for use with Xpert BCR-ABL Monitor assay (Cepheid).
Methods: Maine Molecular Quality Controls, Inc. (MMQCI) manufactured a panel of four secondary standards consisting of BCR-ABL1 b3a2 fusion transcript and ABL1 control gene transcript in a proprietary stabilizing matrix. Transcript ratios were calibrated to yield the same%*IS* results as the four WHO primary standards: approximately 0.01%*IS*, 0.1%*IS*, 1%*IS*, and 10%*IS*. Three lots of MMQCI panels were tested on one lot of Xpert BCR-ABL Monitor contemporaneous with testing WHO primary standards.
Results: Three unique lots of MMQCI secondary standards panels yielded quantitative results in good agreement with one another and with the WHO primary standards panel at each of the four%*IS* levels. MMQCI secondary standards panels demonstrated linearity across the range of%*IS* levels, comparable to linearity of WHO primary standards.
Summary/Conclusions: MMQCI's BCR-ABL1 secondary standards panel shows potential for verifying performance of Xpert BCR-ABL Monitor, thereby contributing to harmonization of minimal residual disease (MRD) measurements to the International Scale. This approach to creating standards secondary to WHO primary standards serves as a model for creation of secondary standards for Xpert BCR-ABL Ultra (Cepheid).

PB1837

LOW DOSE DASATINIB TREATMENT IN ELDERLY CML PATIENTS

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Background: Clinical outcomes and survival of chronic myeloid leukemia (CML) patients treated with ABL tyrosine kinase inhibitors (TKIs) have significantly improved. To achieve a good prognosis, including cessation of TKI treatment, a rapid and deep response to TKIs is important. Elderly patients usually cannot receive the standard dose (100mg QD) of dasatinib because of its adverse effects (AE). Nevertheless, several elderly patients achieve a deep response to very low doses of ABL TKIs.
Aims: We retrospectively analyzed the efficiency of low dose dasatinib in elderly patients with CML.
Methods: We retrospectively evaluated imatinib-resistant and -intolerant CML-CP patients aged 65 or over who had been administrated less than 100mg QD dasatinib between 2010 and 2015 at Saga University Hospital. We estimated therapeutic efficiencies based on bcr-abl mRNA levels measured by RQ-PCR compensated according to international scale (IS) and/or TMA (transcription mediated amplification) method for peripheral blood. In the TMA method, undetectable is considered to be the same as a 4-log reduction (MR^4) of RQ-PCR on the IS. In addition to efficacy, adverse effects at lower doses of dasatinib were also investigated.
Results: Twenty-one elderly CML-CP patients were investigated. Sixteen patients (76.2%) were newly diagnosed. The median age at diagnosis was 72 years old (range, 65–83). Thirty-three per cent were male, and the median follow up time was 20.5 months (range, 4–56). The mean dose of dasatinib was 24.6 mg. The numbers of patients receiving a mean dasatinib dose of 50mg or less and 20mg or less were 19 (90.4%) and 15 (71.4%), respectively. Twenty patients (95.2%) and 16 patients (76.2%) achieved a major molecular response (MMR) and a MR^4, respectively. In addition, 10 patients (47.6%) achieved a $MR^{4.5}$. The median durations of dasatinib treatment to achieve a MMR and an MR^4 were 6 months (range; 2–11) and 12 months (range; 3–50), respectively. All cases achieved a complete cytogenetic response (CCyR) within 12 months. In the case of the 15 patients receiving a mean dose of 20 mg or less, 14 patients (93.3%) achieved a MMR and 11 patients (73.3%) achieved a MR^4. The median durations of MMR and MR^4 were 6 months (range, 3–10) and 9.5 months (range, 3–38), respectively. Additionally, 13 patients treated with a dose of 20mg or less as first line therapy. MMR and MR^4 were achieved in 84.6% and 69.2%, respectively. 53.8% achieved a $MR^{4.5}$. The median durations of MMR and MR^4 were 6 months (range, 3–11) and 10 months (range; 4–38), respectively, in this cohort. The main AE was plural effusion (6 patients; 28.5%). Surprisingly, HBV reactivation occurred in 2 patients treated with 20 mg QD. Except for 1 patient who changed to another TKI because of gastrointestinal bleeding, most AEs became tolerable after dose reduction. Seven patients administered 50 mg or less had increased large granular lymphocyte counts.
Summary/Conclusions: Treatment with dasatinib at lower than standard doses was well tolerated and resulted in an adequate molecular response in elderly CML patients without causing severe AEs. Thus, prospective studies should be performed to determine the effects of administering lower doses of dasatinib to elderly CML-CP patients.

PB1838

A NATIONWIDE OBSERVATIONAL STUDY OF THE ISRAELI EXPERIENCE WITH PONATINIB OUTSIDE CLINICAL TRIALS IN CHRONIC MYELOID LEUKEMIA

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Background: Ponatinib is an oral tyrosine kinase inhibitor (TKI) that has been designed to be effective also in patients who harbor the TKI-refractory threonine to isoleucine mutation at position 315 (T315I). In December 2014 the drug was granted an accelerated approval by the FDA, based on the promising results from the phase II PACE trial. Nevertheless, the wide use of the drug has been limited due to safety matters, in particular arterial thrombotic complications, reported in up to 17% of patients. Currently, there is little real-life information regarding the use of ponatinib outside of clinical trials.
Aims: 1) To characterize patients with chronic myeloid leukemia (CML) who received ponatinib. 2) To determine safety profile of ponatinib outside clinical trials. 3) To assess the efficacy of ponatinib outside clinical trials.
Methods: Between 4.2011 and 7.2015 (51 months) 24 patients in 7 medicals with CML received ponatinib. We reviewed the medical records of these patients and asked their physicians to rank the quality of life of their patients on a 1 to 5 scale.
Results: The median age was 44 years (rang: 23 to 79) and most were in accelerated (21%, N=5) or blast crisis (41%, N=10) phases at time ponatinib was initiated. Prior to ponatinib, the patients were treated for a median of 55 months (range: 1 to 215) with 1 to 3 different TKIs (median: 3). Thirteen received at least one course of chemotherapy or interferon-α, and two underwent allogeneic bone marrow transplantation. Mutations at the bcr-abl DNA binding domain were detected in 11 patients (69%) and T315I mutation in 8 of those. Based on the medical history, 48% were at-risk for vascular complications either because of prior cerebrovascular event or myocardial infarction (18%) or because of vascular risk factors (30%). Baseline ECG and cardiac ECHO studies were available in 11 patients and none had significant structural or functional pathology. Patients were followed between1 to 34 months at time of analysis (median: 6 months) and during this time 4 patients died and 14 were still receiving ponatinib. Patients discontinued the treatment either because of major cardiovascular events (N=3), severe pancytopenia (N=1), planned bone-marrow transplantation (N=1) or because they were lost to follow-up (N=1). Seventeen patients were available for response assessment at time of analysis and the overall response rate was 77% (N=13). Five of six patients at chronic phase achieved either complete or major molecular response. Eight patients at accelerated phase or at blast crisis (8/11, 72%) achieved either hematological (5/11, 45%) or molecular response (3/11, 27%). Two of those, with hematological response, underwent allogeneic bone marrow transplantation. The median subjective scoring of ponatinib contribution to the quality of life of patients was 2.4 (range 1 to 5). Physicians that gave low scoring mentioned Severe weakness, myalgia and cytopenias were mentioned as a major concern.
Summary/Conclusions: In real- life setting ponatinib is generally used as a last resort. In our cohort, it was almost exclusively given to patients who experienced failure to previous TKIs. Only one third of patients tolerated the recommended dose of 45mg. Yet, response rate was still relatively high, suggesting that a daily dose of 30mg might be appropriate. With only 6 months of follow-up we documented 3 cardiovascular events (12%) that prompted discontinuation of the drug. The high response rate and overall contribution to quality of life in patients, who already experienced failure of other TKIs, support the use of ponatinib in this setting.

PB1839

ASSESSMENT OF PLATELET FUNCTIONS IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH TYROSINE KINASE INHIBITORS

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Background: Bleeding is observed time to time in chronic myeloid leukemia (CML) patients using tyrosine kinase inhibitors (TKI). It has been suggested that tyrosine kinase inhibitors can disrupt platelet functions and cause beeding without thrombocytopenia in CML patients.
Aims: In this study, we aimed to evaluate platelet functions of CML patients using tyrosine kinase inhibitors and examine in conjunction with beeding symptoms to find out whether there is a correlation between them.
Methods: In this study, bleeding surveys were performed for 68 CML patients in cronic phase receiving imatinib (n=47), dasatinib (n=15) and nilotinib (n=6). Hematology analyzer LH780 (Beckman Coulter) was used for measurement of complete blood counts. BCS XP coagulation analyzer (Siemens) was used for measurement of PT, aPTT, TT, fibrinogen and factor assays. In our coagulation laboratory, platelet rich plasma pool was prepared and light transmittance agregometry (Chrono-Log) was used for the evaluation of platelet functions. Platelet agregation was induced in *in vitro* conditions by different agonists (ADP, epinephrine, collagen, ristocetin) and the agregation percentage/time graph was analyzed to evaluate platelet functions.
Results: The median age was found as 47 years (range, 18-78 years) in CML patients. Complete blood counts and coagulation test results were in normal range; white blood cell count 6.72,5 $\times 10^3/\mu L$, hemoglobin 13.0±1,7 g/dL, platelets 234.7±74,5 $\times 10^3/\mu L$, PT 12,8±1,4 sn, INR 1,0±0,1, aPTT 27.6±2,8 sec, TT 16.5±2,0 sec, fibrinojen 325.7 mg/dL and Factor VIII levels were 120,8±11,2%. When the bleeding survey results were analysed, the patients who have bleeding score below 3 were accepted as *minor bleeding*. When the relation between bleeding scores and TKI treatment were evaluated; we observed that 25,6% of the patients who use imatinib and 20% of the patients who use dasatinib have minor bleeding symptoms. In Nilotinib treatment group, no bleeding symptom was observed. In total, 22% of CML patients under TKI treatment have bleeding symptoms; 17,6% with bleeding score 1 and 4,4% with bleeding score 2. In platelet function tests, when aggregation was induced by ADP, epinephrine, collagen and ristosetin, *secretion type defect* was observed in 26% of the patients. No correlation was observed between platelet function defects and minor bleeding symptoms in these patients.
Summary/Conclusions: As a result, platelet agregation disorders can be observed in CML patients treated with tyrosine kinase inhibitors. However, platelet dysfunction is not associated with bleeding disorder. Furthermore, the effect mechanism of tyrosine kinase inhibitors on platelet homeostasis warrants further investigation.

PB1840

ELEVEN-YEAR RESULTS OF THE THERAPY OF CML TYROSINE KINASE INHIBITOR IN A LARGE INDUSTRIAL CENTER OF SIBERIA

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Background: Modern method of therapy inhibitors tyrosine kinase (ITK) of chronic myeloid leukemia (CML) allows patients have long-term remission and life expectancy comparable to the general population. The effectiveness of treatment depends on the sensitivity of the malignant clone to a medicine, and from of patient compliance of treatment.
Aims: To evaluate eleven-year results of therapy of chronic myelogenous leukemia treatment with tyrosine kinase inhibitors.
Methods: For the period of January 2004 and up until now the 103 CML patients have been observed at the municipal hematology center of Novosibirsk – capital of Siberia: 39 men (37.9%) and 64 women (62.1%), with age ranging from 16 to 78 years, and the mean value being 43.4±14.15 years, 71.8% patients was included in the analysis (74 patients): 71 people in the chronic phase, 3 - in the acceleration phase. All patients in the chronic phase started taking the TKI in the first 6 months after diagnosis of CML. Patients in accelerated phase and blast crisis were diagnosed before 2003, these patients were significantly pre-treated with various cytotoxic medications and interferons. All patients are receiving treatment by various tyrosine kinase inhibitors (imatinib at a dose of 400-800 mg per day, nilotinib at 800 mg per day, dasatinib 80-100 mg per day). In Russia, imatinib therapy became available only in 2004, as part of the charity program GIPAP (on treatment 10% of patients). All the patients treatment became available in 2005. Today in Russia are using generic medications Philachromin® and Genfatinib®, instead of using original medication Glivec®, their effectiveness requires further observation. New patients - 10 people (9.7%), they are treated by the TKI less than 6 months and have not got the analysis. Also excluded from the analysis of 19 people (18.4%) with low compliance to therapy, they have not received an adequate response to treatment. 22 patients have died: 9 patients in chronic phase, for reasons not related to hematological malignancies, 13 patients with low compliance due to the progression of CML.
Results: Administration of a TKI as a first and second line was followed by a complete clinical and hematological response in 98.5% patients, complete cytogenetic response (CCyR) – in 83,1%, major molecular response (MMO) – in 63,1%. In 3 patients AP - clinical and hematological response. Survival was analyzed in patients administered TKI, as compared to patients not administered TKI (data based on the retrospective review of medical records of CML patients observed at Novosibirsk municipal hematology center for the period of 1999 – 2004). A statistical method of calculating the cumulative fraction of survivals (Kaplan-Meier) was used to evaluate survival, with $p<0,05$ established as the reliability criterion. No medial survival was established in the group administered TKI, the 11-year survival - 84.8%, the estimated 15-year survival was 71,2%. Overall event-free survival rate was 57.8%, in the chronic phase - 69.7%. In the group treated with other cytotoxic agents median survival was 4.1 years, the estimated 15-year survival rate - 3%, $p <0,000001$.
Summary/Conclusions: TKI (original medicine and branded generics) are considered an effective and safe method of treatment for CML in CP, if the patient in compliance with all recommendations of the doctor, associated with a high MMO rate in the chronic phase, which leads to a significant increase in the overall and event-free survival.

PB1841

CML AS PART OF DUAL MALIGNANCIES: POSSIBLE MECHANISMS AND REVIEW OF LITERATURE

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Background: Since the time Imatinib Mesylate has been recommended as standard of care for chronic myeloid leukaemia, the overall survival of CML patients is almost equivalent to general population. Improved survival in CML patients have provided an opportunity to study disease behaviour and its associations in long term basis.
Aims: To study the incidence of secondary malignancies in CML and their outcome.
Methods: We reviewed the medical records of all the CML patients treated with Imatinib at PGIMER, Chandigarh, India from 2001-2014**.**
Results: During a 15 year period (Jan 2001 to Dec 2014), a total of 2183 patients of CML were registered with the adult haematology clinic of PGIMER, Chandigarh of which 1677 patients were included where complete patient details were available. A total of 15 patients were found to have co-existent second malignancy with CML. Four patients had CML as the secondary malignancy. Among 15 patients, three patients had synchronous and 12 had metachronous malignancy. The median age of the patients was 50y (25-66) at the time of diagnosis in this series. Of the 15 patients five were male and rest 10 were female. None of our patients were on 2nd line TKI (Nilotinib and Dasatinib). Two of these 15 patients were in accelerated phase and none in blast phase at the time of diagnosis. History of smoking was present in only one patient who developed bladder cancer and one patient was consumed alcohol in non cirrhogenic doses. None of our patients had history of any carcinogenic exposure. Median duration of imatinib therapy in patients with secondary malignancy is 45.5 months (5 to 130 months). Median duration of follow up was in patients with dual malignancies. Two patients with dual malignancies succumbed to their illness because of blast crisis and one among them was lost to follow up for last 1.5year. None of the patients of CML succumbed to the co-existing malignancy. In patients with CML presenting as primary malignancy (patient no 1-8) the median interval until development of secondary malignancy was 62.3 months (ranged from 8 to 240 months). Whereas, in patients with CML presenting as secondary malignancy the median interval of development of CML was 118.5 months (range 60 to 144 months). None of our patients of co-existent malignancy with CML underwent bone marrow transplantation. Cumulative incidence of second cancer (all categories) in our CML patient cohort is 0.0089. Accordingly, within the limitations of the number of patients which are enrolled in this retrospective analysis, the total number of cases of cancer could be in accordance with the incidence of cancer in the Indian population.
Summary/Conclusions: None of our patients of co-existent malignancy with CML underwent bone marrow transplantation. Cumulative incidence of second cancer (all categories) in our CML patient cohort is 0.0089. Accordingly, within the limitations of the number of patients which are enrolled in this retrospective analysis, the total number of cases of cancer could be in accordance with the incidence of cancer in the Indian population.

PB1842

THE RESULTS OF TYROSINE-KINASE INHIBITORS TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS ACCORDING TO 2013 EUROPEAN LEUKEMIANET RESPONSE CRITERIA: RUSSIAN SINGLE-CENTER STUDY

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Background: The 2013 European LeukemiaNet (ELN) response criteria define when to change therapy in the case of treatment failure, when the treatment with tyrosine-kinase inhibitors (TKIs) of CML patients should be continued (optimal response) and when a careful monitoring is required (warning). To date, no data presented that show the results of the treatment approach according to ELN2013 response criteria.
Aims: The aim of the study was to evaluate the results of TKIs treatment in CML patients according to ELN2013 recommendations.
Methods: The prospective study included 71 adult patients with newly diagnosed CML in chronic phase. Baseline demographics characteristics: median age: 44 years (interquartile range (IQR) 31-57 years); male sex: 51% (n=36); Sokal score: high 23%, intermediate 27%, low 50%. Baseline treatment: imatinib (IM) 400 mg (n=63), nilotinib (NIL) 600 mg (n=7), dasatinib (DAS) 100 mg (n=1). The switching to another TKI or increasing doses was performed at 3,6,12 months or late in case of failure according to ELN2013 recommendations. Therapy was also changed in cases with no early molecular response (EMR) (BCR-ABL ≤10% at 3 months). Overall survival (OS), cumulative incidence of complete cytogenetic response (CCyR), major molecular response (MMR) and deep molecular response (MR4 or deeper) were evaluated (Intention-to-treat analysis).
Results: The failure was recorded in 14 (20%) cases (at 3 months n=6, at 6 months n=3, at 12 months n=2, after 12 months n=2, 1 patient died before 3 month assessment due to progression). Imatinib was a baseline treatment in all failures. Therapeutic options for failures: increasing dose of IM (600 mg) n=2, switching to DAS n=5, switching to NIL n=4, 2 patients are currently being examined before switching. In 6 cases switching or IM dose escalation was performed because of no EMR (out of failure) (NIL n=3, DAS n=1, IM600 mg n=2), other reasons (toxicity, late warning): n=4 (NIL n=2, DAS n=2). Median time to switching after failure detection was 67 days (IQR 36-103 days). Median follow-up: 19 months (IQR 12-31 months). Still alive: n=69; on IM treatment: n=44 (64%), on 2nd TKI: n=25 (36%). The 3-yars OS was 96%. Cumulative incidence of CCyR, MMR and MR4 was 98%, 86% and 73% respectively. Independent of switching, cumulative incidence of MR4 was higher in group with EMR than no EMR: 73% and 22%, respectively (p=0,03) (3 months landmark) (Figure 1).

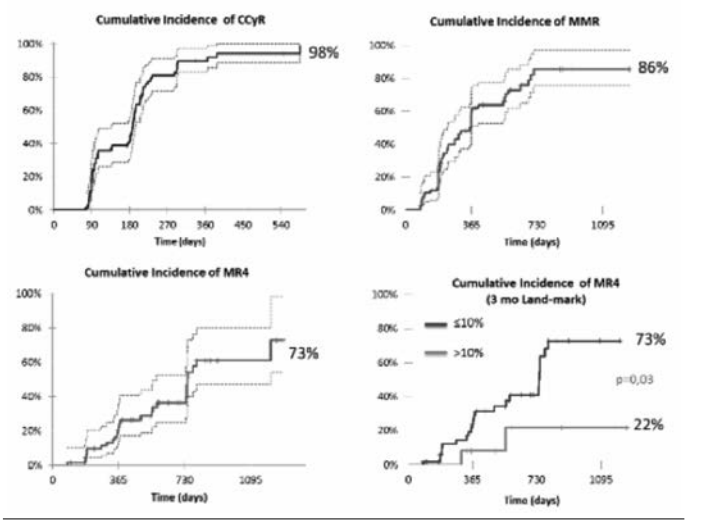

Figure 1.

Summary/Conclusions: The principle of early therapy modification in patients with no optimal response according to ELN2013 response criteria allows to reach CCyR (the main surrogate endpoint for survival) in almost all cases. Early molecular response is a predictor of achieving deeper molecular response not depending on early switching approach.

Gene therapy, cellular immunotherapy and vaccination

PB1843

OUT OF THE BAG – FUNCTIONAL CHARACTERIZATION OF *EX VIVO* EXPANDED MSC

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Background: Bone marrow (BM) derived mononuclear cells (MNC) are the starting point of numerous protocols, such as *ex vivo* expansion of HPC, neuro and mesenchymal stem cells (MSC). We have previously established that the BM collection bag and filter system (which are usually discarded) are an alternative source of MNC, yielding viable and sterile cells equivalent to 50ml of filtered BM, and should not be considered clinical waste. The viability and functionality of the recovered MNC were evaluated by trypan blue exclusion and *in vitro* differentiation into MSC, respectively.
Aims: In order to further validate the applicability of these cells, MSC obtained were tested for morphology, immunophenotype, ability to hamper alloreactivity and to differentiate into adipocytes. tyle='mso-ansi-language:EN-GB'>We have previously established that the BM collection bag and filter system (which are usually discarded) are an alternative source of MNC, yielding viable and sterile cells equivalent to 50ml of filtered BM, and should not be considered clinical waste. The viability and functionality of the recovered MNC were evaluated by trypan blue exclusion and *in vitro* differentiation into MSC, respectively.
Methods: The collection bag and filter system were back-washed and rinsed with RPMI under aseptic conditions and MNC isolated by density gradient centrifugation. To obtain long term MSC cultures, MNC were cultured in DMEM media supplemented with foetal bovine serum, at 37ºC and 5% CO_2. Cultures were replated when confluence was reached. MSC morphology was confirmed with a reverse microscope, and immunophenotype by flow cytometry. To assess adipogenic differentiation capacity, MSC were cultured in the presence of dexamethasone and diclofenac, with adipocytes generation tested by Oil Red staining of lipid deposits. To evaluate their imunossupressive role a one way MLR was performed. Briefly, irradiated MNC (stimulator) and CFSE-dyed MNC (responder) were co-cultured in the presence or absence (control) of MSC. After 7 days of incubation, proliferation of responder cells was measured by flow cytometry.
Results: In all cases, filter recovered MNC viability was superior to 90%, and long term MSC cultures were established, as shown by morphology and immunophenotype (CD105+, CD44+, CD90+, CD73+, CD45-, CD14-, and CD34-). To further characterize our MSC lineage differentiation, a hallmark of MSC, was shown by Oil Red staining of MSC-derived adipocytes. Another characteristic of MSC, down regulation of alloreactivity, was demonstrated *in vitro*, with a consistent 20% reduction of proliferating cells. -family:Calibri;mso-bidi-font-family:"Times New Roman"; mso-ansi-language:EN-GB;mso-fareast-language:EN-US;mso-bidi-language:AR-SA'>MSC morphology was confirmed with a reverse microscope, and immunophenotype by flow cytometry. To assess adipogenic differentiation capacity, MSC were cultured in the presence of dexamethasone and diclofenac, with adipocytes generation tested by Oil Red staining of lipid deposits. To evaluate their imunossupressive role a one way MLR was performed. Briefly, irradiated MNC (stimulator) and CFSE-dyed MNC (responder) were co-cultured in the presence or absence (control) of MSC. After 7 days of incubation, proliferation of responder cells was measured by flow cytometry.
Summary/Conclusions: With this study we demonstrated that, from the usually discarted BM collection bag and filter system, long term MSC cultures can be established. These cells can be expanded *ex vivo,* maintaining an appropriate phenotype and functional capabilities, being suitable for both investigation and clinical settings.

PB1844

FUNCTIONAL CAPACITY OF TUMOR LYSATE-PULSED MONOCYTE-DERIVED DENDRITIC CELLS INDUCING *IN VITRO* IMMUNE RESPONSES

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Background: Dendritic cells (DC) have been shown to be a promising adjuvant to initiate antitumor immune responses. Over the last years, several methods have been developed to isolate DC from cancer patients, *ex vivo* expand and pulse them, aiming to generate highly immunogenic clinical grade infusion products.
Aims: In order to validate our previously established *in vitro* methodology for DC generation, the present study aims to assess the functional capacity of the DC final product inducing immune responses. usion products.
Methods: As part of an authorized pre-clinical study with solid tumors, four experiments were performed using tumor lysates (2 glioblastomas, 1 sarcoma and 1 breast cancer) for DC stimulation. DC were differentiated from monocytes obtained from peripheral blood (PB) and subsequently matured and pulsed with previously prepared tumor lysate, during 8 days of culture in cytokines-supplemented medium. Loaded mature DC (mDC) were evaluated for cell counting, viability, morphology and immunophenotype. DC functionality was evaluated via one-way MLR using peripheral blood mononuclear cells (PBMC) which were isolated from PB by density gradient centrifugation and pre-labeled with the 'green' fluorescent dye CFSE. The $CFSE^+$PBMC (responder cells) were co-cultured with mDC (stimulating cells) in a 96-well plate. PBMC without DC stimulation was used as negative control. In both MLR and negative control, the same tumor lysate used in DC stimulation was added or not. After 7 days of incubation at 37°C, 5% CO_2, proliferation of responder cells was measured by flow cytometry.
Results: The single-cell suspension obtained at the end of culture showed: numerous cells presenting extended and multiple dendrites; up-regulation of the characteristic maturation markers CD83/CD86; and down-regulation of CD14, marker of the precursor cells. Taken together these results prove the achievement of the DC maturation state. These final loaded mDC were assessed for the antigen presentation skills by their *in vitro* allostimulatory capacity of PBMC. The average percentage of proliferating PBMC obtained when co-cultured with mDC was greater than that verified without DC stimulation (47.7±3.0% *vs* 19.3±8.4% and 47.0±5.0% *vs* 8.8±3.5%, with and without tumor lysate, respectively). Despite the small sample size, DC stimulation consistently induced more than double the PBMC proliferation.
Summary/Conclusions: This study demonstrated the functionality of the mDC final product to induce *in vitro* immune responses. With our mDC manufacturing protocol validated, we are motivated to obtain the legal authorization for the implementation of this Advanced-Therapy Medicinal Product in clinical grade. It may be beneficial for the patients to have cell collection, production and administration available in the same hospital setting.

PB1845

CELLULAR IMMUNOTHERAPY: A STORY OF HOPE

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Background: Narrative medicine is a medical approach that recognises the value of people's narratives in clinical practice, research and education. Narrative medicine aims not only to validate the experience of the patient, but also to encourage creativity and self-reflection in the health care provider and researcher.
Aims: Rupert Suply, a young adult diagnosed with Ewing Sarcoma, was included in a study with dendritic cell vaccination to prolong his second remission period. This new therapy slowed down the aggressive tumor growth. Because he is convinced that cellular immunotherapy gave him many extra quality years and he believes in its future possibilities he knows that his story of HOPE is his way of contributing to clinical practice and research.

Figure 1.

Methods: In a documentary short film, directed by Hedwige Daenens, we use narrative medicine to describe our progress of adoptive cellular therapy in diseases where relapse remains a major problem and has an important impact on survival. Rupert tells us his moving story and guides the audience through the different steps in making a cancer vaccine. We filmed at the Center for Cell Therapy and Regenerative Medicine CCRG of the University Hospital Antwerp. This project was made possible by a collaboration with Biological and Medical Art in Belgium BIOMAB, based at the University of Antwerp, and the country's leading organisation to promote interdisciplinary research between scientists and those with an interest in the synergy between Art, Science and Technology.
Results: Leukapheresis, DC generation, antigen loading of DCs by electroporation of WT1-encoding mRNA, cryopreservation of WT1 mRNA-electroporated

DCs, timing of first vaccination, thawing of the DC vaccine, immunisation schedule, route of administration and vaccine dose are described by using documentary methods in a hospital and research setting. Each procedure is explained in detail by a qualified member of staff understandable for a general audience. This movie shows a series of various interactive scenes at different departments, mostly restricted areas, during Rupert's path creating his personal cancer vaccine.
Summary/Conclusions: Narrative medicine is a valuable tool to describe cell collection, processing and therapy. It promotes understanding between researcher, clinician, nurse and patient, encourages additional therapeutic options and generates new hypotheses. The documentary A STORY OF HOPE was presented for the first time at this year's EBMT meeting (Figure 1).

Hematopoiesis, stem cells and microenvironment

PB1846

MAGIC-TT-MEDIATED CELLS TARGET TRANSPLANTATION INTO BONE MARROW WHICH INCLUDES THE STUDY OF CELL DISTRIBUTION *IN VIVO* AND THE EFFECTS OF HEMATOPOIETIC RECONSTRUCTION

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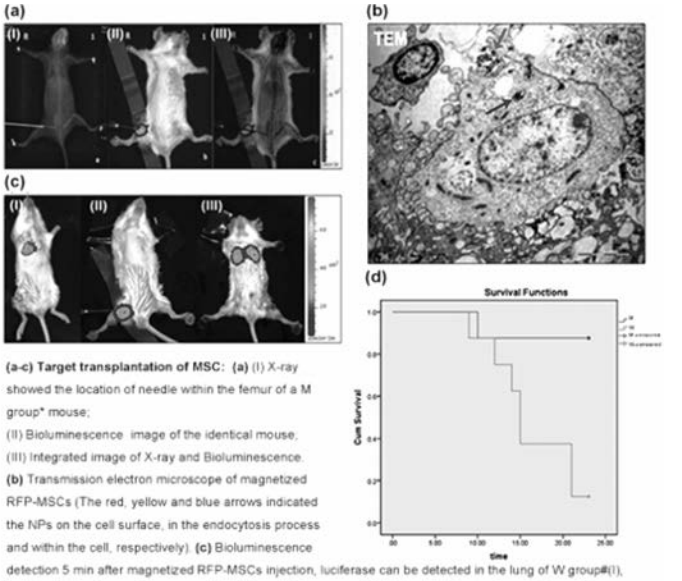
Background: Target cell transplantation to bone marrow (BM) is possible by Magnetism-induced cell target transplantation (MagiC-TT) (EHA2015posterP701).
Aims: RFP-MSC were used to observe the cell's distribution and the following effect, additionally $CD45^+$GFP cells were used to evaluate the promotion of hematopoietic reconstitution of MagiC-TT.
Methods: 1) *Target transplantation of MSC:* Fe_3O_4@PDA@Au nanoparticles (NPs) were synthesized and introduced into Luciferase gene modified RFP-MSC (Luc-RFP-MSCs). Magnetized and wt Luc-RFP-MSCs were compared on the biological feature and their ability of migration *ex vivo*. Then magnetized RFP-MSCs were micro-injected into the femur cavity of the mice with the help of X-ray (Figure 1a), under magnet in M group or without in W group. Bioluminescence, FACS, PCR and histopathological analysis were ued after transplantation. 20 eGFP transgenic mice and another 20 C57 mice were used. 2) *$CD45^+$cells transplantation:* 34 C57 mice were randomly divided into 2 groups evenly. 7.5Gy myeloablative irradiation were given 1d before, then $CD45^+$cells were isolated by MACS from eGFP transgenic mice and freshly micro-injected into right femur, 1×10^6 cells/20uL per mouse, with or without magnet for 24hrs (M or W group). At 0h, 24h, 72h after injection, every 3 mice in both groups were sacrificed at each time point for detection, then remaining 8 mice in both groups were compared with the general conditions, hematopoietic recovery, GFP^+cells in different organs *etc.*
Results: 1) *Target transplantation of MSCs:* NPs exist within or on the surface of magnetized RFP-MSCs (Figure 1b), no obvious change was found. The magnetized RFP-MSCs were capable of target migration under magnetism *ex vivo*. Bioluminescence assay showed that the magnetized Luc-RFP-MSCs appeared in the lung of W group 5 min after cell injection, while fixed in the femur of M group mice. However, on withdrawal of magnet 1h after cell injection, strong fluorescence was observed in the lung of M group gradually (Figure 1c). By pathological examinations, FACS and PCR, large number of RFP-MSCs were observed to reside within the BM in M group while few in W group, thereby demonstrating the specific BM target transplantation of magnetized RFP-MSCs. Those RFP-MSCs were found to survive more than 3m in different organs. 2) *$CD45^+$cells transplantation:* The mice that survived were 7/8 *vs* 1/8 in M or W group respectively (Figure 1d). The GFP% in femurs of both groups (Table 1) also proved the specific BM target transplantation in M group. Platelet recovery in M group is faster than that in W group (12.33d±2.42d *vs* 16.38d±2.39d, P=0.009); and the lowest value of decreased hemoglobin in M group was higher than that of W group (43.75±13.02 *vs* 13.75±5.18, P<0.001).



(a-c) Target transplantation of MSC: **(a)** (I) X-ray showed the location of needle within the femur of a M group* mouse; (II) Bioluminescence image of the identical mouse; (III) Integrated image of X-ray and Bioluminescence. **(b)** Transmission electron microscope of magnetized RFP-MSCs (The red, yellow and blue arrows indicated the NPs on the cell surface, in the endocytosis process and within the cell, respectively). **(c)** Bioluminescence detection 5 min after magnetized RFP-MSCs injection, luciferase can be detected in the lung of W group#(I), but fixed in the femur of M group until withdrawal of the magnet 1 h after (II), 15 min after withdrawal of magnet, fluorescence displayed in the lung of M group gradually (III). **(d) CD45+ cells transplantation:** Survival curves of mice in M group and W group (P<0.05).
* the experimental group with magnetic field. #: the control group without magnetic field.

Figure 1.

Table 1. Comparison of the percentage of RFP-MSCs in M group and W group by flow cytometry.

Group	0h (%)		p	24h (%)		p	72h (%)		p
	*LC	**RT		LC	RT		LC	RT	
BMM	0.017±0.006	0.497±0.151	0.040	0.080±0.026	1.573±0.508	0.030	0.190±0.139	1.960±0.809	0.049
BMW	0.017±0.012	0.050±0.017	0.184	0.013±0.006	0.027±0.015	0.184	0.023±0.015	0.320±0.434	0.368
P	1.000	0.007		0.013	0.006		0.108	0.036	

*: Left control femur; **: Right experimental femur.

Summary/Conclusions: Fe3O4@PDA@Au NPs rendered MSCs their capability of target transplantation by MagiC-TT technique. MagiC-TT also helps in the recovery of PLT after transplantation after CD45+hematopoietic cell transplantation .

PB1847

IMPLICATION OF THE PERCENTAGE OF MACROPHAGES WITH HAEMOPHAGOCYTOSIS IN THE DIAGNOSIS AND PROGNOSIS OF PATIENTS WITH HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Haemophagocytic lymphohistiocytosis (HLH) is a serious condition whose diagnosis is based on clinical,analytical and histopathological criteria established by "The International Histiocyte Society"(IHS) in 2004. Haemophagocytosis is one of the diagnostic criteria. However,haemophagocytosis can also be observed in other disorders in absence of HLH.
Aims: To review a series of patients with HLH and determine the percentage of macrophages and macrophages with haemophagocytosis observed in bone marrow aspirate (BMA). To compare the haemophagocytosis in this group with that observed in patients without HLH. To evaluate the possible impact of both parameters in diagnosis and survival of HLH patients.
Methods: A retrospective study of 31 patients diagnosed with HLH between 2008-2015.Cytomorphological review was carried out by 2 experienced cytohaematologists. The percentage of macrophages was established from a minimum sample of 500 nucleated cells and the percentage of haemophagocytosis from number of macrophages (minimum 30 macrophages).Clinical and analytical data were gathered concerning diagnosis, treatment and follow-up. A control group consisting of patients with haemophagocytosis without HLH criteria was formed. SPSS v19.0 was used for the statistical analysis. Survival analyses were performed using Kaplan-Meier method.
Results: 31 patients were included:median age 41 (range 6 months-80 years),17 male and 14 female. Underlying diseases were:lymphomas 11/31 (36%); infections 10/31 (32%): 3 EBV, 3 leishmanosis, 2 CMV and 2 leishmanosis associated to EBV; autoimmune diseases 5/31 (16%); other causes (solid neoplasm and surgery) 2/31 (6%); idiopathic 3/31 (10%). At diagnosis 30/31 (97%) had fever and 27/31 (87%) had splenomegaly. One cytopenia was observed in 5/31 (16%), bicytopenia 12/31 (39%) and pancytopenia 14/31 (45%). Biochemical parameters:hyperferritinemia in 20/31 (64%),hypertriglyceridemia in 13/31 (42%),high levels of LDH 17/31 (55%), and hypofybrinogenemia in 9/31 (29%).From the total, 12 patients (39%) were treated according to IHS protocol; 10 (32%) with chemotherapy aimed at lymphoma; 3 (10%) steroides; 5 (16%) with antibiotics and 1 was not treated. At median of follow up of 8.2 months,overall survival was 50%. 15 of 31 patients died, 10 of them (32%) within the first 90 days. 40 patients with haemophagocytosis not attributed to HLH were used as control group. Underlying diseases were:lymphomas 11/40 (27.5%);cytopenias 8/40 (20%);leukaemias 4/40 (10%);plasma cell neoplasms 3/40 (7.5%);bone marrow failure 3/40 (7.5%);myelodysplastic or myeloproliferative syndromes 3/40 (7.5%);infections 2/40 (5%);other causes 6/40 (15%).The average of macrophages in patients with HLH was 1.5% (range 0.5-90%) compared to 1% (0.2- 35%) in the control group (p=0.25). The average percentage of macrophages with haemophagocytosis in patients with HLH was 25% (range 0.5-75%) compared to 3.5% (0.5- 60%) in control group (p<0.001). In the group of patients with HLH, those who had a percentage of macrophages >1.5% had a median survival of 99 days compared to 243 days for those with lower percentage (p=0.76). Patients with percentage of macrophages with haemophagocytosis >25% had a survival rate of 107 days compared to 190 days for those who had a percentage lower than 25% (p=0.45).
Summary/Conclusions: The percentage of macrophages observed in BMA in patients fulfilling criteria of HLH is similar to that of patients without HLH. However, there are significant differences in the percentage of macrophages with features of haemophagocytosis. This information could be useful in the differential diagnosis. Among patients with HLH, a trend towards a lower median survival was observed in those with the highest percentage of macrophages as well as in those with a higher percentage of haemophagocytosis.

PB1848

BIOSIMILARS OF FILGRASTIM – ZARZIO® - ASSOCIATED TO PLERIXAFOR ARE EFFECTIVE FOR MOBILIZATION IN LYMPHOMA PATIENTS DEFINED AS HARD TO MOBILIZE

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Background: he biosimilars of G-CSF are the object of debates on their efficiency and their harmlessness princeps medicine. Their use and efficiency for mobilization of the peripheral stem cells (CSP) were less studied. Even less informations are available on the association of biosimilars and Plerixafor in view of the peripheral stem cells harvest in patients defined as hard to mobilize.
Aims: We present our experience on the association of Zarzio ®-Plerixafor for mobilization of lymphoma patients, define as hard to mobilize, with the aim of obtaining of a sufficient graft for an autologous stem cell transplantation.
Methods: Between January, 2012 and December, 2014, we identified 18 patients, 9 mens and 9 women with an average age of 53 years (22-69 years) which benefited from a harvest of the peripheral stem cells at University hospital Brest, France. The patients underwent stimulation by Zarzio ® 30MUI two injections a day, during 5 days (D-4 has D0) and Plerixafor 0.24mg/kg starting at day 0 J0. The stimulation was pursued until the obtaining of a sufficient graft (defined as more than 2.5 millions CD34+/ kg) or for a maximum of 3 consecutive days. We collected for the patients the number of aphaeresis per patient and the number of the CD34+cells harvested by aphaeresis and by patient.
Results: All in all we have made 39 aphaeresis in 18 patients that underwent a double stimulation. The average number of aphaeresis per patient was of 2.16 sessions (1-3/patient). Two patients benefited from a single session of aphaeresis, eleven patients benefited from two sessions of aphaeresis and five patients of three sessions of aphaeresis. For twelve of eighteen patients (66.7%) we achieved the target of a sufficient graft according to the local consensus ($2.5x10^6$ CD34+/ kg). For 14 of 18 patients (77.8%) there was a sufficient according to the recommendations EBMT ($2x10^6$ CD34+cells/kg) on the minimum necessity has the autograft.The average number of CD34+taken by aphaeresis was $1.58x10^6$ cells/kg ($0.22\text{-}6.31x10^6$ CD34+cells/kg). The total average number of the CD34+cells harvested per patient was $3.41x10^6$/kg ($1.5\text{-}6.77x10^6$ CD34+cells/kg).
Summary/Conclusions: We analyzed in a cohort of 18 lymphoma patients defined as hard to mobilize the results of the peripheral stem cell harvest obtained after stimulation by plerixafor associate to a biosimilar of filgrastim - Zarzio ®. We obtained a sufficient graft in 77% according to international recommendation with a limited number of aphaeresis procedure – 2.1 aphaeresis per patient. The results obtained by associating Zarzio® and Plerixafor for peripheral stem cell harvest in lymphoma patients defined as hard to mobilize are comparable with those obtained with products princeps and publish in the literature.

PB1849

THE ROLE OF ENDOTHELIAL CELL-EXPRESSED ALPHA HEMOGLOBIN AND ITS MOLECULAR CHAPERONE AHSP IN BLOOD PRESSURE REGULATION

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Background: Because hypertention is the leading indicator of cardiovascular disease, understanding the blood pressure regulation and regional blood flow to specific vascular beds is of great medical importance. We have recently hypothesized a new mechanism for blood pressure regulation mediated specifically by the alpha subunit of hemoglobin (αHb) [1].
Aims: The α subunit (but not the β subunit of Hb) is expressed in endothelial cells (ECs) within the myoendothelial junction (MEJ), a structure in resistance arteries where ECs and smooth muscle cells (SMCs) cross-communicate to regulate vascular tone. In the MEJ, αHb is proposed to degrade NO via the dioxygenase reaction and thereby increase vascular tone. Moreover, aHb forms a complex with endothelial nitric oxide synthase (eNOS, the major local source of NO production) and cytochrome B5 reductase (CYB5R3) an enzyme that reduces ferric αHb to favor dioxygenase activity [1-3]. Alpha hemoglobin stabilizing protein (AHSP) is a molecular chaperone that binds free αHb, stabilizes its structure and also regulates its redox state, at least in the context of red blood cells [4,5]. Free αHb is extremely unstable and would not be expected to exist without a binding partner in cells. Moreover, we showed previously that AHSP converts bound ferrous αHb into a stable hexacoordinate ferric form that lacks ability to degrade NO [6,7]. Based on this data, we hypothesized that AHSP both stabilizes αHb and regulates its redox state and dioxygenase activity in vascular ECs.
Methods: We investigated this hypothesis by using vascular cell co-culture model composed of human coronary ECs and SMCs, and Ahsp-/- and alpha thalassemic mice strains.
Results: Our studies demonstrate that αHb interaction with AHSP and eNOS

is mutually exclusive and αHb protein expression is disrupted in Ahsp-/- and alpha thalassemic ECs, resulting in reduced arterial tone, lowered blood pressure and abnormal arterial structure.
Summary/Conclusions: Through these mechanisms, AHSP-bound αHb may regulate blood vessel tone dynamically according to ambient conditions and CYB5R3 availability.

References
1. Straub AC, Lohman AW, Billaud M, Johnstone SR, Dwyer ST, *et al.* (2012) Endothelial cell expression of haemoglobin alpha regulates nitric oxide signalling. Nature 491: 473-477.
2. Angelo M, Hausladen A, Singel DJ, Stamler JS (2008) Interactions of NO with hemoglobin: from microbes to man. Methods Enzymol 436: 131-168.
3. Gladwin MT, Lancaster JR, Jr., Freeman BA, Schechter AN (2003) Nitric oxide's reactions with hemoglobin: a view through the SNO-storm. Nat Med 9: 496-500.
4. Kihm AJ, Kong Y, Hong W, Russell JE, Rouda S, *et al.* (2002) An abundant erythroid protein that stabilizes free alpha-haemoglobin. Nature 417: 758-763.
5. Kong Y, Zhou S, Kihm AJ, Katein AM, Yu X, *et al.* (2004) Loss of alpha-hemoglobin-stabilizing protein impairs erythropoiesis and exacerbates beta-thalassemia. J Clin Invest 114: 1457-1466.
6. Feng L, Zhou S, Gu L, Gell DA, Mackay JP, *et al.* (2005) Structure of oxidized alpha-haemoglobin bound to AHSP reveals a protective mechanism for haem. Nature 435: 697-701.
7. Zhou S, Olson JS, Fabian M, Weiss MJ, Gow AJ (2006) Biochemical fates of alpha hemoglobin bound to alpha hemoglobin-stabilizing protein AHSP. J Biol Chem 281: 32611-326.

PB1850

THE "NORMOBARIC OXYGEN PARADOX": INFLUENCE OF NEW MODELS ON THE EVOLUTION OF ERYTHROPOIETIN, RETICULOCYTE COUNT AND HEMOGLOBIN CONCENTRATION IN PATIENTS UNDERGOING BREAST RECONSTRUCTION SURGERY

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Background: The "Normobaric Oxygen Paradox" (NOP) is a physiologic mechanism that induces an increase of endogenous erythropoietin (EPO) production by creating a state of relative hypoxia in subjects previously exposed to a period of normobaric hyperoxia, followed by a rapid return to normoxia. To date, the oxygen exposure duration and the inspired oxygen fraction required to observe a significant increase in hemoglobin or EPO production are not clearly defined.
Aims: This study sought to observe the effect of two models of relative hypoxia compared to a control group on EPO, reticulocytes and hemoglobin stimulation through the NOP theory in patients undergoing breast reconstruction surgery.

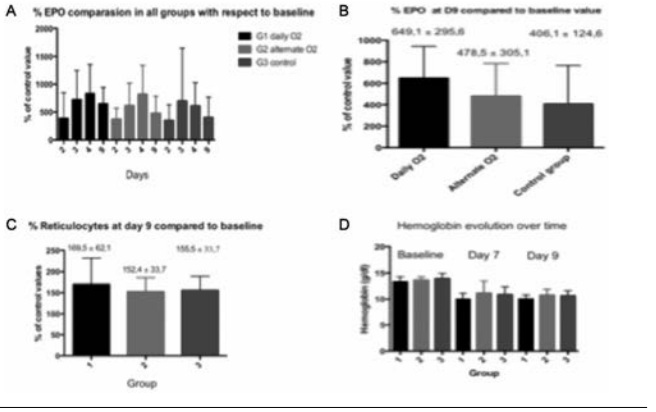

Figure 1.

Methods: After local ethic committee approval and obtaining written informed consent, thirty patients undergoing deep inferior epigastric perforator (DIEP) flap breast reconstruction were prospectively randomized into 3 groups. Nine patients were excluded from analysis for lack of results and/or patient withdrawal. The first group (G1; n=7) was exposed to 5 L O_2/m for 60 minutes per day through a nasal cannula from day one to day nine postoperatively. The second group (G2; n=6) was similarly exposed to O_2, but on alternate days, while the third group (G3; n=8) served as control. All groups recived FiO_2=50% during surgery. Serum EPO was measured on day 0 and postoperatively on days 2, 3, 4 and 9. Serum hemoglobin level and reticulocyte count were measured on day 0 and postoperatively on days 7 and 9. Taking the initial value as 100%, percentage changes in EPO and reticulocytes were calculated, thereby allowing an appreciation of the magnitude on change rather than the absolute values. We used a two-way analysis of variance (ANOVA) for repeated measures to test the variation between groups and over time.
Results: We noticed a pic% EPO elevation at days 3 and 4 with no significant difference between the groups (Figure 1A). At day 9,% EPO in G1, G2 and G3 was 649,1±295,6; 478,5±305,1 and 406,1±124,6 mIU/mL respectively (p>0,05) (Figure 1B). However, the% EPO at day 9 in G1 tended to be more elevated compared to G2 and G3. At day 9, reticulocyte count was more elevated in G1 compared to G2 and G3 (Figure 1C). Hemoglobin level in G1, G2 and G3 was 10,04±0,77; 10,73±1,14 and 10,64±0,96 g/dL respectively (Figure 1D).
Summary/Conclusions: Relative hypoxia seems to be a stimulus for EPO production in all our groups. However, further studies are needed in non-surgical patients with larger sample size and longer follow-up to determine its real impact on hemoglobin.

PB1851

THE RELATIONSHIP BETWEEN BONE MARROW STROMAL FIBROBLASTS, MYELOGRAM PERFORMANCE AND EFFICIENCY OF CHEMOTHERAPY IN CHILDREN WITH ACUTE LEUKEMIA

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Background: Tumor microenvironment plays an important role in cancer initiation and progression. The cancer associated fibroblasts impact on resistance development to chemotherapeutic agents in the treatment of acute leukemia (AL) discussed.
Aims: The aim of the study was to determine the relation between colony-forming efficiency of bone marrow stromal fibroblasts, myelogram parameters and effectiveness of chemotherapy (CT) in children with lympho- and myelovariants of acute leukemia (ALL, AML).
Methods: The study involved 38 children: ALL - 24, AML - 14. Age of children ranged from 6 to 18 years. Treatment was performed by standard protocols. Investigation of patients executed in complete remission phase after full chemotherapy course of primary diagnosis leukemia. Retrospective surveillance was conducted for 15 years. The effectiveness of chemotherapy evaluated by presence/absence of AL relapse. Among patients with ALL the relapse stated in 8 persons, among AML - in 7 children. Efficiency of bone marrow stromal fibroblast colony-forming units (E -CFUf) *in vitro*, myelogram performance were studied. Spearman correlation coefficient, U- test were used.
Results: E-CFUf not depend on the variant of acute leukemia (Uemp.=61.5). In patients with AL we found the inverse correlation between the E-CFUf index and the percentage of erythroblasts (Rs=- 0,52) and monocytes (Rs=- 0,45) in the bone marrow. The direct connection between the number of monocytes and erythroblasts in myelogram defined (Rs=+0,59), probably because of their joint operation in the development of erythroid elements of hematopoiesis. The inverse correlation(Rs=-0,44) between the percentage of monocytes in patients myelogram and AL relapse occurence was found.
Summary/Conclusions: The inverse correlation between stromal fibroblasts and erythroblasts as monocytes in the bone marrow of AL children indicates the regulatory role of stromal microenvironment elements in the functioning of hematopoiesis in the development of malignancy. Discovered correlation between relapse development and the number of monocytes in the AL patients bone marrow after end of protocol treatment opens up further possibilities to determine the mechanisms of chemoresistance formation at AL and review the ways to prognose the effectiveness of treatment of such patients.

PB1852

APHERESIS COLLECTION OF MOBILIZED HEMATOPOIETIC STEM CELLS FROM PERIPHERAL BLOOD IN HEALTY DONORS – 15 YEARS OF EXPERIENCE

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Background: Mobilized hematopoietic peripheral blood stem cell (PBSC) has been widely used for allogeneic transplantation in different hematologic malignancies. Optimal donor and recipient outcomes require maximized stem cell collection efficiency.
Aims: The aim of our study is to present our experience of 15 years in collecting of PBSC in healthy donors.
Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of Macedonia and University Hematology Hospital for period from January 2001 till December 2015. All donors were HLA typed and

matched; they were fully informed on the donation procedure and signed an informed consent for donation. Minimum dose required to ensure successful and sustained engraftment was 2x10^6/kg CD34+cells and 2x10^8/kg mononucleated cells (MNC). PBSC harvesting was performed with continuous flow cell separator Baxter C53000 and COBE Spectra using conventional-volume apheresis processing the 2 – 2.5 total blood volumes per apheresis. A femoral catheter was used for harvesting and Acid Citrate Dextrose formula A is used for anticoagulation. Recombinant human granulocyte colony-stimulating factor (G-CSF) is used to mobilize PBPC for collection. Harvesting of PBSC is usually performed after 4 to 5 days of G-CSF subcutaneous administration at a dose of 10 µg/kg body weight.
Results: All the donors were siblings of the patients treated at the University Hematology Hospital. There were 126 apheresis procedures performed in 74 healthy sibling donors. There were 48 males and 26 females, aged 19-55. The single procedure usually took 3-4 hours and the volume of collected stem cells was 50-220 ml. The needed number of MNC and CD34+cells was successfully collected by 1.7 apheresis (range 1-2). There were 9 ABO incompatible donors. Procedures for mobilization and collection of PBPC from healthy donors are generally well tolerated. The only adverse effects of the apheresis procedure were bone pain as reaction of G-CSF and numbness of the extremities as reaction of ACD-A (hypocalcemia), which occur rarely and were very mild. The collected PBSC were used in allogeneic stem cell transplantation in patients with: acute myeloid leukemia – 61.3%, acute lymphoblastic leukemia – 17.7%, chronic myeloid leukemia – 9%, myeloproliferative disorders – 4.1%, severe aplastic anemia – 2.7%, non-Hodgkin lymphoma – 2.7%, chronic lymphoblastic leukemia - 1.3%, Hodgkin disease – 1.3% and multiple myeloma – 1.3%.
Summary/Conclusions: The apheresis collection of PBSC in healthy donors is an effective and safe procedure. We are developing a National Stem Cell Donors Registry as a part of Bone Marrow Donors Worldwide. In that way we hope we will help widen the world network of stem cell donors and enlarge the possibility for each patient to find the right match.

PB1853

PERFORMANCE EVALUATION OF Q-FLAG OF HEMATOLOGY ANALYZER, SYSMEX XN-20
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Background: The Sysmex XN modular analyzer (Sysmex, Kobe, Japan), which were introduced in 2011, applied several new analysis channels compared to previous analyzer, XE series. White cell precursor channel (WPC), one of the new channels, adopted fluorescence flow cytometry technology and developed especially for detecting myeloblasts or lymphoblasts more accurately than any other channels applied in previous modules.
Aims: Our study aim to investigate the flagging performance of Sysmex XN-20 modular analyzer focusing on the Q values.
Methods: We investigated the performance of Q values flagging according to interinstrumental agreement, intrainstrumental precision, and diagnostic accuracy. Tested suspect flags are “blasts/abnormal lymphocytes”, “atypical lymphocytes” from white cell differential channel, and “blasts” from white cell precursor channel.
Results: In interinstrumental agreement evaluation, two XN modules showed significant Q value correlation for each suspect flags although Pearson’s correlation coefficient for “blasts/abnormal lymphocytes” and “blasts” flags were too low; they were 0.28 and 0.29. However, from Pearson’s chi-square tests, there were significant difference in flagging performance between two analyzers for all of each flags (P<0.01 for all of tested flags). Absolute agreement rates were ranged from 65.0% to 83.5%, and kappa values were ranged from 0.305 to 0.501. In intrainstrumental precision evaluation, for the specimens with Q value of 50-150, standard deviations were ranged from 4.8 to 23.9 for “blasts/abnormal lymphocytes” flag, from 18.7 to 59.1 for “blasts” flag, and from 11.0 to 23.0 for “atypical lymphocytes” flag during 10 replicates. For diagnostic accuracy, in the default Q value cutoff setting (Q value of 100), sensitivities and specificities for “blasts/abnormal lymphocytes” flag were 90.1% and 73.8%, the values for “blasts” flag were 92.1% and 86.3%, and the values for “atypical lymphocytes” flag were 70.0% and 89.9%, respectively.
Summary/Conclusions: We recommend to adjust the Q value threshold setting for “blast/abnormal lymphocyte”, “blast” and “atypical lymphocytes” because of their inadequate diagnostic accuracy of default threshold setting. However, their poor intrainstrumental precision and interinstrumental agreement weaken the reliability of Q value flags.

Hodgkin lymphoma - Clinical

PB1854

SURVIVORSHIP PROGRAM: SECOND MALIGNANCIES IN LYMPHOMA SURVIVORS
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Background: Improvements in the treatment of both Hodgkin’s (HL) and non Hodgkin’s Lymphomas (nHL) have resulted in an increasing number of long term survivors. However this patient’s population is at high risk of developing late therapy related complications that can negatively affect long term survival and quality of life.
Aims: In our institution the HL and aggressive NHL long term survivors are followed up in a dedicated clinic since September 2014. Here we report preliminary data on second malignancies.
Methods: We have collected retrospective data on second tumours in 469 consecutive lymphoma survivors.
Results: We have analyzed data regarding 469 patients coming in our clinic from 15 September 2014 to 18 February 2016, 247 have been successfully treated for HL and 222 for nHL. Two hundred thirty three were females, 236 males; median of age at lymphoma diagnosis was 29 years for HL (range 13-84) and 48 years for nHL (range 12-83). Median age at last observation in the follow up clinic was 50 years for HL (range 21-89) and 62 years for nHL (range 24-88).The median of duration of follow up was 18 years for HL (range 5-40) and 13 years for nHL (range 5-37).Sixty two patients (13%) experienced a second cancer, 4 of them had 2 neoplasms, so we documented 66 second tumours (Table 1). They were: 23 skin (35%) and 43 non cutaneous cancers (65%). The non skin neoplasms were: 10 breast, 9 gastroenteric, 8 thyroid, 6 prostatic, 1 lung, 4 bladder, 1 renal, 1 tongue, 1 testis 1 gynecological metastatic, and 1 cutaneous appendages malignant cancers (Table 2). Four of these tumours (2 colon, 1 thyroid and the metastatic gynecological one) have been diagnosed with our program of early diagnosis of second cancers and thyroid dysfunctions. The median of time between diagnosis of lymphoma and diagnosis of second malignancy was 18 years (range 1-41). Regarding the previous therapies: mediastinal radiotherapy has been administered to 7 out of 10 of the females with breast cancer (70%), mantel or neck radiotherapy to 6 out of 8 (75%) with thyroid cancer; no one of the intestinal and prostatic cancers have received abdominal radiotherapy; one out of 4 of the patients with urinary cancer (25%) had abdominal radiotherapy and MOPP/ABVD regimen; the one with lung cancer had MOPP chemotherapy and mantle radiotherapy. Chemotherapy has been administered for the treatment of lymphoma to 6 with breast (60%), to 7 with thyroid (88%), to all with gastroenteric (100%), and to 4 with bladder cancers (67%). Median of age of breast cancer in our setting was 50 years (range 38-70), of thyroid cancer was 40.5 years (range 24-55), and of gastroenteric cancer was 66 years (range 42-83). Moreover we have documented relapse of their original lymphoma in 3 patients (2 HL and 1 nHL) respectively at 9, 22 and 28 years after initial diagnosis.

Table 1. Features of patients with second neoplasms.

Sex	32 female	30 male
Lymphoma type	40 HL	22NHL

Table 2. Type of second malignancies in our lymphoma survivor patients.

Type of neoplasm	number
Skin	
Basocellular carcinoma	20
Spinocellular carcinoma	3
Breast carcinoma	10
Gastroenteric tract carcinoma:	
Colon-sigma-rectum	7
Gastric	1
Esophagus	1
Thyroid carcinoma	8
Prostatic malignancies	
Adenocarcinoma	5
Sarcoma	1
Bladder	4
Lung carcinoma	1
Tongue carcinoma	1
Cutaneous appendages	1
Testis carcinoma	1
Renal carcinoma	1
Gynecological metastatic neoplasm (histology ongoing)	1

Summary/Conclusions: In our Department we described a significant number of cases of second neoplasms in the lymphoma survivors population: 4 of these (9% of non cutaneous cancers) were detected by tests done for early diagnosis of late complications. These results outlne the importance of a risk adapted plan for early diagnosis of cancers in this setting of patients that would be encouraged by both hematologist and general practitioners.

PB1855

GEMCITABIN, VINORELBINE AND PREDNISOLONE (GVP)±RITUXIMAB COMBINATION IN RELAPSED/REFRACTORY LYMPHOMA CASES: RETROSPECTIVE ANALYSIS

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Background: Lymphomas are heterogenous group of disorders and relapse is not infrequent after standard chemotherapy regimens. Salvage regimens are used to determine the chemosensitivity before stem cell transplantation. GVP combination is one of the salvage regimens used as in cases with relapsed /refractory Hodgkin Lymphoma (HL) or non- Hodgkin Lymphoma (NHL).
Aims: The aim of this study is to evaluate the results of Gemcitabin, vinorelbine and prednisolone GVP plus minus Rituximab regimen in cases with lymphoma treated by GVP.
Methods: Thirty seven cases with lymphoma were included in this analysis. Age range was between 18-77, male female ratio was 20/17. Twenty one of the cases had HL and 16 had NHL. Among NHL, 8 had diffuse large B cell lymphoma (DLBCL), 3 had peripheral T cell lymphoma (PTCL), 3 had indolent NHL (iNHL), 2 had transformed NHL and one had anaplastic large cell lymphoma. All the cases had been treated by standard first line chemotherapy regimens. Results were analyzed retrospectively.
Results: The most commonly used 1st line salvage regimen was DHAP±Rituximab. GVP±Rituximab was used as 1st line, 2nd line and after than 4th line setting in 7, 28 and 2 cases, respectively. Response to 1st line GVP±Rituximab: complete response (CR) was detected in 1 case, partial response (PR) in four cases, minimal response (MR) in one case and progressive disease (PD) in one case. Response to 2nd line GVP±Rituximab: CR, PR and PD were detected in 5, 9 and 14 cases, respectively. Responses according to lymphoma subtypes: 1st line GVP setting CR, PR and PD were detected in 1, 1, 1 cases with HL and PR and MR in 3 and 1 cases with NHL. In 2nd line GVP setting CR, PR and PD were detected in 5, 6 and 7 cases with HL and 1, 2 and 8 cases with NHL, respectively.
Summary/Conclusions: GVP as salvage regimen in second line setting, although the number of the cases treated in 1st line setting is limited, is a reasonable choice with 50% overall response rate in cases with HL but not in NHL.

PB1856

LYMPHADENOPATHY WITH ELEVATED IGG4 PLASMA CELLS IN CHILDHOOD: A POSSIBLE ASSOCIATION WITH MALIGNANCIES OR IMMUNE MEDIATED DISEASES

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Background: IgG4 related disease (RD) is a fibroinflammatory condition, identified in 2003, that can involve every organ and apparatus. The diagnostic criteria for IgG4 (RD) are based on three characteristics of the disease: organ enlargement or nodular lesions in different organs, increase of serum IgG4 and infiltration of IgG4 plasma cells. The most frequent disease manifestations are autoimmune pancreatitis, sialadenitis, dacryoadenitis and retro-peritoneal fibrosis; IgG4 related lymphadenopathy is rare. Differential diagnosis of IgG4 RD are malignancies and autoinflammatory diseases. It is usually diagnosed in middle and senile aged men; only few pediatric cases in literature are described, and the organs involved are orbits, lungs, mediastinum and abdomen. Lymphadenopathy of IgG4 (RD) is often the first manifestation of the disease, that could progress and involve any organ; a disease localized only in lymph nodes is rare and poorely characterized. No pediatric cases of IgG4 related lymphadenopathy or association with malignancies or other immune-mediated disorder are described in literature.
Aims: To describe for the first time two pediatric cases of lymph node histological pattern of IgG4 (RD) with association with lymphoma and immune-mediated disease.
Methods: Data of the two pediatric patients were retrospectively reviewed. The specimens for the histological analysis were centrally reviewed.
Results: Data of two patients with a histological pattern of IgG4 lymphadenopathy were analyzed. Both of them had a history of persistent lymphoadenopathy. Patient #1, a 11 years old male, with normal serum IgG4 concentration, had a first cervical lymph node biopsy with the evidence of progressive transformation of germinal centers and a high number of IgG4 plasma cells; two months later, for increasing of lymph nodes dimension, he had a second biopsy and the diagnosis of nodular lymphocyte predominant Hodgkin Lymphoma (HL) was made. He was treated with three cycles of prednison, vinblastine and cyclophosphamide, obtaining a complete remission (follow-up: 18 months). Patient #2, a 18 years old male, with a personal history of asthma and atopic dermatitis, had a persistent neck lymphadenopathy after 8 years from an infectious mononucleosis. He had high serum IgG4 concentration. Histology showed follicular hyperplasia and increased IgG4 plasma cells in germinal centers. Following analysis evidenced elevated (10%) double negative TCR alpha-beta positive T cells, suggesting the diagnosis of Autoimmune Lymphoproliferative Syndrome (ALPS). Fas-mediated apoptosis assay is currently ongoing.
Summary/Conclusions: We described for the first time 2 pediatric cases with with histological lymph node characteristics usually found in IgG4 (RD); in particular in one patient this finding was associated with a nodular lymphocyte predominant HL, in another patient with clinical and hematological features suggestive of ALPS. The significance of this histological finding in adult patients with isolated lymphadenopathy is still unclear and rare correlations with lymphomas and with autoimmune diseases are reported. Our data suggest that also in childhood this pattern is not specific for IgG4 RD but may be present in different malignant and non malignant disorders. A careful throughout examination and follow-up should be carried out in patients affected by IgG4 RD or with lymph node biopsy showing the IgG4 features, to monitor the possible development of lymphoma and other immune mediated diseases.

PB1857

LATE RELAPSE OF HODGKIN LYMPHOMA – IS IT DIFFERENT IN CLINICAL CHARACTERISTICS AND OUTCOME?

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Background: Relapse of Hodgkin lymphoma usually occur in 20-30% patients. The disease relapse usually occur within the first 5 years after the diagnosis, with the trend of the diminishing after three years ans subsequently, only minority relapses after 5 years.
Aims: The aim of this study was to evaluate the clinical characteristics, prognostic factors, therapy and outcomes of patients with late relapse (>5 years).
Methods: We retrospectively analyzed clinical presentation, the prognostic significance of clinical parameters, therapy and outcome in the group of patients with very late relapse of Hodgkin lymphoma and compare them with patients who relapse earlier.
Results: In group of 102 patients with relapsed Hodgkin disease 16(15.68%) patients had very late relapse of disease. Median time to very late relapse was 86 months (range 61 to 199 months). Most of these patients (11, 68.5%) were in advanced clinical stage (III, IV). 11(68.75%) patients with very late relapse were treated with high dose chemotherapy and autologous bone marrow transplantation. Second complete response was achieved in 13(81.25%) patients. At a median of 4,5 years after therapy for very late relapse, 13(81.25%) patients are still alive and free of disease and 3 patients died: 2 patients from Hodgkin lymphoma, and one patient from brain tumor. There was not noticed significant difference between initial clinical parameters between of patients with very late relapse and patients who relapse earlier. Median survival of patients with very late relapse of disease was significantly longer (p=0.001). However, survival calculated from the moment of relapse of disease was not significantly different between these two group of patients (p=0.83).
Summary/Conclusions: The late relapse is relatively rare event in our group of patients with Hodgkin lymphoma. Patients with very late relapse have longer OS, than the patients who relapse earlier, but survival calculated from relapse was not significantly different. Remains an open question is it necessary to apply high dose therapy and autologous transplantation in all patients with very late relapse of disease. Further clinical trials are needed to characterize best therapy option in patients with very late relapse tailored to disease risk and comorbidities.

PB1858

LYMPHOMA SURVIVORSHIP AND CARDIOVASCULAR DISEASES SURVEILLANCE

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Background: Improvements in the treatment of Lymphomas have resulted in

an increasing number of long term survivors. This patient's population is at risk of developing late therapy related complications that can negatively affect their survival and quality of life.
Aims: In our institution since September 2014 the Hodgkin's (HL) and aggressive non Hodgkin's Lymphomas (nHL) long term survivors are followed up in a dedicated clinic aimed to detect long term clinical problems that can affect people undergone previous radiation therapy and/or chemotherapy.
Methods: We had planned electrocardiographic and echocardiografic evaluation in patients without known cardiological problems.
Results: We have analyzed data regarding consecutive 469 patients coming in our clinic from 15 September 2014 to 18 February 2016, 247 have been treated for HL and 222 for nHL. Two hundred thirty three were females, 236 males; median of age at diagnosis was 29 years for HL (13-84), 48 years for nHL (12-83); median of age at last observation in the follow up clinic was 50 years for HL (range 21-89) and 62 years for nHL (range 24-88). The median of duration of follow up was 18 years for HL (range 5-40) and 13 years for nHL (range 5-37). Thirty seven of our patients had cardiovascular disorders before the diagnosis of lymphoma. The cardiac function has been studied in three hundred sixty one of our patients. Two hundred twenty five out of 361 (62%) developed cardiac abnormalities of different type and severity in the period following treatment for their lymphoma (Table 1 and Table 2). They were 124 females (55%) and 101 males; 126 had previous HL (56%) and 99 nHL.The median age at cardiovascular disease detection was 54 years (range 20-83). The median time between diagnosis of lymphoma and diagnosis of cardiovascular diseases was 14 years (range 0-43).The most common cardiac disturbances described were valvulopathies of different type and grade (95), arterial hypertension (76) and ischemic cardiopathy (32); some patients had more than one alteration. Moreover 77 out patients showed a diastolic relaxation abnormality without ventricular dysfunction which whose the only alteration detected in 33 cases.
In 114 patient abnormalities were first detected during screening in asymptomatic condition: in fact we had planned electrocardiographic and echocardiografic evaluation in 206 patients without known cardiological problems. One hundred fourteen out of 206 screened (55%) showed previous unknown cardiac disturbance. Regarding the previous therapies: mediastinal radiotherapy had been administered in 64 of patients with valvulopathies (and in all with only calcification) (67%), in 13 with ischemic cardiopathies (41%) and in 33 with diastolic relaxation abnormality (43%); chemotherapy antracycline-based had been receveid by 77 with valvulopathies (81%), by 19 with ischemic cardiopathies (60%) and by 73 with diastolic relaxation abnormality (95%).

Table 1. Feature of survivors patients.

Sex	233 females	236 males
Lymphoma	247 HL	222 nHL
Median age at diagnosis	29 years for HL (13-84)	48 years for nHL (12-83)
median of age at last observation in the follow up clinic	50 years for HL (range 21-89)	62 years for nHL (range 24-88)
The median of duration of follow up	18 years for HL (range 5-40)	13 years for nHL (range 5-37)

Table 2. Type of cardiovascular disorders in the lymphoma survivors (225 patients).

Type	Patients
Diastolic relaxation abnormality	77
Valvulopathies	
Insufficiency	78
Sclerosis	10
Calcification without dysfunction	5
Mitral prolapsed	2
myocardic thickness	2
arterial hypertension	76
pulmonary hypertension	6
Cerebrovascular disease	
Chronic	1
Acute	5
Atherosclerosis SAT	3
Arrhythmias	
Ventricular fibrillation	2
Atrial Fibrillation	8
Other	10
Ischemic cardiopathy	32
Dilatative cardiomyopathy	2

Summary/Conclusions: Our analysis confirms that a high percentage of patient survived to lymphomas can develop cardiovascular diseases and outline the importance of cardiac surveillance.Their monitoring would be promote because can detect asymptomatic structural and functional anomalies as diastolic relaxation abnormality considered an early sign of cardiomyopathies.

PB1859

OSTEONECROSIS DETECTED BY WHOLE BODY MAGNETIC RESONANCE IN PATIENTS WITH HODGKIN LYMPHOMA TREATED BY BEACOPP

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Background: The treatment of Hodgkin Lymphoma (HL) has led to the progressive achievement of high survival rates over the last years . ABVD regimen has been considered for a long time the standard treatment for advanced-stage HL patients, due to well documented efficacy and a favourable toxicity profile. Several studies demonstrated the increase of progression-free survival rates by using BEACOPP regimen compared to ABVD, especially in advanced-stage disease, at the cost of increased toxicity. Osteonecrosis (ON) is a complication of chemotherapy that may compromise the patient's quality of life and it is often identified in advanced stages when the only available treatment is joint replacement. The use of corticosteroids is one of the most common risk factors, responsible for 10-30% of cases of ON.
Aims: The purpose of this retrospective study was to assess the frequency of ON with the Whole Body MRI (WB-MRI) scans performed on patients with HL treated by different chemotherapy regimens, including protocols with high doses of corticosteroids.
Methods: We evaluated the WB-MRI scans performed on 42 patients with HL treated by three chemotherapy regimens (6 ABVD, 2 ABVD+4 escalated BEACOPP, 2 ABVD+4 escalated BEACOPP+4 standard BEACOPP), excluding those patients with main risk factors for ON. All the patients underwent WB-MRI without contrast agent administration and FDG-PET/CT before treatment, after two cycles of ABVD, after four cycles of escalated BEACOPP and a month after the end of chemotherapy.
Results: Six out of 7 patients (85.7%) who received 2 ABVD followed by 4 cycles of escalated BEACOPP+4 standard BEACOPP and 1 out of 5 patients (20%) who received 2ABVD followed by 4 BEACOPP escalated presented ON, with a statistically significant difference of frequency between the two groups of patients ($p<0.05$); whereas no injury has been reported in patients treated by only ABVD. The ON lesions identified in those patients treated with eight BEACOPP were all absent on the WB-MRI scans performed after four escalated BEACOPP, so they developed ON during the last four cycles of chemotherapy. Among a total of 48 ON lesions observed, 23 (48%) were detected in the knee; multifocal ON were detected in six out of seven patients (86%).
Summary/Conclusions: The development of ON seems to be strictly related to the chemotherapy protocol adopted and the number of cycles received, strengthening the hypothesis of a correlation between the dose of corticosteroids included in the BEACOPP scheme and this complication. WB-MRI can be considered as a helpful tool that allows, not only for staging and follow patients with lymphoma, but also to detect ON in patients treated with corticosteroids, avoiding radiation exposure or contrast administration.

PB1860

MORPHOLOGIC PATTERN AND PROGNOSTIC SIGNIFICANCE OF CD30 DISTRIBUTION WITHIN NEOPLASTIC CELLS IN HODGKIN LYMPHOMA

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Background:
Hodgkin's lymphoma (HL) is a highly curable disease and 5-year survival is improving, being currently 86%. Cure rates of more than 90% for early HL and more than 70% for those with advanced HL are expected. CD30 protein is expressed on Hodgkin and Reed-Sternberg (HRS) cells in nearly all cases of classical Hodgkin lymphoma (HL). CD30 is present on a cell membrane and on the Golgi complex of the endomembrane cell system. Given the growing clinical application of CD30 targeting we were interested to see if there is variability in distribution of CD30 protein between the cell surface and the cytoplasm and the impact on outcome.
Aims: Given the growing clinical application of CD30 targeting we were interested to see if there is variability in distribution of CD30 protein between the cell surface and the cytoplasm and the impact on outcome.
Methods: To further assess the presenting features and the prognostic significance of CD30 expression in HL we performed a retrospective single institution study of 179 cases with a median follow-up of 5 years. The median ages were 40 years (range, 14–83 years) for men and 34 years (range, 16–77 years) for women; Histological subtypes of HL were nodular sclerosis in 155 (87%), mixed cellularity in 19 (11%), lymphocyte rich in 5 (3%) of cases. Stages III-IV were present in 116 pts (65%), bulky disease in 68 pts (38%), extranodal disease in 21 (12%) and 73 (41%) had a score >2 (intermediate-high-risk). Combined radio-chemotherapy was administered in 116 pts (65%) and chemotherapy alone in 63 (35%).The CD30 protein was evaluated in formalin-fixed paraffinembedded (FFPE) tissue sections by immunohistochemistry (IHC) with monoclonal mouse antihuman antibody CD30 (clone Ber-H2) (Dako). Morphological pattern (localization of CD30) of CD30 expression was evaluated in each case under a microscope examination.
Results: FFPE tissue specimens from 179 HL patients were examined. Immunophenotype was typical of classical HL, and HRS cells were positive for CD30 in all cases. Immunostaining for CD30 was present only on the Golgi body in 147 (82%) of cases, and it was demonstrated on the Golgi body and

on the cell membrane in 32 (18%) of cases. The 32 patients with golgi and surface CD30 expression had poor prognosis compared with the patients with CD30 surface alone (5 year progression-free survival [PFS], 20% *versus* 86%; p<0.001).
Summary/Conclusions: In our series of 179 patients with classical HL, predominant pattern of CD30 expression was cytoplasmic (Golgi body), and only 18% of cases demonstrated cell surface and cytoplasmic expression, with the evidence of poor prognosis.

PB1861

EFFICACY AND SAFETY OF APREPITANT FOR PREVENTION OF CHEMOTHERAPY INDUCED NAUSEA AND VOMITING IN PEDIATRIC PATIENTS

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Background: Chemotherapy-induced nausea and vomiting (CINV) are common side effects for cancer patients with a considerable impact on quality of life. Traditional regimens to prevent CINV commonly contain a combination of corticosteroid plus ondansetron, a 5-hydroxytryptamine receptor antagonist. Nevertheless, CINV persist in 20-30% of patients and in 40% remains even after of chemotherapy (delayed nausea and vomiting). The NK-1 receptor inhibitor aprepitant, in addition to usual anti-emetic therapy, seems to improve both acute and delayed nausea and vomiting in adults. Preliminary studies have shown good efficacy and tolerability also in adolescents; however, trials of using aprepitant in pediatric hemato-oncologic disease are limited.
Aims: Here, we report our experience about the safety and the efficacy of aprepitant in children and adolescents with Hodgkin Lymphoma (HL), treated with highly emetogenic chemotherapy.
Methods: Pediatric patients aged >10 years and with diagnosis of HL received aprepitant as part of triple antiemetic prophylaxis during a cycle of chemotherapy. Aprepitant was administered orally at a dose of 125 mg on Day 1 and 80 mg on Days 2 and 3; at the same time, patients received ondansetron at a dosage of 4 mg/mq and dexamethasone (0.5-2 mg/kg). The efficacy of the drug was evaluated through a questionnaire given to the patient in the next cycle, after obtaining informed consent. Toxicity was evaluated according to CTCAE criteria (*v 4.02: Sept. 15, 2009)*.
Results: Ten patients were enrolled between January 2015 and January 2016; nine received a first line chemotherapies (COPP/ABV, ABVD), while only one of them underwent a fourth-line therapy (Bendamustine); mean age was 13.6 (range 11-16 yrs) and mean number of cycle administered was 3.8 (range 1-6). Five of ten patients reported nausea with a variable intensity from 2 to 6, measured with a scale from 1 to 10; only two patients reported vomiting (2 and 4 episodes). All patients experienced a grade III-IV neutropenia and a a slight increase in transaminases, effects likely related to chemotherapy; no other side effects were registered.
Summary/Conclusions: In our experience, aprepitant, in combination with ondansetron and dexamethasone, significantly decreased the incidence of CINV in children receiving highly emetogenic chemotherapy. We obtained a complete response rate of 50% with a good toxicity profile. This results make us continue to use aprepitant in our patients, since the reduction of nausea and vomiting sensation represents a considerable advantage on quality of life and therapeutic efficacy.

Indolent Non-Hodgkin lymphoma – Clinical

PB1862

TCL1A EXPRESSION IN SPLENIC MARGINAL ZONE LYMPHOMAS (SMZL) AND CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN (CBL-MZ): CORELLATION WITH IMMUNOPHENOTYPE, MYD88L265P EXPRESSION AND CLINICAL DATA

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Background: SMZL and CBL-MZ display significant similarities. TCL1A expression may be a valuable tool for the diagnosis of SMZL. TCL1A has been recently shown to be highly expressed in Waldenstrom macroglobulinemia, although data is limited.
Aims: The aim of the present study was to evaluate TCL1A expression in a series of bone marrow samples involved by SMZL and CBL-MZ and correlate the findings with other immunophenotypical, morphological, and clinical data, as well as with the presence of MYD88 L265P mutation.
Methods: 45 patients were retrospectively analyzed: 20 with SMZL and 25 with CBL-MZ. Immunohistochemical staining was performed using the following antibodies: CD5, CD20, Ki67, cyclin D1, TCL1, T-bet, DBA44, CD23, CD27, IRTA1. MYD88 L265P mutation was detected by allele specific PCR.
Results: The main clinical characteristics as well as immunohistochemical and molecular findings are summarized in the Table 1.TCL1A staining was negative in 17/20 cases of SMZL (85%) and 22/25 (88%) cases of CBL-MZ.DNA-44 was positive in 17/24 (71%) of cases in both groups. None case expressed IRTA1. T-bet was positive in 55% and 36% of SMZL and CBL-MZ cases, respectively. No correlation was found between TCL1A and DBA44 expression. Regarding SMZL cases, all TCL1A-positive cases were also positive for CD27. 8/25 (28%)CBL-MZ cases were positive for the presence of MYDL265P mutation. In SMZL cases only 1/11 patient was positive for the presence of MYD88. No correlation was found between TCL1A expression and the presence of MYD88 mutation. None of the mutated cases were TCL1A positive. Among TCL1A-positive SMZL cases, 1/3 presented disease progression with histologic transformation to double-hit lymphoma. All TCL1A-negative SMZL cases were treated with rituximab-monotherapy and are alive without disease.2/3 TCL1A-positive CBL-MZ cases progressed and required therapy due to the presence of cytopenias and one of them died of the disease. None of the TCL1A-negative CBL-MZ cases presented disease progression.

Table 1. Characteristics of SMZL and CBL-MZ cases.

Feature	SMZL # (%)	CBL-MZ # (%)
Male sex	11/20 (55)	5/25 (20)
Age	65 (49-85)	71 (46-81)
Splenomegaly	20 (100)	0
Lymphadenopathy	7/20 (35)	0
ALCs (median)	5200 (690-32100)	2800 (1000-10010)
Hb<10gr/dl	4/20 (20)	0
PLTs<100.000/μL	2/20 (10)	0
HCV (+)	0	0
LDH elevated	9/20 (45)	0
Paraproteinemia	5/20 (25)	16/25 (64)
%BM infiltration	40 (5-85)	30 (15-80)
Pattern		
Mixed	11 (55)	15 (60)
Nodular	14 (70)	10 (40)
Intrasinusoidal	10 (50)	12 (48)
TCL1A (+)	3/20 (15)	3/25 (12)
MYD88 L265P (+)	1/20 (5)	7/25 (28)
DBA44 (+)	9/12 (75)	8/12 (67)
IRTA 1	0/20	0/25
T-bet	11/20 (55)	9/25 (36)
CD27	6/7 (86)	2/2 (100)

Summary/Conclusions: Both SMZL and CBL-MZ display similar pattern of TCL1A, DBA44, IRTA1 and T-bet expression. TCL1A is rarely expressed in SMZL and CBL-MZ cases. No correlation was found between TCL1A and DBA44 expression. No correlation was found between TCL1A and MYDL265P mutate. CD27 and TCL1A were not mutually exclusive. TCL1A expression may be associated with worse outcome, although the number of TCL1A positive cases are small in order to reach safe conclusions.

PB1863

DESCRIPTION OF LATE ONSET NEUTROPENIA IN PATIENTS TREATED WITH BENDAMUSTINE PLUS RITUXIMAB

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Background: Bendamustine (B) is an alkylating drug indicated for the treatment of patients with chronic lymphoid leukemia (CLL) who are not eligible to Fludarabine. In addition, Rummel *et al.* (Lancet 2013) described B associated with Rituximab (R) as a therapeutic alternative to R-CHOP in follicular and other indolent lymphomas. Although hematoxicity of B is largely described in the literature, both late onset neutropenia and lymphopenia occurring after the last cycle of immunochemotherapy are not described.
Aims: We report a cohort of patients treated with B+R showing late onset neutropenia after the end of lymphoma treatment.
Methods: This is an observational retrospective study (Anticancer Center Antoine-Lacassagne, Nice and General Hospital, Antibes, France) including patients treated from January 2009 to June 2015. The study included patients with CLL and indolent NHL (follicular, mantle cell and marginal zone) having received at least one cycle of the association of R (375mg/m²) and B (70 or 90mg/m²x 2 D) as part of their first or second line treatment. Assessment of hematotoxicity was performed according to CTCAE v4.0. We excluded patients with neutropenia preexisting to immunochemotherapy or relapsing within 3 months after the end of induction treatment.
Results: 287 patients received one to six cycles of B+R. We noted a total of 83 episodes of late onset of neutropenia (51 grade I/II, 32 grade III/IV) in 36 patients (follow-up 12 months). The characteristics of those 36 patients are the following: sex ratio (M/F) 0.61; median age 73 years (48 to 93); 94% PS 0/1; 36% CLL, 42% follicular lymphomas (FL), 14% mantle cells lymphomas (MCL) and 8% other indolent lymphomas (including marginal lymphoma). Dose intensity is 100% for B and R with an average of 5 cycles (92% of patients received more than 4 cycles of R+B). Among 15 patients with FL, 13 received a maintenance treatment after B+R, with R monotherapy (mean number of cycles: 5.9) that was interrupted in 8 for at least grade II neutropenia. Grade III/IV neutropenia occurred in 9 patients (including 2 patients with FL treated with R maintenance). There was no event of febrile neutropenia within the study period. Four patients had ongoing grade I/II neutropenia one year after the last cycle of immunochemotherapy. Bone marrow smears weren't performed systematically in our patients (n=13).
Summary/Conclusions: In our retrospective study we describe late onset neutropenia in lymphoma patients treated with R+B. The incidence reported is 12,5% (11% for grade ≥3). Although we did not observe febrile neutropenia in those patients, late onset neutropenia directly impacts on the strategy of lymphoma treatment especially in FL patients receiving R maintenance. We cannot determine separately the impact of R or B for late onset neutropenia but it is hypothesized that the association of R+B increases late onset neutropenia when compared to each treatment alone. Exploratory sub-group analysis is under way. Considering those results, we suggest a close hematologic follow-up of patients treated with R+B immunochemotherapy in order to identify and treat late onset neutropenia.

PB1864

IS THERE A REAL INCRESED PREVALENCE OF HEPATITIS B REACTIVATION IN PATIENTS WITH CD20+INDOLENT NON HODGKIN LYMPHOMA DURING MAINTENANCE WITH RITUXIMAB?

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Background: Hepatitis B reactivation is a potentially serious complication of anti CD20 antibody (Rituximab) based chemotherapy regimens although sporadic HBV reactivation cases are reported in patients on maintenance with single therapy Rituximab.
Aims: The aim of this study is to assess the prevalence of HBV reactivation among patients HBsAg-/HBcAb+undergoing maintenance chemotherapy with Rituximab.
Methods: We have analyzed 148 patients treated for non Hodgkin's lymphoma according to standard maintenance therapy with Rituximab (375 mg/mq every 2 months for 2 years) from January 2007 to February 2016. Patients received different chemotherapy regimens during induction: 15.5% (23/148) with RCHOP, 27% (40/148) with R-FN, 22% (23/148) R-Bendamustine, 4% (6/148) with R-Fludarabine, 4% (6/148) with R-Leukeran, 19% (28/148)with R-CEOP, 2% (3/148) with R-CVP, 0.6% (1/148) with R-FC, 0.6% (1/148) with R-C, 1.2% (2/148) with R-FN and Bendamustine and 3% (5/148) with Rituximab monotherapy. We performed blood tests for HBV (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) and liver function tests in all patients at diagnosis and before each administration of maintence with Rituximab. Patients HBsAg-/HBcAb+with increased level of transaminases perfmormed HBV-DNA test. Six patients with HBsAg+received prophylactic therapy with antiviral drugs during induction and maintenance therapy.
Results: 69.6% of the patients (103/148) completed therapy with Rituximab and 30.4% of the patients (45/148) are still in maintenance therapy : 32% of the all patients (47/148) were HBcAb+positive. Only 4.2% (2/47) of these patients occurred HBV reactivation.
Summary/Conclusions: In clinical practice patients HBsAg-/HBcAB+treated with Rituximab in single therapy are normally considered for prophylaxis with lamivudine. In our experience HBsAg-/HBcAB+patients don't received therapy with antiviral drugs during maintenance therapy with Rituximab. Only two of our patients experienced HBV reactivation during maintence therapy. In terms of cost benefit analysis there is an advantage in the monitoring approach that was used in our patients in respect to universal prophylaxis, with a savings of about € 3.400,00 for each patient and a total cost of 159.000€ for all our patients. This study is the first study which analyzes HBV reactivation among HBcAb positive patients which underwent to maintenance therapy with Rituximab for indolent NHL.

PB1865

THE ABSOLUTE LYMPHOCYTE COUNT AS INDEPENDENT PARAMETER MAY PREDICT PROGNOSIS OF FOLLICULAR LYMPHOMA PATIENTS

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Background: The role of absolute lymphocyte count (ALC), absolute monocyte count (AMC) and lymphocyte to monocyte ratio (LMR) in follicular lymphoma (FL) as potential surrogate markers of gene expression profiling analysis has been investigated with consequently reported contradictory data.
Aims: The aim of this study was to evaluate the role of clinical and laboratory parameters, including ALC, AMC and LMR, on the overall survival (OS) and event free survival (EFS).
Methods: A total of 185 patients (106 females/79 males) with median age of 55 years (range 30-88 years) were analyzed. Majority of patients (87.6%) were in advanced Ann Arbor clinical stage (III-V), while 8.1% had leukemic phase of the disease. Bone marrow infiltration had 61.1% patients and histopathological grade 3 of disease had 25.4% of them. Low FLIPI (Follicular Lymphoma International Prognostic Index) had 22.2% of patients, intermediate 28.1% and high 44.3%. Pre-therapy median ALC was $1.83x10^9$/L (range 0.10-$123.9x10^9$/l), AMC $0.52x10^9$/l (range 0.06-$12.39x10^9$/l) and LMR 4.0 (range 0.04-$97.0x10^9$/l). Cut off values were set on $1.1x10^9$/l, $0.63x10^9$/l and $4.7x10^9$/l for ALC, AMC and LMR, respectively, according to the previously published data. All patients were treated with immunochemotherapy.
Results: Complete remission (CR) was achieved in 64.9% of patients, partial remission (PR) in 27.0% and 8.1% of patients had primary resistant disease. The disease relapse was verified in 39.5%. The following variables didn't influence neither OS nor EFS: gender, Ann Arbor disease stage, presence of B symptoms, bulky disease, and leukemic phase of disease. However, the FLIPI had significant impact on the OS (Log Rank=11.75, p=0.003) and EFS (Log Rank=9.14, p=0.01). Furthermore, the patients with lymphoma grade 1-3 had superior OS (Log Rank=10.70, p=0.001) and EFS (Log Rank=6.89, p=0.009) compared to the patients with grade 3. The patients with ALC≥$1.1x10^9$/l had better OS (Log Rank=4.135, p=0.042) and EFS (Log Rank=3.9, p=0.049) compared to those with lower ALC values. However, AMC and LMR were not in correlation with the outcome. Multivariate Cox regression analysis among FLIPI, lymphoma grade and ALC, has sorted over lymphoma grade as the most important parameter that influenced OS (HR=6.57, 95% CI 1.49-6.58, p=0.003) and EFS (HR=2.56, 95% CI 1.24-5.32, p=0.011) along with the FLIPI (OS, HR=1.50, 95% CI 1.09-2.05, p=0.012; EFS, HR=1.4, 95% CI 1.03-1.92, p=0.03).
Summary/Conclusions: In FL patients ALC, as individual parameter, markedly influenced survival. Moreover, in the rituximab era FLIPI still retains prognostic significance along with the histological grade of FL illustrating biologically different course of disease.

PB1866

PEGFILGRASTIM IN PRIMARY PROPHYLAXIS OF FEBRILE NEUTROPENIA DURING RITUXIMAB-BENDAMUSTINE TREATMENT IN INDOLENT NON HODGKIN LYMPHOMA : A REAL-LIFE EXPERIENCE

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Background: Febrile neutropenia (FN) is a serious side effect of chemotherapy, and even when it does not result in significant morbidity, mortality and costs, it normally leads to a delay in chemotherapy treatments.
Aims: Pegfilgrastim is a pegylated long-acting recombinant form of G-CSF which extends the half-life, requiring less frequent dosing. The objective was to evaluate the efficacy and safety of pegfilgrastim in newly diagnosed patients

with indolent NHL, in treatment with R-Bendamustine (RB), in order to determine whether a single injection of pegfilgrastim is as effective as daily injections of filgrastim, in terms of toxicity, febrile neutropenic episodes, antibiotic usage, and hospitalization duration.
Methods: 59 patients (31 M/28 F), median age 48.7 years (range 33-87), since first course of treatment performed blood counts twice weekly and received, d8-d19, prophylactic oral quinolones and anti-fungal drugs. During neutropenia, filgrastim (5 µg/kg/d for at least 3 days) was given "on demand" if neutrophils count was <1000x10^9 cells/L. Median number of filgrastim administrations was 3.4 (r.3-5); nadir neutropenia was registered after a median of 9.1 d. (r.8-15); median of nadir neutrophil count was 1.27x10^9 cells/L (range 0.3-1.7x10^9 cells/L), with maximum duration of 11 days. From the second course, all patients switched to pegfilgrastim (6 mg) prophylaxis, injected subcutaneously with a single administration on day+4.
Results: During pegfilgrastim, neutropenia was never longer than 7 days, with a consequent reduction of risk of infections. Median nadir neutrophil count, evaluated for at least 3 courses of therapy (r.3-6) registered at d+11, was 1.626 (range 0.88-2.11x10^9 cells/L); only 9 patients (15.2%) needed, after pegfilgrastim, a supplement of 3 administrations of filgrastim. During pegfilgrastim, neutropenia, when present, was shorter than during filgrastim treatment (median of 3.3 d, range 3-8). Pegfilgrastim was well tolerated: main side effects were mild fever and bone pain (8/59 : 13.5%). Moreover, no hospitalization was needed during pegfilgrastim, while two hospitalization for pneumonia were needed during filgrastim. During observation, no patient died during filgrastim or pegfilgrastim.
Summary/Conclusions: In patients affected by newly diagnosed patients indolent NHL, in treatment with RB, pegfilgrastim seems to reduce the incidence of neutropenia, is better tolerated and may increase the possibility to maintain the schedule of treatment.

PB1867

RISK OF HISTOLOGIC TRANSFORMATION (HT) IN SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) PATIENTS TREATED WITH RITUXIMAB

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Background: SMZL is an indolent lymphoma with long survival. The risk of HT is not well defined, ranging between <5% to >20% in various reports. Rituximab has shown significant activity in the treatment of SMZL. In the rituximab era there is no study assessing the incidence of HT in SMZL patients.
Aims: To analyze the incidence and risk factors for HT in a large series of SMZL patients homogeneously treated with rituximab.
Methods: The studied cohort included 89 SMZL patients diagnosed and treated between 2003 and 2015 exclusively with rituximab monotherapy as first line treatment. 41% were males with a median age of 65 years (range, 41-95). 6/89 (7%) presented with B-symptoms and 43% with elevated LDH. According to IPI, 31% were classified as intermediate high and high risk. No patient was HCV(+). The response rate to rituximab was 95%. HT was confirmed by detailed immunomorphologic study and defined by a biopsy-proven transformation of previous SMZL into an aggressive entity. Overall survival (OS) and progression free survival (PFS) were estimated by the Kaplan-Meier method.
Results: 7-year PFS and OS of the whole population were 69% and 87%, respectively. After a median follow-up of ….years, HT was observed in 4(4%) patients. HT occurred at a median interval of 40 months (range: 32-114) after diagnosis. The main features at diagnosis of the patients who experienced HT are summarized in the Table 1. Transformation into diffuse large B cell lymphoma (DLBCL) was documented in all patients. In one of them a "double hit lymphoma" was reported due to the presence of the rearrangement of c-MYC, and BCL2. In one case a coexisting low-grade component was present in the context of the same specimen.Risk of HT was ?% at 5 and 10 years after diagnosis. At the time of HT 2 patients had high LDH and one B-symptoms. No patient had splenomegaly, while BM was infiltrated by HT in one patient (Table 1). The site of transformation was the lymph nodes in all cases, and additionally BM in one case (the one with the double hit lymphoma). Immunohistochemistry showed positivity of CD30 in 3/4 cases. Treatment at the time of HT included RCHOP in 3 and R-Bendamustine in one case. Two patients achieved CR, one PR, while the fourth did not respond and is alive with progressive disease. At a median follow-up of 10 months (range, 5-13) after HT, 3 patients are alive, 2 in CR, 1 in PR and one with PD.
Summary/Conclusions: To our knowledge, this is one of the largest series of consecutive SMZL patients treated homogeneously with rituximab monotherapy, evaluating the incidence and risk factors for HT. HT occurred in 4% of the cases as a relatively early event during disease course. Post-transformation survival cannot be safely assessed due to short follow up after HT.

Table 1. Main features at diagnosis and at the time of HT in the four patients who were transformed in DLBCL.

Features	Case #1	Case #2	Case #3	Case #4
AT DIAGNOSIS				
SEX	Female	Female	Man	Man
Age	53	72	65	65
Spleen (cm)	22	17	18	22
Lymphadenopathy	No	No	Yes	No
Hb (gr/dl)	9.1	11	10.8	13.3
PLTs (/µL)	190.000	286.000	97.000	160.000
ALCs (/µL)	21.000	1500	14.100	14.000
LDH elevated	No	Yes	Yes	No
Paraproteinemia	No	No	IgMλ	No
Prognostic group	B	B	B	A
% BM infiltration	10	10	60	50
Response to rituximab	CR	CRu	CR	SD
AT HISTOLOGIC TRANSFORMATION				
Site of HT	Axillary LN	Axillary, mediastinal, intraabdominal LN	Intraabdominal LN	Generalized lymphadenopathy and BM
Histology	SMZL+ DLBCL	DLBCL	DLBCL	Double hit DLBCL
Immunohisto-chemi-stry	CD30, Bcl2, bcl6, MUM-1 (+)	CD30, bcl-2,bcl-6, MUM-1(+)	CD30, bcl-2,bcl-6, MUM-1(+)	BCL-2 (+) c-MYC (+)
Stage	IA	IIIB	IIA	IVA
Time to HT (months)	35	32	46	114
Hb (gr/dl)	15	8.6	11	13.6
ALCs x10^9/l	1.65	1,2	1,575	24,1
PLTs x 10^9/l	218.000	568.000	179.000	182.000
Elevated LDH	No	No	Yes	Yes
Paraproteinemia	No	No	IgMλ	No
Treatment	RCHOP	R-BENDAMUSTINE	RCHOP	RCHOP
Response	CR	?	CR	PD
Follow-up (months)	48	43	54	119
Follow-up after HT (months)	13	11	8	5
Curent Status	Alive in CR	Alive with disease	Alive in CR	Alive with disease

PB1868

OUTCOMES OF INDOLENT LYMPHOMAS (INHL) IN THE ELDERLY - AN OBSERVATIONAL STUDY OF KROHEM, THE CROATIAN COOPERATIVE GROUP FOR HEMATOLOGICAL DISEASES

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Background: New treatment approaches have improved outcomes in patients with indolent NHL. However, only limited data exist on outcomes of elderly patients.
Aims: We performed this observational study to analyse disease characteristics, treatment patterns, outcomes and prognosis of elderly patients with indolent B-NHL.
Methods: Data were generated from a country-wide observational study of patients treated with rituximab in 2007 and 2008 and from the KroHem Lymphoma registry. Only those receiving front-line treatment were included; watch-and-wait patients were excluded.
Results: We identified 172 patients older than 60 years. Median age was 72, the oldest treated patient was 95 years old; 58% were women. 82 patients had follicular lymphoma (FL), 59 marginal zone lymphomas (MZL), 16 small lymphocytic lymphoma (SLL) and 15 lymphoplasmacytic lymphoma (LPL). Median follow-up of the whole cohort was 44 months. Most patients received R-CHOP (45%) or R-CVP (39%). Selected patients with stage 1 disease were treated with radiotherapy only and those with splenic lymphomas with surgery. Only 22 received rituximab maintenance. 5-year overall survival (OS) was 61% and progression-free survival (PFS) 46%. Extranodal MZL had best OS and PFS (72% and 66% at 5y respectively). FL had better OS but not PFS (64% and 42% at 5y respectively) than non-FL iNHL. Nodal MZL, SLL and LPL had similar outcomes (OS at 5y 53%, 48% and 47%; PFS at 5y 44%, 38% and 33%, respectively). Best outcomes were seen in the group of patients amenable to local therapy: surgery or irradiation. R-CHOP did not improve OS in comparison

to R-CVP but there was a trend to better PFS, especially in patients with FL (p=0.07). When compared to younger patients registered in the observational study, patients older than 60 had somewhat inferior OS and PFS, but the difference in outcomes between those younger and older than 75 was striking (p<0.0001) (Figure 1). All types of deaths were more frequent in the very elderly, but deaths during treatment especially so; 22% of deaths in the group between 60 and 75 occurred during front-line treatment in comparison to 37% in those above 75. In contrast, 58% of deaths in those between 60 and 75 occurred after relapse in comparison to only 37% in the older group.

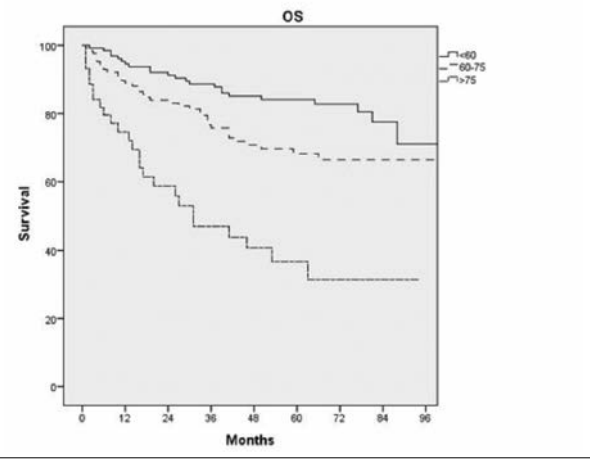

Figure 1.

Summary/Conclusions: It seems that the prognostic influence of iNHL types has remained unchanged from the pre-rituximab era with extranodal MZL having the best prognosis, followed by FL. Patients with stage one disease have an excellent outcome. The impact of anthracyclines seems limited. Currently used regimens are too toxic or not effective enough in patients older than 75. In this group of patients new treatment approaches are needed that would reduce mortality during front-line treatment without compromising efficacy.

PB1869

RESPONSE ASSESSMENT USING IWG2007 AND DEAUVILLE CRITERIA IN B-CELL NON-HODGKIN LYMPHOMA PATIENTS TREATED WITH 90Y-IT

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Background: 90Y-Ibritumomab-tiuxetan (90Y-IT) has become an efficient alternative therapy in non-Hodgkin Lymphoma (NHL). The staging and response assessment have changed and PET/CT scan with Deauville/Lugano criteria have become the recommended tool.
Aims: To analyse our updated information of patients treated with 90YIbritumomab/tiuxetan in our institution comparing the IWG2007 Chenson and Lugano/Deauville criteria to analyse treatment outcome.
Methods: 120 NHL patients were included in a clinical protocol conducted by a multidisciplinary team and treated in the same centre. For this sub study 54 patients have been included, all of them followed by more than 12 months and the response assessed with PET/CT scan after 12 weeks out therapy using the same machine, the images were available for re-assessment retrospectively according Deauville/Lugano criteria. Endpoints: objective response rate (ORR), time to relapse (PFS) overall survival (OS).
Results: Until August 2015, 56 patients had receive therapy with 90Y-IT and completed at least 1 year of follow-up were considered to analysis; M/F 29/27 mean age 59.7 years (31-86); ECOG 0-1 94.4%. According WHO classification: follicular-NHL 44 (78.6%), mantle cell-NHL 2 (3.6%), DLCB-NHL 7 (12.5%) and MALT-NHL 3 (5.4%). Score risk distribution: low 19 (33.9%), intermediate 24 (42.9%) and advanced 13 (23.2%). 54.5% of patients were stage IV. The majority of patients received ≤2 (90.7%) previous therapy schedules and >2 (9.3%). The mean follow-up time: 53.1 months (95% CI: 12; 145). 31 (57.4%) patients received 90Y-IT as consolidation of first line therapy and 23 relapsed/refractory (42.6%). According IWG2007 criteria: ORR was 98.2% CR: 50 (92.6%); PR 3 (5.4%) and 1 failure (1.9%) in relapsed/refractory disease. According Lugano following the recommendation of Deauville criteria about the presence of residual mass and the scale of 5 points, the response were: CR (1-2 scores or 3 scores with a reduction respect the baseline and no presence of residual disease) 46 (85.2%), presence of residual disease (scores 4-5 or mass with score 3 without a reduction respect baseline): 8 (14.8%). During follow-up 13 patients (24.1%) had relapsed, 10 were categorized as CR by IWG2007, 9 CR by Lugano and 7 CR by Deauville with a higher negative predictive value. The mean PFS: 109.6 months (95% CI: 94.8; 124.4) median NR. However for patients classified as CR by Deauville, the mean PFS was 117.1 month (102.2-132.0), median NR, and 40.7 (16.4-65.2), median 16 (6.2-25.7) months for the rest, (Figure 1). During follow up eight patients have dead, 5 of them after 55 or more months after 90Y-IT therapy. Dead were related to disease in 7 patients.

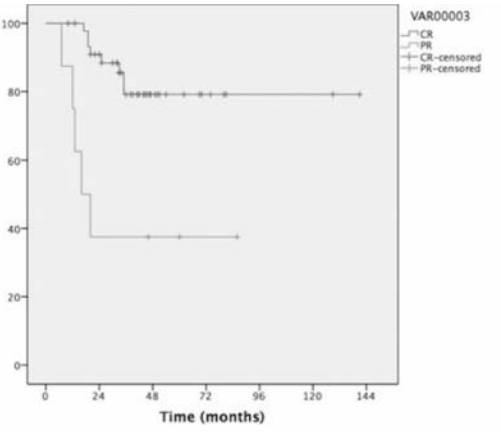

Figure 1. PFS according deauville classification after 90Y-IT therapy.

Summary/Conclusions: In our series of NHL patients treated with 90Y Ibritumomab tiuxetan, the re-assessment of response by PET/CT scan according Deauville criteria at the end of therapy shows more restrictive for CR when scale 3 combining with residual mass is considering as residual disease, improving the accuracy of CR in 7.4% and prolonged PFS in 7.5 months, respect IWG2007.

PB1870

SHOULD STAGE I AND II FOLLICULAR LYMPHOMA BE TREATED EVEN IN THE RITUXIMAB ERA?

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Background: The optimal management of stage I and II follicular lymphoma (FL), according to consensus guidelines, is not well defined, there are heterogeneous criteria to choose the option of treatment and the decision used to be based on uncontrolled experiences. Diverse treatment approaches are used because the most of guidelines recommended a broad spectrum of alternatives that includes from observation to radiotherapy or chemotherapy, without clear indications to choose one. It is still widely accepted to observe advanced stage FL in case of asymptomatic and stable disease, nevertheless, this watch-and-wait policy is not too well established in early stages. The clinical outcome of patients with follicular lymphoma has been improved since the introduction of rituximab, nevertheless the guidelines still recommend the radiotherapy as a first option.

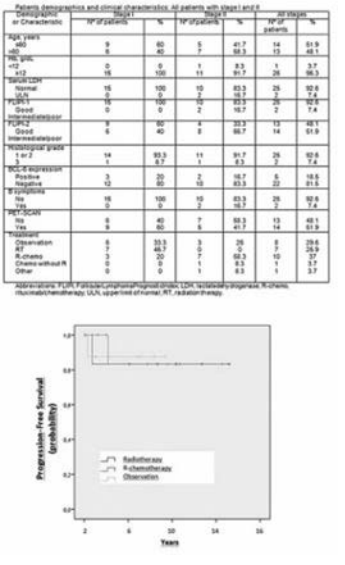

Figure 1.

Aims: This study was launched to evaluate the time to relapse with the three more common approaches in the Rituximab era.
Methods: All patients with histologically confirmed diagnosis of follicular lymphomas grade I-II were selected from our data base starting from January 2000 to June 2015.
Results: From January 2000 to June 2015, 179 patients were diagnosed of follicular lymphoma in our Institution. 35 patients were staged as I and II, 5 patients were excluded from the analysis because of not rigorous staging as defined by bone marrow biopsy and an imaging study either with computed tomography [CT] scan of the whole body or a positron emission tomography [PET]/CT scan, and 3 patients were excluded because of bulky stage II. The median age at diagnosis was 58 years (range 29-78). PET/CT was done in 52% patients. Treatments given to staged patients were rituximab/chemotherapy (R-chemo; 37%) that includes regimes such as R-CHOP (cyclophosphamide [C], doxorubicin, vincristine [V] and prednisone [P]) R-CVP, R-FC (fludarabine [F], cyclophosphamide), chemotherapy without immunotherapy (3.7%), RT (25.9%), observation (29.6%), and other (3.7%). With a median follow-up of 70 months for PFS, there were 3 progression events (11,11% of patients). PFS was statistically significant improved with observation compared with patients treated either receiving R-QT or RT (p=0.042). There were nostatistical differences in PFS according to FLIPI 1 or 2 score, histological grade or BCL-6 expression in univariate analysis. There were not differences in overall survival (Figure 1).
Summary/Conclusions: This retrospective monocentric study suggest that observation could be a valid approach for patients of FL stage I and not bulky II, not only for stage III or IV. PFS did not improve with treatment, even with rituximab-containing chemotherapy regimens. It is necessary prospective and randomized studies in these cases to get stronger conclusions.

PB1871

18FDG-PET ROLE OF IN THE EVALUATION OF BONE MARROW INFILTRATION IN PATIENTS WITH NHL

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Background: ^{18}FDG PET/CT is now the standard procedure to evaluate indolent and non indolent non-Hodgkin lymphoma (NHL). It plays an important role in staging, restaging, prognosis and planning treatment. On the other hand bone marrow infiltration affects stage, prognosis and therapeutic approach
Aims: To compare three basal ^{18}FDG PET/CT parameters to detect bone marrow infiltration in patients with newly diagnosed non Hodgkin lymphoma (NHL).
Methods: 25 patients with newly diagnosed NHL from February 2012 to February 2016 were retrospectively analyzed. All patients received diagnosis both ^{18}FDG PET/CT contrast enhanced scan and bone marrow biopsy in the posterior iliac crest. There were three evaluation parameters of ^{18}FDG PET/CT to detect bone marrow infiltration: visual analysis, maximal standardized uptake values (SUVmax) in bone marrow of iliac crest (>2.5) and ratio of maximal standardized uptake values of iliac crest bone marrow to liver intensity (Deauville score positive when ratio >1). All results were compared with the bone marrow biopsy. Twelve patients (48%) had an aggressive NHL (9 DLBCL, 1 Burkitt Lymphoma, 1 T cell lymphoma, 1 mantle cell lymphoma,); 13 (52%) had indolent NHL (7 follicular lymphoma, 2 marginal zone lymphoma, 4 small lymphocytic lymphoma)
Results: We divided patients in two different groups: indolent and aggressive NHL. For each group we analyzed the accuracy, sensitivity and specificity of the three parameters (visual analysis, SUVmax and Deauville score). Aggressive NHL: Accuracy, sensitivity and specificity accord to visual analysis were 60%, 75% and 25%, respectively. Accuracy, sensitivity and specificity accord to SUVmax were 50%, 71% and 20%, respectively. Accuracy, sensitivity and specificity according to Deauville score were75%, 89% and 33%, respectively Indolent NHL. Accuracy, sensitivity and specificity accord to visual analysis were 53%, 14% and 100%, respectively. Accuracy, sensitivity and specificity accord to SUV max were 46%, 43% and 50%, respectively. Accuracy, sensitivity and specificity according to Deauville score were77%, 57% and 100%, respectively. Taking into consideration the aggressive NHL group the three parameters compared to standard bone marrow biopsy point to high sensitivity and low specificity. In indolent group the three parameters point to low sensitivity and high specificity. In particular, in this later group, the presence of 4 CLL patients (31%) commonly not FDG avid could have reduced the sensitivity of the imaging procedure compared to bone marrow. According to preliminary observation Deauville score seems to be more accurate in evaluation of bone marrow infiltration both in indolent and in aggressive NHL patients.
Summary/Conclusions: Although obtained in a small group of patients, our results show a significant correlation between bone marrow infiltration verified by biopsy procedures and peculiar aspects of bone marrow FDG uptake, in particular when they are analyzed by the Deauville score.

PB1872

SUCCESSFUL SWITCH FROM INTRAVENOUS TO SUBCUTANEOUS RITUXIMAB AT THE ULSTER HOSPITAL, BELFAST, NORTHERN IRELAND

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Background: Chemo-Immunotherapy is currently widely used in treating lymphoproliferative disease. There is ever increasing indications for the monoclonal antibody, rituximab, with varied chemotherapy regimens. In our chemotherapy day unit there has been a significant increase in patients requiring chemo-immunotherapy. This increase has led to increasing waiting times for patients to start their chemotherapy. In 2005, a group at the Ulster Hospital completed a project examining the use of rapid intravenous (IV) rituximab infusion over 90 minutes as opposed to standard 4 ½ hours administration at this time, for continuing therapy as long as the first infusion had been tolerated without any reaction (El-Agnaf, *et al.*, 2007). With the availability of subcutaneous (SC) rituximab, the group decided to switch to this product for patients with DLBCL and Follicular Lymphoma being treated with a combination of rituximab and chemotherapy.
Aims: - Investigate the safety profile of SC Rituximab in treatment of DLBCL/Follicular lymphoma. - Impact of SC Rituximab on the resource utilisation within our department. - Convenience of adminstration of SC Rituximab for both patients and nursing staff.
Methods: We examined the notes of all patients who received subcutaneous Rituximab either in combination with CHOP, CVP or alone as maintenance treatment. The first dose of rituximab was given IV as per the product monograph. The second and subsequent doses for eligible patients were given over 5-6 minutes SC at a fixed dose of 1400 mg. All eligible patients received standard oral premedication of prednisolone, chlorphenamine and paracetamol. Patients were monitored closely and any adverse events recorded using National Cancer Institute (NCI) common terminology criteria for adverse events. Vital signs (Blood Pressure (BP), temperature, pulse, digital O2 saturation and respiratory rate) were checked before the first Rituximab infusion and 15 minutes after the infusion commenced. If normal at these time points, they were then checked 30 minutes later and at infusion end. For SC rituximab, vital signs were measured prior to first injection, and one hour post administration. If the patient had no adverse reaction to the first SC injection, subsequent SC therapy was given with vital signs monitored prior to injection only. A sample of patients were contacted by telephone and asked about their treatment preference. The nursing staff were also asked about their preference.
Results: There were no reported adverse reactions to the first infusion in all patients. SC rituximab was well tolerated apart from mild local skin reactions. None of these reactions requires specific therapy and did not preclude further SC treatments. The service change from IV to SC was smooth with no issues noted by staff involved. In total 29 patients (7DLBCL and 22 Follicular lymphoma) received 135 vials of 1400mg SC rituximab from 1st January 2015 until 31st December 2015. If all 29 patients had rapid IV infusion over 90mins, they would have taken 202hours 30mins (if each infusion went smoothly). Our SC rituximab patient sample took 37hours 30mins to infuse (allowing 10mins for injecting the SC rituximab and 1hour observation post 1st SC rituximab). This equated to a minimum saving of 165 nursing hours. Our pharmacy takes 31mins to manufacture IV rituximab as opposed to 7mins for SC rituximab. In 2015 we saved our pharmacy a minimum of 55hours. Patients uniformly preferred SC Rituximab over IV, as did the nursing staff.
Summary/Conclusions: A number of studies (Salar, *et al.*, 2014) (Davies, *et al.*, 2014) showed that there was non inferiority of SC rituximab (1400 mg) compared with IV rituximab (375 mg/m^2) for treatment outcomes and that the only safety considerations relates to a higher incidence of administration related reactions. Overall response rate and complete response rate indicate that switching to SC rituximab has no effect on anti-lymphoma activity, but decreases overall administration time to 5-6 minutes without significantly affecting patient safety, and is acceptable to patients. Switching from IV to SC Rituximab has had a significant impact on the time spent for the patient in the unit, the time pharmacy saved in Rituximab preparation and storage of the product and the time saved by nursing staff administering the drug.

PB1873

THERAPY AND LONG-TERM RESULTS OF HEPATITIS C-ASSOCIATED INDOLENT NON-HODGKIN LYMPHOMA

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Background: The incidence of chronic hepatitis C (HCV) in patients with indolent B-cell non-Hodgkin's lymphoma (IL) is approximately 15%. In those cases where the tumor cell NHL patients were in the presence of hepatitis C virus protein by immunohistochemistry (IHC), we define diagnosis of hepatitis C associated indolent lymphoma (IL+C).

Aims: Since 2004 to 2014 81 patients were observed with IL+C. The median age 50 years, the disease is equally common in men and women. Almost all patients (97%) were determined III-IV st of the disease. In 25% of patients were primary extranodal variants IL+C . Most often determined by the follicular lymphoma, 74%, 32% - cell lymphoma marginal zone. HCV RNA was determined in 82% of patients in the blood, the median viral load was $2.3x10^5$ copies/mL. Increased ALT and LDH was diagnosed in 71% and 82%, respectively.
Methods: As a first-line therapy of 52 patients was only antiviral treatment by interferon and ribaverin . If tumor remission was achieved, the antiviral therapy lasted for 2 years. 29 patients as a first line therapy was immunochemotherapy (R-CHOP, R-CVP).
Results: Complete and partial remission of antiviral therapy were obtained in 88% of patients, on immunochemotherapy - in 64% of patients. Median progression-free survival in patients with IL+C treated with antiviral treatment was 42 months, during immunochemotherapy - 19 months (p=0.00001). Five-year overall survival was 67% and 32%, respectively (p=0.0003). After immunohimoterapy in 19 patients was the relapse of the disease . All this patients was treated by antiviral therapy as second-line therapy . Complete and partial remission was achieved in 92% of patients. Median progression-free survival in these patients was 31 months.
Summary/Conclusions: Antiviral therapy in first-line and relapse of disease better then immunochemotherapy in the overall effectiveness, of the disease-free survival and overall survival in patients of NHL associated with hepatitis C. Antiviral therapy is a priority for patients with hepatitis C-associated lymphoma.

PB1874

DURABLE RESPONSES WITH IDELALISIB MONOTHERAPY IN PATIENTS WITH RELAPSED OR REFRACTORY SMALL LYMPHOCYTIC LYMPHOMA (SLL)

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Background: SLL, an indolent non-Hodgkin lymphoma (iNHL), is often diagnosed at an advanced stage, and patients (pts) with relapsed or refractory (R/R) disease have a poor prognosis.Idelalisib (IDELA), is a selective, oral PI3Kδ inhibitor which as monotherapy (IDELA-mono) showed considerable anti-tumor activity in pts with R/R iNHL in phase 1 (Study 02; NCT00710528) and phase 2 studies (Study 09; NCT01282424; Gopal *et al. NEJM*. 2014;370:1008-18).
Aims: The objective of this post hoc analysis was to describe the efficacy and safety of IDELA-mono specifically in pts with R/R SLL enrolled in Study 02 or Study 09.
Methods: Eligible pts with previously-treated SLL were those with R/R disease (disease status defined by response to last prior regimen) treated with IDELA-mono across 7 dose cohorts (minimum dose 50 mg BID; maximum dose 350 mg BID) in Study 02, and those with double-refractory disease (to both rituximab and an alkylating agent) treated with IDELA-mono (150 mg BID) in Study 09. In both studies, IDELA was continued until progressive disease (PD) or unacceptable toxicity. Radiographic and pertinent clinical data were reviewed by an independent review committee (IRC) to determine response based on standard criteria for lymphomas (Cheson *et al.* J *Clin Oncol*. 2007;25:579-86).
Results: A total of 39 pts with R/R SLL participated in the 2 trials, 11 in Study 02, (5 on IDELA doses <100 mg BID; 6 on IDELA doses >100 mg BID), and 28 in Study 09 (IDELA 150 mg BID). The median age of pts was 69 and 65 yrs [range 34-87], and 73% and 75% were male, in Studies 02 and 09, respectively. In both studies, 82% of pts had Ann Arbor Stage IV disease at baseline and had received a median of 4 prior regimens [range 1-9]. In Study 02, 9 of the 11 pts (82%) had refractory disease; all 11 pts had received prior regimens containing fludarabine. In Study 09, 27/28 pts (96%) were refractory to both rituximab and an alkylating agent (1/28 pts had received insufficient cycles of bendamustine). Common prior therapies included: rituximab (n=28), cyclophosphamide (n=25), bendamustine (n=21), vincristine (n=19), and fludarabine (n=18). The overall response rate (ORR) was 6/11(55%) in Study 02, with all 6 responders achieving a partial response (PR) and 3/11 pts (27%) having stable disease (SD). In Study 09, the ORR was 17/28 (61%), with a complete response (CR) reported in 1/28 pts (4%), a PR in 16/28 pts (57%) and SD in 10/28 pts (36%); 1 pt (3.6%) was not evaluable. Among responders, median time to response was 0.9 mo and 1.9 mo, respectively; median duration of response (DOR) was 2.3 mo and 12.5 mo. Median progression-free survival (PFS) was 3.7 mo (Study 02) and 11.4 mo (Study 09). Median (KM-estimated OS was not reached for Study 02; OS was 88.9% at 12 mo, 88.9% at 24 mo and 59.3% at 36 mo. Median KM-estimated OS was 22.5 mo in Study 09; OS was 89.1% at 6 mo, 74.3% at 9 mo and 70.4% at 48 mo. In Study 02, grade ≥3 AEs and laboratory abnormalities occurring in ≥20% of pts included pneumonia (n=5), febrile neutropenia (n=3), and neutropenia (n=4). In Study 09, these AEs and laboratory abnormalities included pneumonia (n=7) and neutropenia (n=8).
Summary/Conclusions: IDELA-monotherapy provides clinical evidence of efficacy in pts with R/R SLL, with a safety profile similar to that described in other larger clinical trials.

PB1875

INITIAL TREATMENT RESPONSE PREDICTS THE OUTCOME OF ELDERLY FOLLICULAR LYMPHOMA PATIENTS TREATED WITH IMMUNOCHEMOTHERAPY

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Background: Advanced age is considered as an unfavorable prognostic factor for follicular lymphoma (FL). The optimal treatment approach for elderly FL patients is still not clearly defined.
Aims: The aim of this study was to compare the outcome of FL patients between by age (50-60 years and more than 60 years) two adjacent patients groups treated with immunochemotherapy. The second aim was to identify high risk patients for poor outcome.
Methods: A retrospective study was performed on 101 FL patients older than 50 years who had to be treated, including the patients who were previously on watch and wait. Forty-eight patients were older than 60 years. All the patients were treated with immunochemotherapy R-CHOP or R-CVP in the period 2002-2014. Totally 10 patients received R-CVP, of whom 5 were older than 60 years. The examined parameters in statistical analysis were gender, FLIPI score, the presence of B symptoms, bulky disease, bone marrow involvement, ECOG PS, FL grade and response (complete remission or not) on initial treatment.
Results: The median age of the patients was 58 (range 51-82). Complete remission (CR) was achieved in 65 (65.3%) patients. None of the examined parameters was more often present on diagnosis in elderly patients compared to patients 50-60 years old. Patients older than 60 yrs had significantly shorter overall survival (OS) compared to patients 50-60 years old (log rank=4.855, $p<0.05$), while there was no difference in event free survival (log rank=0.015, $p>0.05$). Elderly patients with ECOG PS >1, as well as the patients who didn't achieve CR had significantly worse OS (log rank=4.892, $p<0.05$; log rank=33.980, $p<0.01$; respectively), while none of the examined parameters had an influence on EFS. In patients 50-60 years old, the patients who didn't achieve CR had significantly worse EFS (log rank 13.977, $p<0.01$), while none of the examined parameters had an influence on OS. The multivariate Cox regression analysis identified treatment response as an independent prognostic factor for OS in elderly patients and for EFS in patients 50-60 years old.
Summary/Conclusions: The patients older than 60 years with FL had worse outcome when compared to 50-60 years old patients. According to our results, achieving CR on initial treatment should be one of the goals in treating elderly FL patients.

PB1876

DURABLE RESPONSES WITH IDELALISIB MONOTHERAPY IN PATIENTS (PTS) WITH RELAPSED OR REFRACTORY MARGINAL ZONE LYMPHOMA (MZL)

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Background: MZL is a group of indolent non-Hodgkin lymphomas (iNHL) arising from marginal zone B cells in lymph nodes and/or extranodal tissues, is classified per the World Health Organization as splenic MZL (SMZL), nodal MZL (NMZL) and extranodal marginal zone B-cell lymphoma (mucosa-associated lymphoid tissue; MALT). Given the rarity of MZL, few randomized trials have directly compared treatment options. Thus, there is a lack of consensus about best practices. Idelalisib (IDELA) showed considerable anti-tumor activity in pts with relapsed/refractory (R/R) iNHL in a phase 1 (P1) dose-escalation study (NCT00710528; Flinn, 2014) and in pts with refractory iNHL in a phase 2 (P2) study (NCT01282424; Gopal, 2014).

Aims: The objective of this post hoc analysis was to describe the safety and efficacy of IDELA in a subset of pts with MZL.
Methods: The P1 and P2 studies were single-arm monotherapy trials that assessed safety and efficacy of idelalisib in R/R hematologic malignancies (P1) or iNHL (P2). Eligible pts with MALT, NMZL or SMZL were those with R/R disease (disease status defined by response to last prior regimen) in the P1 study and those with double-refractory disease (to both rituximab and an alkylating agent) in the P2 study. IDELA was given as monotherapy (P1: minimum dose 50 mg BID, maximum dose 350 mg BID; P2: 150 mg BID) until progressive disease (PD), death or unacceptable toxicity. Radiographic and pertinent clinical data were reviewed by an independent review committee (IRC) to determine the response based on standard criteria for lymphomas (Cheson *et al. J Clin Oncol.* 2007;25;579-586).
Results: In all, 21 pts with MZL were enrolled, 6 in the P1 and 15 in the P2 study: 12 with MALT, 7 with NMZL and 2 with SMZL. Median age was 74 and 72 yrs [range 50-91], and 50% and 80% were male, in the P1 and P2 studies, respectively. Pts had received a median of 4.5 prior regimens in the P1 study [range 1-10] and a median of 2 prior regimens in the P2 study [range 2-9]. The median (range) IDELA exposure was 5 mo (0.4-16.4) and 6.4 mo (1.8-21.6) in the P1 and P2 studies, respectively. In the P1 study, Gr≥3 adverse events (AEs) occurring in ≥20% of pts included diarrhea (3/6), and chills, fatigue, pyrexia, urinary tract infection and dizziness (each 2/6); key Gr ≥3 laboratory abnormalities occurring in ≥20% of pts included thrombocytopenia (4/6), and anemia, neutropenia, and ALT and AST increases (each 2/6). In the P2 study, Gr≥3 diarrhea and Gr≥3 neutropenia laboratory abnormalities each occurred in 3/15 pts. Of the 21 pts, 9 (43%) achieved a response. Outcomes by MZL subtype are shown in the Table 1. Median times to first response were 1 mo and 3.5 mo in the P1 and P2 studies, respectively. In the P1 study, the median duration of response (DOR) was 8.2 mo, and in the P2 study 18.4 mo. In the P1 study, with median follow-up time of 5 mo, median progression free survival (PFS) was 7.4 mo; in the P2 study, with median follow-up time of 20 mo, median PFS was 6.6 mo. Overall Survival (OS) was 100% at 1 yr in the P1 study, and in the P2 study was 86% at 6 mo, 79% at 9 mo, and 72% at 1 yr.

Table 1.

Best Overall Response, n	CR	PR	SD	PD
MALT	1	4	6	1
NMZL	0	2	4	1
SMZL	0	2	0	0

Summary/Conclusions: Although the sample size was small, IDELA showed promising activity in pts with R/R MZL. IDELA was well tolerated with no apparent disease-specific safety signals.

PB1877

THE STRUCTURE AND EFFICACY OF B-CELL LYMPHOMA'S REAL LIFE TREATMENT ACCORDING DATA OF THE REGIONAL SEGMENT OF THE RUSSIAN FEDERATION'S FEDERAL REGISTER

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Background: The Russian federal program of "7 nosology" since 2008 has given the chance of stable treatment CD20+B cell lymphoma patients with rituximab, B cell chronic lymphoid leukemia patients with rituximab and fludarabin and multiple myeloma (MM) patients with bortezomib in combination with conventional chemotherapy. The obtaining medications provide patient's inclusion in the federal register and exception from the register in case of death and other actions.
Aims: To analyze the federal register's regional segment of the of patients with B-cell mature lymphomas, residents of the Nizhny Novgorod Region of RF (Population of the Nizhny Novgorod Region in 2008 of-3359,8 thousand and in 2015 – 3164,2 thousand people) for receiving demographic data and efficacy of real life treatment with using rituximab without or with fludarabin and conventional chemotherapy on the basis of overall survival (OS) by Kaplana-Meyer's method.
Methods: Materials and methods: from Jan 2008 to Dec 2015 2094 patients are allocated, the analysis has included 2051(98%) with diagnoses: C82 Follicular lymphoma – 103 (5%), C83.0 – Lymphoma from small lymphocytes, Nodal marginal zone lymphoma, Lymphoplasmacytic lymphoma (excepting Waldenstrom macroglobulinemia) – 183 (9%), C83.1 – Mantle cell lymphoma -46 (2%), C83.3 – Diffuse large B-cell lymphoma (all variants) – 532 (26%), C91.1 – Chronic lymphocytic leukemia – 578 (28%), C90 – multiple myeloma - 609 (30%). Date of death is determined in 713 (35%) cases, other censored for date of the last data in the register. The OS of patients is defined from the date of inclusion in the program of 7 nosology. In the groups the OS is defined depending on a sex, age group with the step in 10 years, of a year of inclusion in the program of 7 nosology. To analyze the Excel and Statistica 9 software are used.
Results: The probability of OS from the moment of inclusion in the register within 12 months for patients with C82 was 87%, C83.0 - 84%, C83.1 - 67%, C83.3 - 77%, C91.1 - 86%. The probability of OS for patients with C82 till 96 months is 72%, C 83.0 till 93 months – 29%, C 83.1 till 34 months – 59%, C 83.3 till 96 months - 61%, C 91.1 till 96 months - 45%, (Chi-square=23,2, p=0,0001). In groups except C91.1 women prevailed, distinctions of OS according sex aren't received. Only in the group C 91.1 men (315) prevailed (women 154), the median of OS for women isn't reached, for men – 62 months (p <0,003). Depending on age with a step in 10 years in all groups distinctions in OS aren't received. The maximum number of patients with the diagnosis C82, C83.1, C83.3, C91.1 were in age 60-69 years, with the diagnosis C83.0 – in age 50-59 years. Only patients with the diagnosis C83.3 are registered in all age groups. During 2009-2015 the number of the patients included in the register in a year with diagnoses of C82 has made from 7 to 24, on average 13; C83.3 – from 32 to 96, on average 68, C91.1 – from 30 to 83, on average 63; C83.0 – from 16 to 27, on average 21, C 83.1 from 1 to 15, on average 9. In each group of distinctions of survival depending on a year of inclusion in the program of 7 nosology it isn't received (Figure 1).

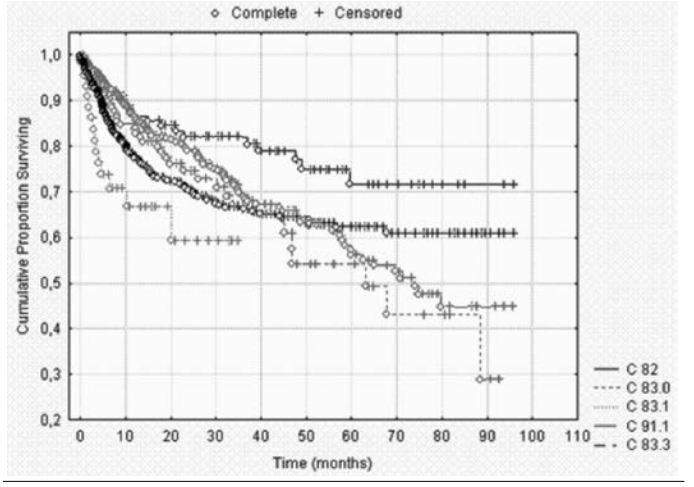

Figure 1. Overall surviving patients with mature B-cell lymphomas (Kaplan-Meier).

Summary/Conclusions: The rituximab for all CD20+lymphomas and fludarabin+rituximab for BCLL improve the outcome patients in real life clinical practice. The register interacted to the mechanism of the provision of medication is the effective resource allowing to have demographic data and data on efficacy treatment in real life medical practice.

PB1878

DETECTION OF MYD88 (L265P) SOMATIC MUTATION IN SPLENIC MARGINAL ZONE LYMPHOMA (SMZL)

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Background: MYD88 L265P is a somatic mutation that has been identified in about 90% of Waldenstrom macroglobulinemia (WM)/ lymphoplasmacytic lymphomas (LPLs). Several reports demonstrated the presence of the mutation in other lymphomas mainly DLBCL of activated type and SMZL. In SMZL, the frequency of MYD-88 mutation ranges between 0-21% in various studies.
Aims: The aim of the present study was to determine the presence of L265P MYD-88 mutation in a well characterized series of SMZL cases along with an extended analysis of the clinical, morphologic, immunophenotypic and histologic features.
Methods: We retrospectively evaluated 37 SMZL cases. The identification of MYD 88 L265P mutation was performed in blood or BM mononuclear cells with allele specific PCR. The sensitivity of the method was 10^{-3}.
Results: The main clinical and laboratory characteristics are shown in the Table 1. 11/37 (30%) were males with a median age of 62 years. All cases presented with splenomegaly and one with B-symptoms. Lymphadenopathy was evident in 7 cases (19%). Bone marrow (BM) was infiltrated in all cases, with a mixed (nodular and intrasinusoidal) pattern of infiltration in most cases. Paraproteinemia was present in 9/29 (31%), 6 of the IgG and 3 of the IgM type.None of the studied cases had positive serology for the hepatitis C virus. Anemia and thrombocytopenia were found in 28% and 8%, respectively. MYD-88 L265P mutation was detected in one case (1/37, 3%). This was a case of a 62-year old man with IgMκ paraproteinemia of low level (550mg/dl), lymphocytosis

(8000/μL), no anemia, mild to moderate splenomegaly (max 15.6 cm), small hilar lymphadenopathy and 60% BM infiltration with a nodular and intrasinusoidal pattern. At the time of CR documentation, disease was not detectable including the disappearance of the IgMκ monoclonal fraction in the blood, except the presence of the MYD-88 mutation in the BM mononuclear cells which in the same specimen was IgVH negative. The other two patients with an IgM monoclonal band were negative for the MYD-88 mutation, as were those with IgG monoclonal gammopathy.

Table 1. Characteristics of the SMZL cases studied for the presence of MYD-88 L265P mutation.

Feature	# (%)
Total #	37
Male sex	11 (30)
Age (median)	62
(range)	(49-85)
B-symptoms	1 (3)
Splenomegaly	37 (100)
Lymphadenopathy	7 (19)
IPI	
Low	11/33 (33)
Low/intermediate	13/33 (39)
High/intermediate	9/33 (27)
HB<10gr/dl	10/35 (28)
PLTs<100.000/μL	3/35 (8)
ALC>5000/μL	18/35 (51)
%BM infiltration	40
(median) (range)	(0-85)
Paraproteinemia	9/29 (31)
IgG	6/9 (67)
IgM	3/9 (33)
HVC (+)	0
MYD-L265P (+)	1/37 (3)

Summary/Conclusions: Based on our findings, MYD88 L265P mutation is very rare in SMZL. In our series of 37 SMZL cases, MYD88 L265P mutation was detected in only one case (3%), which a monoclonal IgMκ was present. Our findings support the view that the presence of an IgM monoclonal gammopathy in the serum, with a lymphocytic – lymphoplasmacytic infiltration of the BM by no means document the diagnosis of WM/LPL.

PB1879

SIGNIFICANT ROLE OF FLOW CYTOMETRY IN THE DIAGNOSIS AND FOLLOW UP OF GASTRIC LNH

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Background: The diagnosis of lymphoma is mainly based on morphology, but the diagnostic accuracy is greatly increased by the use of ancillary techniques like immunophenotyping, cytogenetic and molecular tests. This multiparametric approach is recommended by WHO classification.
Aims: To evaluate the role of flow cytometry in the study of gastric biopsies with suspect of NHL. We have assessed the validity of integration of histological and cytometric assays to optimize diagnosis of lymphoproliferative diseases and to lower the number of inconclusive cases at histological exam. The aim of flow cytometry was to define lineage and clonality of lymphoid proliferation, thus giving a support to final diagnostic assessment.
Methods: 33 patients with primary gastric lymphoma underwent, from January 2007 to December 2015, to double gastric biopsy at diagnosis or during follow up. Diagnosis was MALT in 19/33 (58%), DLBCL in 9/33 (27%) and other histological type in 5/33 (15%). Every patient underwent complete staging and the stage was I/E in 18/33 and II/E in 15/33. Therapy was antibiotic eradication of HP in 8/33 pz and chemo-immunotherapy in accordance with guidelines. Samples for histology were fixed with formalin and stained with HE, then tested by antibodies for CD3, CD5, CD10, CD20, CD21, CD23, CD30, CD38, CD43, CD45RO, CD79a, CiclinaD1, BCL-2, BCL-6, Ki67, EMA, k e l. Samples for flow cytometry were put in saline, sent to laboratory within thirty minutes and processed with standard methods. A cytometric pattern was defined as pathological if there was an evident atypical assembly of B or T lymphoid antigens along with clonal restriction for Ig light chains or TCRVb repertoire.
Results: We have carried out 44 biopsies in a population of 33 patients and we obtained a final diagnosis in all cases. Diagnosis was: NHL in 11/42 (9 MALT,1 follicular lymphoma,1 DLBCL), plasmacytoma in 1/42, and benign conditions in the remaining 32/44. There was strict concordance between the two methods, but in one case flow cytometry was negative and histology was positive for lymphoma, in other one positivity of flow cytometry led to histological revision and, finally, to full concordance.
Summary/Conclusions: This study demonstrated that flow cytometry is an easy and reliable diagnostic tool for gastric lymphoma: assumed that its goal is to assess lineage and clonality, it is fast, sensitive and specific. It is synergistic to histology, especially in difficult cases like biopsy performed during a revaluation after chemotherapy or antibiotic therapy, or reactive conditions where lymphoid infiltrates can mimic lymphoma. Histological diagnosis certainly remains the gold standard for lymphoma diagnosis but flow cytometric typing can usefully support histology to reach a correct diagnosis in 100% of cases.

PB1880

RESULTS OF LAPAROSCOPIC SPLENECTOMY IN SPLENOMEGALY

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Background: Surgical approach to splenectomy depends on clinician's experience and laparoscopic technique. However, splenectomy in patients with splenomegaly remains a challenge. Due to the lack of randomized controlled studies the main sources of data to evaluate surgical approach to splenomegaly are case-control series.
Aims: Our goal was to determine criteria for laparoscopic approach to splenomegaly
Methods: Since 1999 1027 patients underwent laparoscopic splenectomy (LS) at our department. Spleen size was considered to determine surgical approach in connection with other factors such as abdominal cavity volume. Splenomegaly was detected in 506 cases of 1027 LS performed. In this group the mean spleen size was 21.25 sm (range 14-34 sm), mean spleen weight was 1016 g (range 300-2650 g). The mean age was 37 years (range 14-72 years). Indications were same as for the open procedure. Mean operative time was 137 min *versus* 115 min in open procedure. Mean blood loss was 54 ml *versus* 664 ml in open procedure. Conversion rate was 19.7% (100 cases). Spleen hilum infiltration and perisplenitis were the factors most commonly leading to conversion. There were no severe complications and no deaths.
Results: Splenomegaly is not considered a crucial factor to determine surgical approach. We've separately analyzed 250 LS performed before 2003. Conversion rate in this group was 22%, and it was as high as 31% in cases of splenomegaly. Since 2003 gaining experience resulted in conversion rate decrease to 18.7% in patients with splenomegaly. The conversion rate decreased with the increasing surgical experience. Apparently excessive spleen size is relevant if analyzed in proportion to limited working space in abdominal cavity. Conversion rate in lymphoproliferative disorders was 28% and for benign disorders – 13%. LS has proved to have advantages over open procedure such as minimal tissue injury, improving exposure to the superior pole and splenic pedicle and minimization of pancreatic injury. Even if excessive spleen size leads to conversion, laparoscopic approach facilitates dissection as the first operative step.
Summary/Conclusions: LS is being increasingly used in patients with splenomegaly. Spleen size is not the most important factor to determine approach to splenectomy. There is a decrease in conversion rate with the increasing surgical experience. Laparoscopic approach to splenomegaly is preferable considering sufficient knowledge and skill of the operating surgeon.

PB1881

BRAF GENE MUTATIONS AND PROGNOSTIC SIGNIFICANCE OF CLINICAL FINDINGS IN CASTLEMAN DISEASE

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Background: Castleman's Disease is a quite rare lymphoproliferative disorder. The association with IL-6 secretion, HIV/HHV-8 is well described, but the pathogenesis is not fully understood. BRAF is a serine/threonine kinase that has an important role in the mitogen activated protein kinase (MAPK) pathway. Dysregulation of this pathway and gene mutations lead to development of malignancy. BRAF mutations are present in malignant melanoma, colorectal cancer, ovarian carcinoma, papillary thyroid carcinoma and hairy cell leukemia.
Aims: We aimed to determine the presence of BRAF gene mutation in Castle-

man's Disease and to assess clinical data of disease and the relationship with prognosis.
Methods: This retrospective study included 34 patients who were diagnosed as Castleman's Disease between 2007-2014 from 8 different medical centers in Turkey. Clinical and laboratory findings of the patients have been evaluated from medical records. All tissue samples were characterized for the presence of the BRAF mutation by pyrosequencing. Statistical analysis was performed to determine any associations among histological variants,clinical type and clinical features of the patients including age, gender, survival, and laboratory findings.
Results: There were 17 men and 17 women. Hyaline vascular variant was the most common subtype (75.6%). The study included 21 (61.8%) cases with unicentric Castleman's Disease (UCD) and 13 (38.2%) cases with multicentric Castleman's Disease (MCD). Hyaline vascular variant was more common in the UCD group ($p<0.05$). Cases were divided in 3 sites according to involvement; thoracic, abdominal and cervical. The majority of patients (61.7%) had cervical involvement. 10 of patients (29.4%) had more than one region involvement. The plasma cell variant was significantly higher in men ($p=0.006$). The mean age was significantly higher in the plasma cell group and MCD ($p<0.05$). CRP levels were found significantly higher in plasma cell variant compared to hyaline vascular variant ($p<0.05$). The BRAF gene mutation was not detected in all tissue samples. Hepatomegaly and/or splenomegaly were presented significantly common in patients with multiple site involvement ($p<0.05$). Splenomegaly was presented significantly common in MCD ($p=0.001$). 3-year overall survival was significantly higher in patients treated with only radiotherapy or chemotherapy in addition to radiotherapy when compared to only chemotherapy ($p=0.001$). Mean survival was significantly higher in patients with hyaline vascular variant and UCD ($p <0.05$).
Summary/Conclusions: This study is the most comprehensive study in our country about Castleman's Disease and we obtained important information about clinical, histopathological and prognostic features of the disease. We suggest combined therapy with radiotherapy is superior to chemotherapy alone, but as we know the efficiency of radiotherapy in the treatment of unicentric Castleman's Disease and we may have got this outcome due to some of the unicentric patients in our study were treated by radiotherapy alone.

PB1882

OCULAR ADNEXAL LYMPHOMAS: A CASE SERIES
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Background: Ocular lymphomas comprise only 1% of all non-Hodgkin lymphomas (NHL)[1]. However, it is a common extranodal site (5-15% of the extranodal cases)[2]. Lymphoma arises from the localized lymphoid tissue affecting the orbit, the eyelids, the conjunctiva and the lacrimal gland. The high curative potential of the local disease makes prompt diagnosis clinically important.
Aims: Case series involving 16 patients with ocular adnexal lymphoma and study of their treatment outcomes.
Methods: Retrospective review of 16 patients' records from Royal Victoria Eye and Ear Hospital Dublin and University Hospital Waterford with a diagnosis of ocular adnexal lymphomas (OAL).
Results: In our case series, we found that follicular lymphomas were the most common subtype (50%), followed by extranodal marginal zone B-cell lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma (37.5%) and diffuse large B-cell lymphoma (12.5%). Majority of the patients were between the ages of 70-80 years. Male to female ratio was 1:1.3. Nine patients (56.2%) had primary OAL, 6(37.5%) had concurrent systemic involvement and one (6.25%) presented with ocular relapse of systemic disease. The most common presenting complaint was swelling of the eye or visible lump (62.5%) and the most common site was the orbit (37.5%) followed by conjunctiva (25%) and eyelid (25%).
Summary/Conclusions: Ocular site of the tumor does not influence the natural history of NHL. Localized disease was controlled in all cases. 10 patients (62.5%) had localized disease; radiotherapy remained the standard of treatment. Patients with localized disease who were treated with 4 cycles of monoclonal antiCD20 antibody (Rituximab) alone responded well to the treatment and sustained remission. Treatment with Rituximab alone averts potential toxicity of radiotherapy (*e.g.* cataract formation). Systemic disease accounted for 37.5% of the cases and were treated with systemic chemotherapy.

References
1. Fitzpatrick PJ, Macko S. Lymphoreticular tumors of the orbit. Int J Radiat Oncol Biol Phys. 1984; 10:333-340.
2. Lambo MJ, Brady LW, Shields CL. Lymphoid tumors of the orbit. In: Alberti WE, Sagerman RH, eds. Radiotherapy of Intraocular and Orbital Tumors. Berlin, Germany: Springer-Verlag. 1993; 205-216.

Infectious diseases, supportive care

PB1883

RISK FACTORS AND MICROBIOLOGICAL FEATURES OF BLOODSTREAM NONTYPHOIDAL SALMONELLA INFECTION IN ADULT PATIENTS RECEIVING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: In patients receiving allogeniec hematopoietic stem cell transplantation (allo-HSCT), it is not uncommon to encounter post-transplant infection and/or acute or chronic graft-versus-host disease (aGVHD or cGVHD) which commonly involve gastrointestinal tract and liver. Thus, post allo-HSCT patients might be vulnerable to foodborne pathogens, such as nontyphoidal salmonella (NTS) infection. To the best of our knowledge, very few reports discuss this issue of post allo-HSCT bloodstream NTS infection. We retrospectively analyzed the clinical risk factors and microbiological features of bloodstream NTS infection among adult allo-HSCT recipients during a 12-year period in our institution.
Aims: To determine the survival outcomes, the risk factors and microbiological features of bloodstream nontyphoiidal salmonella infection in adult allo-HSCT recipients.
Methods: We retrospectively reviewed adult (age ≥18 years) allo-HSCT recipients between 2003 and 2014 with regular follow-up till October 2015. Pretransplant and transplant-related clinical data were collected. Myeloablative conditioning included busulfan (4mg/kg/day for 4 days) and cyclophosphamide (60mg/kg/day for 2 days), or total body irradiation (TBI) of 12 Gy combined with cyclophosphamide (60 mg/kg/day for 2 days). Fludarabine-based conditioning were administered to elderly patients or with comorbidities. Standard protocol with cyclosporin (i.v 3.0 mg/kg/day in 2 split doses with dose being adjusted to maintain trough plasma level at 100-250 ug/L) and short-term low dose methotrexate were adopted for GVHD prophylaxis. Recipients of unrelated donor transplants also recieved anti-thymocyte globulin (2mg/kg/day for 3 days). The overall survival (OS) in patients with or without bloodstream NTS infection was analyzed by Kaplan-Meier method. A log-rank test was used to compare survival curves for statistical significance. Odds ratios (ORs) and the 95% confidence interval (CI) were calculated using logistic regression models. We used multivariate logistic regression models to calculate odds ratios while adjusting for possible independent confounding factors. All risk factors with $p<0.1$ in the univariate model further entered into the multivariate analysis. All statistical testing was performed using 2-tailed tests; $p<0.05$ was considered statistically significant.

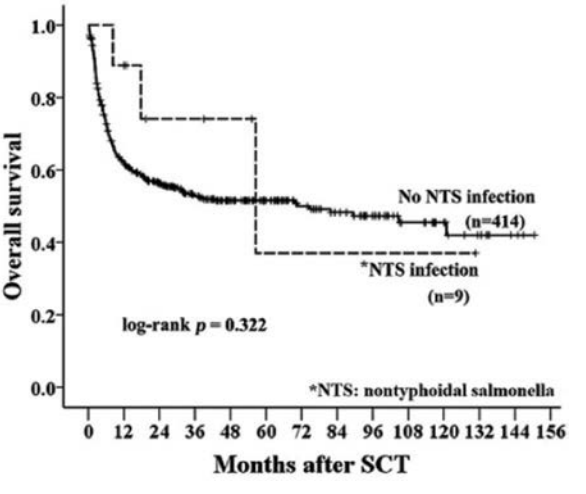

Figure 1.

Results: Total nine patients (2.13%) of 423 patients recieving allo-HSCT experienced post-tansplant bloodstream NTS infection in a median onset of 315 days after HSCT. As for the nine with NTS infection, fever was a majorly presenitng symptom. Metastatic infection of knee joint occurred in 2 patients. One suffered from concurrent bloodstream NTS infection and spetic arthritis and the other one had subsequent septic arthritis after 42 days of bloodstream NTS infection. *Salmonella* group *D* was cultured in eight out of nine patients (89%). Of note, among the 9 bloodstream NTS infection, 7 patients had cGVHD, 5 of whom suffered from extensive cGVHD. Fortunately, all patients recovered with

antibiotic therapy, which comprised of a at least third-generation cephalosporin or a quinolone or both. In addition, in patients with or without bloodstream NTS infection, there was no statistical difference of post-transplant survival (p=0.322; Figure 1). After multivariate analysis, there were statistical significances in patients receiving non-myeloablative conditioning (OR: 4.604; 95% CI: 1.098-19.308; p=0.037) and post-transplant extensive cGVHD (OR: 8.054; 95% CI: 2.048-31.674; p=0.003).
Summary/Conclusions: In a cohort of 423 adult patients recieving allogeneic hematopoietic SCT, 9 patients (2.13%) developed post-transplant NTS infection, including two patients had subsequent or combined metastatic infection. Multivariate analysis revealed that extensive chronic graft-versus-host disease (GVHD) (OR 8.054, p=0.003) and non-myeloablative transplant conditioning (OR 4.604, p=0.037) were significant risk factors for NTS infection. Our study determined the risk factors and microbiological features for this infection.

PB1884

THE REAL WORLD USE OF BISPHOSPHONATES IN MULTIPLE MYELOMA AT UNIVERSITY COLLEGE HOSPITAL (UCH), LONDON: A COMPARISON WITH LATEST GUIDANCE

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Background: The use of intravenous bisphosphonates in patients diagnosed with myeloma is regarded as standard of care, irrespective of the presence of bone lesions. The only exceptions may be patients in advanced renal failure, or those with osteonecrosis of the jaw (ONJ). Zoledronic acid is the bisphosphonate of choice given evidence of an anti-myeloma effect, including survival. Optimal duration of bisphosphonate usage is less clear but the recommendation from the International Myeloma Working Group (IMWG) is to continue for a minimum of 1-2 years after which discontinuation can be considered if complete response (CR) or very good partial response (VGPR) is achieved. This guidance is recommended but not always followed. We review clinical practice surrounding bisphosphonate usage at a tertiary centre.
Aims: To describe the real world use of bisphosphonates in patients attending a specialist myeloma clinic and how it compares with published guidance.
Methods: A sample of 60 patients with symptomatic myeloma was taken sequentially from clinic lists in December 2015. Data was collected for these patients from clinical case record (CDR) and electronic prescribing system (Chemocare) in regards to current disease status, chemotherapy, bisphosphonate use and complications and renal function. A literature search was performed using Pubmed in order to collate the latest guidance.
Results: 60 patients with myeloma attending the myeloma clinic were identified. Of these, 55% were male. 10% patients were <50 years old, 72% patients were aged 50-70 and 18% were >70 years old. 27% were diagnosed with myeloma within the last 2 years. Disease isotypes were: 52% IgG, 12% IgA, 17% kappa light chain, 13% lambda light chain and 6% other disease type. 52% were on active treatment; 17% on Bortezomib regimens, 25% on Lenalidomide, 8% on Pomalidomide and 2% on other regimens. 58% of patients had abnormal renal function (GFR<90ml/min). 57/60 were commenced on a bisphosphonate at diagnosis, which was Zoledronic acid in 77% of all cases but just 46% in patients with eGFR <60ml/min. In all other cases, it was Pamidronate. All patients had renal function monitored via a blood test prior to each bisphosphonate infusion but urine albumin was not measured. DEXA scans were not routinely done to assess bone density. 88% of patients diagnosed within 2 years were still on 4-weekly bisphosphonates. After 2 years, 12% had the bisphosphonate stopped whilst 48% had the frequency reduced to 2-3 monthly. The remaining 40% remained on monthly treatment. Of the 40% on monthly bisphosphonate treatment more than 2 years from diagnosis, most (88%) were being actively treated for their myeloma. Of the 31 patients on active treatment, 71% were still on 4 weekly bisphosphonates. Of patients with an eGFR of <60ml/minute, 64% had their bisphosphonate dose reduced. One patient had a reduction in treatment frequency as a consequence of ONJ. A further 4 patients had dental intervention documented during bisphosphonate treatment, one of whom had their bisphosphonate treatment held for 2 months as a result.
Summary/Conclusions: Compliance with IMWG guidance on initiation of bisphosphonates occurred in 95% of cases. Zoledronic acid is used less frequently in patients with abnormal renal function. Whilst frequency of bisphosphonate use often reduces after 2 years (61%), there is marked variability in the time point and disease status at which this occurs. Further work is required to clarify optimal duration of bisphosphonate use and scheduling, and the adjustments for osteonecrosis and dental intervention.

PB1885

ANTIFUNGAL PROPHYLAXIS WITH MICAFUNGIN IN PATIENTS UNDERGOING AUTOLOGOUS AND ALLOGENIC STEM CELL TRANSPLANTATION. A SINGLE CENTRE EXPERIENCE

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Background: Micafungin is an echinocandin antifungal approved for prophylaxis in patients undergoing allogenic hematopoietic stem cell transplantation. It has a broad spectrum against yeasts and filamentous fungi. In our institution, we employ Posaconazole as antifungal primary prophilaxis for allogenic transplantation and Fluconazole for autologous procedures but there are different situations that require us to change the protocol such as tolerance or toxicity to azoles or interactions with other drugs such as rapamycin.
Aims: Analyze the efficacy and safety of the use of Micafungin for antifungal prophylaxis in patients undergoing autologous and allogenic hematopoietic stem cell transplantation.
Methods: Between January 2013 and December 2015, 77 patients have received Micafungin at doses 50 mg daily. 51 (66.2%) of them were male. The mean age at transplantation was 43.7 years-old (20-61). Diagnoses were 23 acute leukemias (29.8%) twenty-two (28.5%) Non-Hodgkin Lymphoma, twelve (15.5%) Hodgkin Disease, six (7.7%) myelodisplastic síndrome, five (6.4%%) Multiple Myeloma, five (6.4%) philadelphia-negative chronic myeloproliferative neoplasms, three (3.8%) aplastic anemia and one case of Chronic Myeloid Leukemia. Most of the stem cell transplantation were allogenic (85%). Only seven (8.8%) and five (6.5%) were autologous and haploidentical procedures respectively. Major of Conditioning regimen was myeloablative (71.3%), 17.5% undergoing reduced intensity conditioning regimen and only 11.3% no myeloblative regimen. 46.3% of the patients presented absolut neutrophils count (ANC) less than 1000/mm^3. The average duration of treatment with micafungin was 20.7 days (5-150) and the median duration was 14 days. The reason to start micafungin treatment was primary prophylaxis in ten cases (10%), bad gastrointestinal tolerance to azoles in fifty-four cases (70.1%), farmacological interactions with azoles in seven patients (9%) and toxicity to azoles in only five patients (6.4%).
Results: Reason to finish prophilaxys with micafungin was the improve of the gastrointestinal syntoms and, consequently, the end of prophilaxys period in fifty-two cases (67.5%). Six patients (7.8%) presented breakthrough fungal infection. Other causes were positive antigen galactomannan (2 patient), persistent fever (5 patient), exitus not attributable to IFI (5 patient), toxicity to micafungin (2 patients) and unknown cause in four patients. Six patients (7.8%) presented breakthrough fungal infection (all probably invasive fungal infection according to the EORTC criteria). Isolated fungi were Geosmithia argillaceae, Aspergillus Fumigatus, Aspergillus Flavus and Aspergillus spp. Mortality due to Fungal infection was 3.8% (three patients). With regards to tolerance, there were no reports about any infusional reaction. Only 2 patient referred micafungin liver toxicity grade II without any problem when the micafungin was suspended.
Summary/Conclusions: 1. Micafungin at doses of 50 mg/24 hours, is an effective and safe option for yeast prophylaxis in patients undergoing autologous and allogenic stem cell transplantation. 2. In our series, although they have identified six breakthrough infections (7.8%), it must be noted that these were patients with active refractory GVHD to several lines of immunosuppressive therapy, including, of course, high-dose steroids. Maybe, we have to use prophylaxis protocol based on liposomal anfotericin, with a better anti-mold spectrum, in this group of high risk patients. 3. The safety of the treatment was excellent, without tolerance problems. The kidney and liver function was not altered by treatment with micafungin.

PB1886

THE POSITIVE IMPACT OF ANTIMICROBIAL STEWARDSHIP IN THE BIGGEST PEDIATRIC HAEMATOLOGY ONCOLOGY CENTRE, RUSSIA

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Background: Multidrug resistant organisms (MDRO) are the emerging problem across the world, and one of the most effective and mandatory steps in solving it is optimization of antibiotics use. Here we present the first results of the fight against MDRO at the Dmitry Rogachev Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russia (Center).
Aims: The Infection Prevention and Control Department (IPCD) has made a number of steps to rationalize the use of anti-infectives.
Methods: On January 1st, 2014, the algorithm for empirical anti-infective therapy in febrile neutropenia was implemented. It included recommendations for first line (empiric) antibacterial therapy (in accordance with the patient's risk group), spectrum of recommended evaluation tests and rules for treatment modification. For example, fluoroquinolones and ceftazidime were excluded from empiric therapy and reserved for the targeted one only. The same recommendations were made regarding tigecycline and ertapenem. Another step was the implementation of guidelines for perioperative antibiotic therapy, with cephalosporins I-II regarded as the drugs of choice in most of cases. Along with those measures, precise control of antibiotics and antifungals discontinuation was established. For the evaluation of the effectiveness of the results we have calculated the use of the most common classes of antibiotics and antifungals (per 100 bed-days) before the intervention and a year after it. Microorganisms'-wise drug resistance index (DRI) was also calculated.
Results: Having compared the respective rates before and after the intervention, we detected the overall decrease of use in vancomycin (64%), piperacillin/

tazobactam (15%), aminoglycosides (15%) and fluoroquinolones (7%) in all the Center's departments. Calculated separately, the rates were significantly higher for the intensive care unit, where interaction compliance with the IPCD was the highest: reduction of aminoglycosides use -58%, fluoroquinolones -66%, cephalosporins III-IV-8%, vancomycin-82,5%, piperacillin/tazobactam -52%, carbapenems-20%, linezolid-47%. The use of colistin remained on the same level, while the use of tigecycline raised 35%. As for antifungals, the analysis showed a 75% reduction of use in amphotericin B and an 18% reduction in case of voriconazole. Posaconazole raised 25%. Although echinocandins were used as the drugs of choice in empiric antifungal therapy in febrile neutropenia algorithm (in accordance with the ECIL recommendations), their use remained on the same level. During the surveillance period DRI levels for *P.aeruginosa* and *E. coli* dropped down (graph 1). In terms of money, the reduction of anti-infectives use have saved the Center around €500,000 in 12 months. It is important to mention that the rates of infections-related mortality has not increased since the intervention (26 in 2013 compared to 16 in 2014).

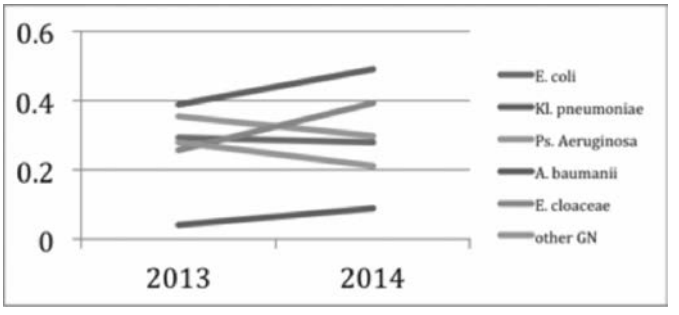

Figure 1.

Summary/Conclusions: The reduction of antibiotics and antifungals use and as a result - the decrease of microbial resistance and money savings in hematology/oncology hospital is an achievable goal. Every hospital should make the effort to initiate and sustain an effective Antimicrobial Stewardship Program.

PB1887

INVESTIGATION OF RELATIONSHIP BETWEEN NEUTROPENIA AND SERUM CHITOTRIOSIDASE AND NEUTROPHIL GELATINASE RELATED LIPOCALIN ENZYME ACTIVITY IN CHILDREN UNDERGOING CHEMOTHERAPY

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Background: Human chitotriosidase (ChT) is a member of the chitinase family and it is synthesized and secreted by activated macrophages and neutrophilic granulocyte progenitors. Neutrophil gelatinase related lipocalin (NGAL) is situated in secondary granules of granulocytes and is specific to neutrophils.
Aims: The aim of this study was to investigate the relationship between neutropenia and serum ChT and NGAL levels in patients with hematologic malignancy and hospitalized because of febrile neutropenia.
Methods: This study included 27 children between 1-15 years old, who have hematologic malignancies and whose chemotherapies were still ongoing and were known not having a disease that leads to macrophage activation. Complete blood count (CBC),serum levels of c-reactive protein (CRP), lactate dehydrogenase (LDH), ChT and NGAL were determined before the initiation of chemotherapy while the patients were not neutropenic. The same tests were studied also in the severe neutropenic period and after the recovery of neutropenia. Serum ChT and NGAL levels of the patients during these periods were compared. Whether there is a correlation between the number of neutrophils and these enzyme levels were also investigated.
Results: Total granulocyte count (TGC), CRP, LDH, NGAL and ChT levels were statistically significant different between the periods of severe neutropenia, improving neutropenia and recovered neutropenia (p:0,0001). There was a statistically significant positive correlation between ChT and TGC levels during the period of severe neutropenia (p:0.034, Pearson's r: 0,418). There was not a statistically significant relationship between NGAL and TGC levels during the period of severe neutropenia.
Summary/Conclusions: The results of our study exhibiting a statistically significant difference, between serum ChT and NGAL levels, during the periods of severe neutropenia, improving neutropenia and recovered neutropenia, supports the data suggesting that these enzymes are secreted by neutrophils. Especially, statistically significant positive correlation between ChT and TGC levels suggests that levels of this enzyme can be used as a marker to determine the period of recovery from neutropenia. Further studies are needed to investigate the relationship between febrile neutropenia prognosis and NGAL and Cht levels.

PB1888

EPIDEMIOLOGY OF FEBRILE EVENTS (FE) IN NEUTROPENIC PATIENTS (PTS) WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) TREATED BY «ALL-2009» PROTOCOL

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Background: Hematological patients with acute leukemia undergoing intensive myelosupressive treatment are at high risk for severe, life-threatening infections.
Aims: To evaluate the epidemiology of FE in neutropenic pts with ALL on different chemotherapy phases (CP).
Methods: Single-center, prospective observational study in adults with newly diagnosed ALL treated by «ALL-2009» protocol (NCT01193933) was performed from Jan 2013 till Jan 2016. Pts were followed up for 180 days.
Results: Total of 44 pts were enrolled (22 - male, 22 - female; median age 26 (17-61). On admission hyperleukocytosis was in 25% (11/44) of pts, ECOG score ≥3 had 61% (27/44). These pts had 271 CP (induction I-43, induction II-42, consolidation I-42, consolidation II - 41, consolidation III-40, consolidation IV - 33, consolidation V - 30). Neutropenia was in 33% of CP, median duration 9 (2 - 45) days, more frequent and prolonged in induction then in consolidation (58% *vs* 22%, p<0.0001; 19 *vs* 6 days, p<0.000001). FE occurred in 18% (48/271) of CP, more often in induction than in consolidation (36% *vs* 9%, p<0.0001). FE (48) reasons were: fever of unknown origin (FUO) in 29% (14), clinically documented infection (CDI) in 48% (pneumonia - 17, cellulitis - 6) and bloodstream infection (BSI) in 23% (11). Among BSI pathogens Gram-negative bacteria were in 77% (*E. coli*-3, *Salmonella* spp.-3, *K. pneumonia*-2, *E. asburae*-1, *C. youngae* - 1), Gram-positive bacteria were in 23% (*B. cereus*-1, *S. aureus*-2). BSI was polymicrobial in 18% (2/11). Rate of invasive mycoses (IM) was 14% (6/44): 2 - invasive aspergillosis, probable (IA), 1 - mixed IA, probable plus mucormycosis, 3 - hepatosplenic candidiasis. All IM occurred in induction. Nobody had IM in consolidation (p=0.0002). Overall 180 days mortality was 5% (2/44), 1 patient died in induction (1-IA plus mucormycosis) and 1 in follow-up period (BSI due to *Salmonella* spp.).
Summary/Conclusions: Our study showed moderate rate of infections (18%) in ALL pts treated by «ALL-2009» protocol. FE were more frequent in induction than in consolidation (36% *vs* 9%). Most pts (71%) had CMI and BSI. IM occurred only in induction. Overall 180 days mortality was relatively low (5%).

PB1889

THE CLINICAL SIGNIFICANCE OF PROCALCITONIN MONITORING IN INFECTED PATIENTS WITH HEMATOPATHY

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Background: Due to the application of the drugs such as high doses of chemotherapy, immune inhibitors and hormone, hematopathy patients are becoming more likely to get granulocyte lack and the resistence to infection. For those patients with the application of the peripheral venipuncture in granulocyte lack period, infection incidence can be as high as 53% ~ 80%. Accordingly, infection is one of the most common life-threatening complications, and therefore the early diagnosis and treatment of infection is very important to better prognosis. But now there is no sufficient sensitive markers to reflect the level of inflection in hematopathy patients. Traditional markers such as the number of white blood cells, C - reactive protein have no obvious specificity in the diagnosis of hematologic malignancies with infection. Positive bacteriology of blood culture is the only gold standard in the diagnosis of patients with bloodstream infections.However, it can't meet the requirements for early and rapid diagnosis, because the blood culture positive rate is only 10% ~ 20%, and pathogen isolation and culture time is long; Calcitonin original protein (PCT) is the precursor of calcitonin,which has been proven to be high in a variety of disease infection since it was found in 1993. Furthermore, it is a sensitive and specific indicator in early granulocyte lack with severe bacterial infections.
Aims: To evaluate the clinical value of procalcitonin (PCT) measurement and its dynamic monitoring in hematopathy patients undergoing chemotherapy or transplant-related infections.
Methods: The clinical data were collected and analyzed retrospectivey in 497 hematopathy patients with serum PCT above 0.5μg/L. The procalcitonin were compared between the patients with septic shock and those without. The procalcitonin were also compared between the patients with positive blood culture and those with negative blood culture. Further, theprocalcitonin were compared between the patients with gram-positive bloodstream infection and those with gram-negative bloodstream infection. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of procalcitonin in predicting sepsis. PCT values were collected at the onset of fever and 3, 7, 14, 21, 28 days after antimicrobial therapy. The dynamic change of PCT after operation

was compared between the patients whose infection was successfully controlled and those without adequate control.
Results: There were totally 497 hematopathy patients with serum PCT value >0.5μg/L, 421 (84.7%,421/497) cases among whom were diagnosed with infection based on clinical symptoms and microbiological assays. Out of these 421 patients, we found that 296 (70.3%, 296/421) cases' PCT values increased (>0.50μg/L) within 24 hours after having a fever. The median (range) procalcitonin level [6.29 (0.505-100) μg/L]was significantly higher in 59 (14.0%, 59/421) patients with septic shock than in those (86.0%, 362/421) without [1.16 (0.502-100) μg/L,Z=-7.726,P<0.01].408 patients received blood culture examination. The median (range) procalcitoninlevel [2.47 (0.508-100) μg/L]was significantly higher than in 167 patients with positive blood culture than in 240 patients with negative blood culture [1.15 (0.501-100) μg/L, Z=-5.762, P<0.01]. Additionally the median (range) procalcitoninlevel [4.315 (0.508-100) μg/L] was significantly, higher in 108(64.7%, 108/167) patients with gram-negative sepsis than in 37 (22.2%, 37/167) patients with Gram-positive sepsis [1.39 (0.521-55.44) μg/L, Z=-2.024, P<0.01]. The area under the curve (AUC) for PCT was 0.670, whereas a cut-off value of 2.05μg/L, provided the best sensitivity and specificity with a value of 54.3, and 70%, respectively. The dynamic changes of 289 monitoring PCT patients reflected the infection course. No significant difference was found between the patients with deteriorate and persistent infection and those whose infection was successfully eradicated properly within 72 hours (*P*>0.05). However,the PCT after 72 hours were significantly different among the three group (All *P*<0.01).
Summary/Conclusions: Procalcitonin monitoring could be used as a rapid and supplementary diagnostic marker of infection,especially in blood culture-negative infected patients.Additionally,its dynamic change could also reflect the infection course,which provides a better guide for the use of antibiotics.

PB1890

PREVALENCE OF CYTOPENIAS IN PATIENTS LIVING WITH THE HUMAN IMMUNODEFICIENCY VIRUS. AN OBSERVATIONAL COHORT STUDY
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Background: HIV-induced immunosuppression was an important reason for the increased morbidity and mortality. Disorders in haematopoiesis are encountered far less frequently in the current era of highly active antiretroviral therapy (*HAART*). The expected survival of people living with HIV who are diagnosed early in the course of infection and who have access to modern antivirals now almost equals that of the general population. Ineffective haematopoiesis from direct suppression of bone marrow progenitor cells by HIV infection or indirectly through excessive secretion of inflammatory cytokines induced by HIV, can blunt haematopoiesis. So too can nutritional deficiencies, infiltrative bone marrow disease of infectious or neoplastic origin, and adverse drug effects. Cytopenias play a significant role in the morbidity associated with HIV. Although cytopenias may be poorly documented, they can also impact negatively on quality of life.
Aims: To analyze the prevalence of cytopenias in patients with HIV infection and evaluate the impact of HAART in our patients.
Methods: This is a retrospective study of 108 patients older than 16 years, HIV infected, diagnosed between 2002-2014. Their medical histories were analyzed. Data was collected from laboratory at the moment of diagnosis, one year later and two years of follow-up. 98 patients were included in this analysis. We did not include patients with pediatric HIV infection or a history of vertical transmission and patients with loss of follow-up (follow-up less than 2 years).

Table 1.

Table 1: Cytopenias in patients with and without AIDS at the diagnosis, at 0 (dx), 12 (12m) and 24 months (24m) of follow up.

n(%)	No AIDS	AIDS	Total
N	67	31	98
Sex F/M	15/52	10/21	25/73
Anemia (dx)	20 (28%)	23 (61%)	43 (44%)
Thrombocytopenia (dx)	21 (29%)	18 (54%)	39 (40%)
Leukopenia (dx)	1 (2%)	4 (16%)	5 (5%)
Anemia (12m)	17 (20%)	11 (30%)	28 (25%)
Thrombocytopenia (12m)	4 (7%)	-	4 (5%)
Leukopenia (12m)	-	-	-
Anemia (24m)	17 (19%)	3 (8%)	20 (17%)
Thrombocytopenia (24m)	6 (8%)	-	6 (7%)

Results: The selected 98 patients showed a median age of 37.7 years (r16-65), 74% were male. At the time of diagnosis, 44% had anemia, 40% thrombocytopenia and 5% leukopenia (table 1). Only 8% of the patients had neutropenia. At the beginning, anemia was present in 48% of female and 36% of male patients. At the time of diagnosis, 31.6% (n31) were diagnosed with AIDS (according to WHO criteria). Out of these 31 patients, 61% had anemia basal *vs* 28% in those without AIDS at the start (p:0.001). At 12 months, only 29% of the patients showed anemia, with improvement in both male and female patients compared to the time of diagnosis as well as in the group of patients with initial AIDS. The presence of thrombocytopenia was observed in 5% of the patients. At 24 months of follow-up, 21% had anemia, and only 7% of those who initially had a diagnosis of AIDS. The thrombocytopenia appeared in 7%. None of the patients presented leukopenia at 12 or 24 months. As regards HAART, out of the 98 patients, 64.2% (n:63) received treatment since the diagnosis. Within this group, the emergence of anemia at 1 and at 2 years was 22% and 15% respectively, compared to 24% and 16% in patients who received no treatment at the beginning. Twenty-three of the patients who received zidovudine (AZT) developed anemia after one year of treatment. Those who did not receive AZT, the 27% presented anemia at 12 months. None of the patients who received AZT treatment showed leukopenia at one year of follow-up.
Summary/Conclusions: In our experience, the most frequent hematological alteration was anemia, mainly at the time of diagnosis and in patients with AIDS. The haemoglobin level evolved favorably in all patients, regardless of the presence or absence of antiretroviral therapy at the beginning. The thrombocytopenia was more frequent at the time of diagnosis, but it decreased with time. The less frequent cytopenia was the leukopenia, showing no association with HAART. HIV associated cytopenias are very frequent and its appearance occurs mainly at the time of the diagnosis. When faced with this type of cytopenias, it is mandatory to have this infectious disease in mind within the diagnosis algorithm.

PB1891

IMMUNOGLOBULIN SUPPLEMENTATION IN ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT IN A SINGLE PEDIATRIC INSTITUTION: COMPARISON BETWEEN TWO CONSECUTIVE AIEOP CLINICAL TRIALS
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Background: The intensification of chemotherapy in childhood acute lymphoblastic leukemia (ALL) has dramatically improved the 5-years overall survival but, at the same time, has increased the site effects therapy related. The most common complication remains the immunosuppression that causes increased risk of infections. The recent introduction of a pegylated formulation of L-Asparaginase (Peg-L-Asp), with more prolonged half-life and immunosuppressive effect than *native* E. Coli L-Asp, could result in a greater injury on ALL patients' humoral immunity.
Aims: We compared the rate of supportive Immunoglobulin (Ig) infusion during two consecutive therapeutic protocol of the Italian Association of Hematology and Pediatric Oncology (AIEOP) in a single pediatric center. The first trial, ALL 2009, contained Peg-L-Asp and the second, ALL 2006, *native* L-Asp; the remaining chemotherapy was similar.
Methods: Seventy-three patients, stratified in high-risk (HR; n=19), medium-risk (MR; n=32) and standard risk (SR; n=22), enrolled in ALL 2009 protocol, were compared with 68 patients (15 HR, 30 MR and 23 SR), of ALL 2006 trial. Patients submitted to a bone marrow transplantation or early relapsed were excluded by the analysis. Number of Immunoglobulin infusions, type and number of L-Asp administrations, risk stratification, IgG levels at diagnosis and episode of infections were collected by clinical records of both protocols. Possible correlations between Ig supplementation rate and the other parameters were analyzed using t-Student test and linear regression. Immunoglobulins were supplemented for Ig levels beyond 5.0 g/L at the standard dose of 0.5 g/kg.
Results: In ALL 2009 protocol**,** mean of Ig infusions were 6.2±2.9 SD (range 1-12) in HR, 2.8±2.0 SD in MR (range 0-8), and 2.2±2.1 SD in SR (range 0-4), while in ALL 2006, mean of Ig administrations were 2.9±1.7 in HR (range 0-6), 1.2±1.3 in MR (range 0-6) and 1.5±1.6 in SR (range 0-7), respectively. A higher rate of Ig infusions was observed in ALL2009 trial but this cumulative rate is not statistically significant. However, the difference between HR and MR risk stratification for the average of Ig infusions was significant (p <0.05) comparing ALL2009 and ALL 2006 trials. No correlation was found between Ig supplementations and risk of infections, number of L-Asp administrations, and IgG level at diagnosis.
Summary/Conclusions: In our experience, an higher Ig supplementation was observed in the very intensive protocol (HR and MR), mostly in ALL2009 trial. Further studies, with more extensive series of patients, need to understand the reason to the increased demand of immunoglobulin support in HR and MR patients treated with Peg-L-Asp.

PB1892

CANDIDAEMIA SPECTRUM: RESULTS OF A THREE-YEAR ANALYSIS AT THE PEDIATRIC HAEMATOLOGY ONCOLOGY CENTRE, RUSSIA
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Background: Invasive infections due to Candida spp. are among the leading

causes of morbidity and mortality of immunocompromised pediatric patients. Candidemia levels are still high, despite the ongoing improvements in diagnostics, prophylaxis and treatment possibilities. It not only affects the outcomes of cancer treatment, but also shifts the treatment costs. As for now, there are no data available on the epidemiology of invasive candida infections for pediatric hematology/oncology in Russia.
Aims: The study's aim was to determine the rate and spectrum of candidemia in Russia's largest pediatric hematology/oncology center.
Methods: We present a retrospective analysis of candidemias occurred at the Dmitry Rogachev Center for Pediatric Hematology, Oncology and Immunology, Moscow, Russia. All blood cultures positive for Candida spp. taken from 22 March 2013 to 22 November 2015 have been identified, and all first patient-episode pairs have been included. Spectrum of species, patient's characteristics, treatment strategies and outcomes have been analyzed.
Results: During surveillance period 34 episodes in 32 patients occurred. 36 Candida spp. were identified. 2 patients had 2 separate candidemia episodes, and in 2 cases more than one Candida spp. was cultured. Age distribution was 0-21 years (58% 0-3 years, 16% 5-10 y., 26% 11-21 y.). The majority of patients had some sort of hematological malignancy (39%), 29% had primary immunodeficiency, 26% had solid tumors and 3% had severe congenital neutropenia. 6 patients among all those with candidemia were after hematopoietic stem cells transplantation (18.7%). *C. parapsilosis* was responsible for the majority of episodes (53%) (graph 1). In 11 of 18 isolates the ecchinocandines susceptibility testing was performed: 10 (90.9%) were susceptible, 1 had intermediate susceptibility. As the first line therapy, 19 patients (59%) received echinocandins, 6 - amphotericin B, 3 - voriconazole, and 3 - fluconazole. In all cases antifungals were given within 24 hours from the time of execution of the blood cultures. One patient did not receive any therapy (although the central line was removed immediately). Central line was removed in 29 (88%) cases. Speaking of the outcomes, 72% patients were alive at the end of the episode. Mortality attributed to Candidemia was 12.5% (4 cases), with the time median of 21 day (3-37 days).

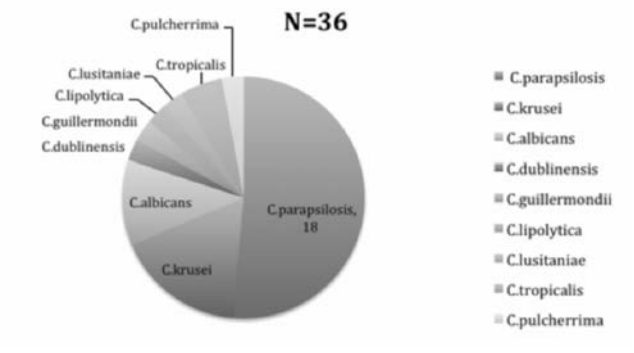

Figure 1.

Summary/Conclusions: Invasive candidiasis is a common complication in pediatric hematology/oncology patients. In our hospital in 2013-2015 candidemia accounted 8.6% of all bloodstream infections. *C. parapsilosis* was responsible for the majority of the cases, while mixed infections were rare. The attributive mortality was as high as 12.5%.

PB1893

RISK FACTORS FOR INFECTION AND HIGH MORTALITY RATE AMONG PATIENTS WITH KPC PRODUCING KLEBSIELLA PNEUMONIAE BACTERAEMIAS IN A HAEMATOLOGY UNIT

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Background: Emergence of carbapenem resistant enterobacteriae, especially Klebsiella spp, causing nosocomial infections is growing worldwide and has become a great challenge especially for clinicians and the Public Health System. Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae (KPC-Kp) infections in Haematology Unit patients are associated with increased mortality.
Aims: This study was carried out to determine risk factors for KPC-Kp infections and predictors of mortality in Haematology Unit patients with KPC-Kp bacteraemias.
Methods: In this retrospective study of a 3-year period, all patients with hospital-acquired KPC-Kp isolation were included and were compared with other non bacteraemic patients. K. pneumoniae were identified by Vitek 2 technology, antibiotic susceptibility was determined by agar disk diffusion method and minimum inhibitory concentrations were determined by Etest. The presence of the *bla*KPC gene was confirmed by PCR. Molecular typing was performed by pulsed field gel electrophoresis (PFGE) of XbaI-restricted genomic DNA.
Results: Eighteen episodes of infections were identified and KPC-Kp strains were isolated from 16 patients. Acute myeloid leukaemia was the underlying disease in 10 patients, non-Hodgkin lymphoma in two and acute lymphoblastic leukaemia, chronic lymphoblastic leukaemia, aplastic anaemia and multiple myeloma, one each. Seven of them were males and nine were females and their median age was 70 years (range: 25-83). The medical records of patients were retrospectively collected for demographic, clinical, and microbiological data. Clinical infections *versus* hospitalization and the outcome of infected patients were defined. The median duration between admission and the isolation of KPC-Kp strains was 24 days. Twelve patients were neutropenic during the bacteraemias. Colonization or bacteraemia from multiple pathogens, including multidrug resistant P. aeruginosa, K. pneumoniae (ESBL), vancomycin resistant Enterococcus and various fungi were concurrently identified in 12 patients with bacteraemia from K. pneumoniae. All patients had either multiple or prolonged hospitalizations and all of them had received antibiotics for prophylaxis or treatment prior to the isolation of the pathogen. Infection due to KPC- Kp was the leading cause to death in 11/16 patients (68,8%). One patient died of brain hemorrhage (not attributed to his infection), three died of infections caused by other bacteria, after having survived the KPC infection, and three remained alive. Risk factors for KPC-Kp bacteraemia were the number of intravenous catheters or ports, prior prolonged administration of high spectrum of cephalosporins and prolonged hospitalization. In multivariate analysis the age, performance status, prolonged severe neutropenia, resistance to gentamicin and septic shock were independent predictors of mortality.
Summary/Conclusions: We found many risk factors involved in KPC-Kp infections among Haematology Unit patients. The high mortality rate in these patients reinforces the urgent implementation of appropriate infection control measures to prevent the spread of carbapenemases producing Enterobacteriaceae.

PB1894

PLASMA POSACONAZOLE CONCENTRATION DURING CONCOMITANT TREATMENT WITH PROTON PUMP INHIBITHOR OMEPRAZOLE IN ADOLESCENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANT

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Background: Posaconazole (PCZ) is a broad-spectrum triazole approved for primary antifungal prophylaxis in patients (pts) with acute myeloid leukaemia or myelodisplastic syndrome during chemotherapy, in pts who develop a graft *versus* host disease (GVHD) after allogeneic stem cell transplant (allo-SCT) and for salvage therapy of pts with invasive fungal diseases (IFD). Concomitant use of PCZ suspension (PCZ-susp) and proton pump inhibitors (PPIs) has been associated with a significant reduction in the bioavailability of the antifungal drug, however, whether and in what extent this reduction affects the probability in reaching the target levels should be defined. Furthermore, few data are available in the paediatric population.
Aims: Omeprazole is a PPI often prescribed to allo-SCT recipients who receive steroids for prophylaxis or treatment of GVHD, therefore concomitant PPI and PCZ-susp are commonly administered. In this study the impact of omeprazole on plasma PCZ concentration (PPC) in 4 adolescents who received PCZ-susp after allo-SCT was evaluated.
Methods: This study represents a sub-analysis of a large, prospective, multicenter trial which included adult and paediatric allo-SCT pts. The trial was approved by Ethic Committee, written informed consent was obtained from each parent prior to any study-related activity. They were 3 males and 1 female (aged 11-13-16 and 17 years) and they were affected by sickle cells disease and β-thalassemia (2 pts each) and underwent allo-SCT from related donors. Two pts received PCZ-susp for second line therapy of proven *Aspergillus Terreus* infection and possible IFD respectively, and other 2 pts received PCZ-susp for primary prophylaxis during GVHD. PCZ-susp was administered at standard dose (600 mg/die or 12 mg/Kg regard to the weight). Plasma samples were stored at -80°C. The PPC was measured by high-pressure liquid chromatography at a reference pharmacology laboratory in Florence. All pts received concomitant treatment with omeprazole during the entire PCZ-susp therapy. At steady state (after 5 days of treatment) 0.7 mcg/ml was considered the therapeutic cut-off value.
Results: Overall, 2, 2, 3 and 6 samples were obtained, respectively. In two pts PPC was always above the cut-off value (mean 2.34 and 1.19 mcg/ml respectively), while in two pts the first PPC was inadequate (0,27 and 0,49 mcg/ml respectively) but reached higher values at a second evaluation (0,50 and 2,05 mcg/ml respectively), without changing the dosage of the drug. The patient with persistent sub-therapeutic PPC suffered of gastro-intestinal GVHD with diarrhoea.

Summary/Conclusions: PPC monitoring is considered a standard good practice in pts treated with PCZ-susp. However, considering that a timely PPC monitoring is difficult to perform in several centers, clinical conditions which may impact on the bioavailability of the drug can be considered for the safe use of PCZ-susp without PPC monitoring. This small experience in adolescent SCT pts doesn't seem to show a significant impact of omeprazole in the bioavailability of PCZ-susp. In the only patient with inadequate PPC, diarrhoea, and not concomitant PPI, presumably was the cause of reduced absorption. While diarrhoea has been associated to impaired intestinal absorption in several experiences, concomitant use of PPI doesn't seem to significantly impact on PPC and doesn't seem to be considered an absolute indication to PPC monitoring.

PB1895

A NOVEL MARKER FOR DIFFERENTIAL DIAGNOSIS BETWEEN HEPATITIS B AND HEPATITIS C USING ROUTINE COMPLETE BLOOD COUNT AND CELL POPULATION DATA

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Background: Final diagnosis of hepatitis B or hepatitis C requires various immunologic and molecular diagnostic tests, and accordingly takes a significant amount of time prior to initiation of treatment. There is a high incidence of hepatitis in Korea, incurring high medical costs for hepatitis B (HBV) and hepatitis C (HCV). Reports have recently come that propose screening markers using CBC with cell population data (CPD) for infectious diseases such as sepsis and tuberculosis. If such screening markers for hepatitis are developed, it will contribute to the reduction of time prior to treatment and medical costs.
Aims: The study aims to find any possible screening marker or a combination of parameters detecting HCV from HBV among hepatitis patients using CBC with CPD.
Methods: We analyzed 3787 CBC data from 3721 individuals including patients with hepatitis (325 HBV and 84 HCV), anemia, malignancy, infection other than hepatitis, and health check-up at Seoul St. Mary's Hospital, Korea, between December 2012 and July 2014. We used an automatic hematology analyzer (DxH 800, Beckman Coulter Inc., Miami, FL, USA) to obtain data including CBC-diff and CPD from using direct current impedance to measure cell volume (V) for accurate size of all cell types, ratio frequency opacity to characterize conductivity (C) for internal composition of each cell, and a laser beam to measure light scatter (S) for cytoplasmic granularity and nuclear structure. Each CPD parameter value for WBC, neutrophils, lymphocytes, eosinophils, monocytes, NN RBCs (non-nucleated RBCs) is first stored in Excel, and the values are divided by the mean value from the normal health check-up individuals. HBV and HCV cases are colored red and blue, respectively, and sorted in ascending order in Excel. After sorting, we identify grouping of HBV or HCV cases for each parameter. If grouping is found, the parameter is taken as a marker; otherwise, e.g., even distribution, the parameter is of no value as a marker. The cutoff value for each candidate marker is set when the combination of them yields over 75% both of sensitivity and specificity.
Results: A combination marker composed of four parameters with cutoff values for detecting HCV in the mixture of HBV and HCV is as follows: SD C NNRBC >0.93; MN NNRBC C/AL2 >0.55; MN AL2 EO (Eosinophils) >0.97; SD V Ly >1. The new combination marker detected 142 cases which consist of 64 from 84 HCV cases (76.2%) and 78 from 325 HBV cases (24.0%).
Summary/Conclusions: A new set of CPD marker is proposed with 76.2% sensitivity and 76.0% specificity to differentiate hepatitis C from hepatitis B within less than 1 hour by simple CBC test, reducing time and costs as a screening measure.

PB1896

PREVENTION AND TREATMENT OF INVASIVE FUNGAL INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES - RESULTS FROM A SINGLE HEMATOLOGICAL CENTRE

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Background: Invasive fungal diseases could influence morbidity and early treatment-related mortality in patients of malignant hematological disorders treated with aggressive chemotherapy,especially in acute leukemias or after hematopoietic stem cell transplantation.
Aims: In an observational survey during 2010-2015 we investigated in our hematological department occurence of invasive systemic fungal infections (aspergillosis, systemic candidiasis, other species), methods of antifungal primary and secondary preventions and therapy of patients with acute myelogenous and lymphoblastic leukemias, lymphomas and transplant recipients according tot the criteria of EORTC/MSG, as well.
Methods: During suspect mycotic infection identification of pathogenic fungi specimens taken from blood and/or stool, bronchoalveolar lavage and histological analysis by autopsy, moreover chest/abdominal CT were performed (proven, probable and possible infections). Within period of survey 61 patients (pts) with acute myelogenous leukemia (AML), 32 ones with acute lymphoblastic leukemia (ALL) treated with aggressive cytostatic regimens, in small number other patients of hematological malignancies with systemic fungal infections were also analysed. During prolonged neutropenia patiens were getting antimycotic prevention (in myelogenous leukemia rather posaconazol, in lymphoblastic leukemia mainly fluconazol) in probable or proven mycotic infections such as in Candida infections first line liposomal AmphotercinB, echinocandins, or other second-line drugs (caspofungin), in suspect or manifest Aspergillus infection mostly voriconasol was given (empiric, pre-emptive or targeted treatment strategy).
Results: Systemic Candida infection proven by autopsy in four pts with AML (4/61), in three with ALL(3/32), Mucormyces in one with AML were diagnosed post mortem, probable candidiasis in five others (myeloma, lymphomas, chronic lymphocytic leukemia). Mold infection (Aspergillosis) proven by autopsy in 14/61 pts with AML, in 2 pts with ALL,and in two posttransplanted one, they survived after voriconazol therapy. In invasive Candida sepsis from 10 pts five expired, 5 recovered,out of 25 pts with Aspergillosis 13 ones (chiefly pts with AML) died despite antimycotic treatment, mortality in both fungal infections was equal (50-50%). In remaining pts getting antimycotic prophylaxis during neutropenic episodes invasive fungal infections did not occured.
Summary/Conclusions: Incidence of Candida infections was higher in ALL, whilst of Aspergillosis in AML, however mortality rate was high and same in both groups.but recently due to the early introduction of pre-emptive antifungal therapy fatal outcome could be diminished. Antifungal prophylaxis in prolonged neutropenia reduced frequency and severity of systemic fungal manifestation resulted in better survival data in our leukemic patients.

PB1897

EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS OF BLOODSTREAM INFECTIONS IN HEMATOLOGICAL CANCER PATIENTS

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Background: Bloodstream infections (BSI) are life-threatening illness for immunocompromised patients with hematological malignancies.
Aims: To compare epidemiology, causative pathogens and outcome of hospital-acquired BSI.
Methods: During the period 2007-2014 239 blood samples obtained from patients (pts) with hematological malignancies were studied. All blood cultures were incubated in the continuous monitoring system for 7 days before discard. The real-time PCR was used for indication DNA of human herpesviruses: Herpesvirus 6 (HHV-6), Cytomegalovirus (CMV) and Epstein-Barr virus (EBV). All patients, who showed fever or signs/symptoms of infection underwent at least two sets of blood culture. In this study 65 pts (with 79 episodes of BSI) who fulfilled criteria of systemic inflammatory response syndrome with positive peripheral blood cultures were investigated. All pts received empirical anti-infectious therapy with subsequent correction based on the bacteriological, virological and mycological analyses results.
Results: A total Gram-positive (G+) accounted for 62.7% of BSI, Gram-negative (G-) for 33.3% and mixed (G+ and G-) for 4,0%. Among G+ BSI Coagulase Negative Staphylococci and Staphylococcus aureus were the most frequent pathogens (92.0%), among G- BSI Escherichia coli (42.9%) was predominant. Fungi (Candida spp (5), Rhodotorula spp. (1), Aspergillus fumigatus (3)), including mixed infections, were responsible in 11.4% of BSI. It is shown that the incidences of BSI were significantly more frequent at the background of detectable CMV and EBV specific DNA in blood (38.1% and 51.1% respectively *vs* 4.8 and 24.9% in pts without BSI, $p<0.05$) With regard to the outcome 30 days mortality was 15/79 (19.0%) (7/47 (14.9%) in G+ *vs* 6/25 (24.0%) in G- BSI).
Summary/Conclusions: The empirical anti-infectious therapy showed high efficacy. Further epidemiological surveillance is warranted in order emerging resistant strains and related mortality. Reactivation of CMV and EBV is significantly associated with higher incidence of bacterial BSI.

PB1898

SIMULTANEOUSLY MONITORING OF SERUM AND URINE CYTOMEGALOVIRUS DNA IN PATIENTS UNDERWENT HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Hemorrhagic cystitis (HC) is one of the most common complications after hematopoietic stem cell transplantation (HSCT). Early diagnosis of HC is very important to improve the disease prognosis.
Aims: The aim of this work is to explore the significance of simultaneously monitoring serum and urine cytomegalovirus(CMV) DNA on diagnosing HC after HSCT.
Methods: 57 patients were included in this study and median age was 32 years

(range 10-54). 40 patients were diagnosed of acute leukemias, 6 were lymphoma, 5 were severe aplastic anemia, 4 were chronic myelocytic leukemias, 1 was myelodysplatic syndrome and 1 was multiple myeloma. Among the 57 patients, 30 patients received graft from HLA-identical relative donors, 10 patients received graft from HLA- identical unrelated donors, 10 underwent auto-HSCT and 7 underwent unrelated cord blood transplantation. Serum CMV DNA was tested weekly by real-time PCR from one week before conditioning to 100 days after HSCT. For patients with CMV viremia or lower urinary tract symptoms, urine CMV DNA was also tested.
Results: Within 100 days after transplant, 23/57 (40.35%) patients developed CMV viremia. Among the 23 patientsa, 8 developed CMV uremia. Only 2 (5.88%) patients presented CMV uremia in the 34 patients with normal serum CMV DNA. All of the 10 patients with CMV uremia had complaints of lower urinary tract symptoms and were found abnormal urine routine result and CMV related HC was considered. 10 patients received anti-virus treatment and all of them obtained complete remission.
Summary/Conclusions: The incidence of CMV infection is still high during HSCT. For patients with CMV viremia, urine CMV-DNA surveillance is necessary in order to diagnose HC early and improve the disease prognosis.

PB1899

MORTALITY PREDICTORS IN PATIENTS WITH BLOOD MALIGNANCIES IN INTENSIVE CARE UNIT AFTER CHEMOTHERAPY
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Background: The leading role in the treatment of malignant blood diseases belongs to high-dose chemotherapy. However, despite the high rates of successful treatment of hemoblastosis, the complications such as suppression of the immune system, septic neutropenia, DIC, multiple organ failure threaten the patient's life. The patients are transferred to the intensive care unit (ICU) for the treatment of such complications. The practical application of new treatment regimens and medications can successfully treat and stabilize patients in critical conditions. Despite this, currently there are groups of patients (especially those who have the extended lung ventilation and multiple organ failure) with poor prognosis of therapy outcome.
Aims: The identification of adverse outcome predictors allows to determine in time the treatment approach, which significantly reduces the risk of developing complications, the cost of treatment, significantly improving survival.
Methods: There was made a retrospective analysis of 138 adult patients in State Financed State Institution "Republican Clinical Hospital named after GG Kuvatova of Ufa" with malignant blood diseases, who received different chemotherapy protocols. However the complications developed, which required treatment of these patients in the intensive care unit. Critical analysis was made using the scales SOFA, LODS, ODIN, SAPS II, APACHE II, ICNARC model. Predisposing causes of hospital mortality for all patients were calculated according to the method of Knaus *et al.* Statistical analysis was made using Statistica package, 9.2. (StataCorp. LP, College Station, TX). The average age of patients - 28.7 years, AML (acute myeloid leukemia) - 88 patients (63.8%), ALL (acute lymphocytic leukemia) - 34 patients (24.6%) and 16 patients (11.6%) with other blood system diseases.
Results: The average time of a patient's staying in the unit before being transferred to the intensive care unit: 7 days. At average, duration of stay in the ICU was 15.5 days. According to the results of staying in ICU the patients were divided into two groups. The first group included patients with fatal case - 47 patients. In this group 6 patients got artificial pulmonary ventilation (12.8%), 11 patients had neutropenia (23.4%), 14 patients had inotropic support (29.8%), 8 patients had a fungal infection (17.0%), 13 patients had a confirmed sepsis (27.7%) and 35 patients had trombocytes less 50x109/ l (74.5%). 35 patients had more than 2 of target affected organs (25.4%). The second group included patients who were transferred from ICU with physical condition improvement - 91 patients, 17 patients have neutropenia (18.7%), 4 patients have fungal infection (4.4%), 26 patients have thrombocytes less 50x109/L (28.6%), and 18 patients have sepsis (19.8%). 31 patients have more than 2 of target affected organs (22.5%). According to the analysis of the first and second groups, the average score on APACHE II - 32 and 12, respectively, on-scale SAPS II - 76 and 34, SOFA - 20 and 5, LODS -4.05 and 6.4, ODIN -1.1 and 1.49.
Summary/Conclusions: The mortality predictors of patients in intensive care unit after chemotherapy are multiple organ failure (loss of 2 or more organ systems), as well as prolonged mechanical ventilation. Many other proposed criteria (eg, age, neutropenia, and data of physiological disturbance rating scales) should not be considered as an absolute outcome predictors of ongoing intensive care.

PB1900

INVASIVE FUNGAL INFECTIONS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA: SINGLE CENTER EXPERIENCE IN TURKEY
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Background: Invasive fungal infections (IFI) are a major cause of morbidity and mortality in patients with hematological malignancies. The risk of IFI increase in prolonged febrile neutropenia and stem cell transplantation.
Aims: We aim to present the data of patients with acute lymphoblastic leukemia (ALL), who developed IFI between the years of 2010 and 2015 at the pediatric hematology department of Erciyes University's Medical Faculty.
Methods: We assessed 166 patients with ALL, aged between 2 and 18 years (mean age of 5 years) in this retrospective study. There were 73 female and 93 male patients. They were grouped as IFI and oral candidiasis. Serum galactomannan, computerized tomography scanning and cultures of tissue, blood or fluids were used to make a definitive diagnosis of IFI.
Results: Among 166 patients with ALL, 21 (12.6%) developed IFI. Furthermore, 110 patients (66.2%) had oral candidiasis. Ten patients (47.6%) with IFI had relapsed/ refractory ALL and three of them underwent haploidentical stem cell transplantation. Five patients (23.8%) also had high-risk ALL. We found Candida spp in 7 patients (33.3%) with IFI, 10 Aspergillus spp (47.6%), 1 Zygomycetes (4.76%), 1 G. capitatum (4.76%), 1 A. strictum (4.76%) respectively. One patient (4.76%) had candidiasis and aspergillosis. All patients received fluconazole as proflactic antifungal therapy during chemotherapy. Effective antifungal drugs were started after diagnosis of IFI. Nine patients received granulocyte suspensions in addition to antifungal therapy. Eleven patients (52.8%) died because of IFI despite all the therapy efforts.
Summary/Conclusions: Since invasive fungal infections (IFI) are a major cause of morbidity and mortality in patients with ALL, further studies are needed to investigate new diagnostic and therapeutic strategies to identify and prevent IFI.

PB1901

EPIDEMIOLOGY AND PROGNOSIS OF PATIENTS WITH HEMATOLOGIC MALIGNANCIES ADMITTED TO INTENSIVE CARE
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Background: The hospitalisation in intensive care unit of a patient with malignant haematology constitutes a difficult problem for haematology and intensive care teams.
Aims: -carry out an assessment of activity of the medical intensive care unit of Farhat HACHED hospital in Sousse. -studying demographic and haematological characteristics of these patients and their specific care in the ICU, mostly in terms of organ failure -study the short-term survival of the population -Finally, this study will attempt to identify prognostic factors for ICU mortality.
Methods: Single retrospective study, in the medical intensive care unit of Farhat Hached hospital Sousse on 20 years (1994-2014).this study included 105 patients with haematological malignancies.
Results: During the study period, 6000 patients were hospitalized in the intensive care unit of which 105 (1.75%) had hematologic malignancies. the median age of the study population was 49 years (15-86 years). the sex ratio was 1.56. the 2 most frequent haematological diseases were non-Hodgkin lymphoma and acute myeloid leukaemia. 33,3% of patients were in the diagnosis stage; 51.4% were in relapse or progression stage and 18.1% were in complete remission. the median scores of APACHE and SAPSII scores were respectively 48 and 32. The reason for admission of patients was acute respiratory failure in nearly half the cases. The cause of acute respiratory failure was pneumonia in 54% and an acute oedema of the lungs in 35% of cases. 45% of patients were intubated and ventilated; the median duration of invasive mechanical ventilation was 2 days. 27 patients had received non-invasive ventilation therapy (25.5%) at admission. forty two (40%) patients needed treatment with catecholamine's. the median length of ICU stay was 4 days and the mortality rate in ICU was therefore 73.3%. Independent predictors factors of mortality in intensive care were severity at admission (score SAPSII) (p=10-3), the use of invasive mechanical ventilation (p=0.015), and the use of catecholamine's (p=0.038).
Summary/Conclusions: The short-term prognosis, according to this study, is exclusively predicted severity at admission, the use of invasive mechanical ventilation, and the use of catecholamines. According to recent data, discussion between haematologist and intensive care, the very early admitted patients and non invasive ventilation seem to improve survival.

PB1902

USEFUL CLINICAL FEATURES AND HEMATOLOGICAL PARAMETER FOR THE DIAGNOSIS OF DENGUE INFECTION IN ACUTE FEBRILE ILLNESS PATIENTS
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Background: Dengue infected patients are presented with acute febrile illness without localizing signs and symptoms. Clinical presentations may mimic other

infections. The serology (Dengue nonstructural protein 1 (NS1) antigen or Dengue IgM antibody) for definite diagnosis is costly and inaccessible in many hospitals. There was no study to identify these daily changes to distinguish dengue infection from the other causes in acute febrile illness patients.
Aims: The aim of the study was to identify the clinical features and hematologic parameters from complete blood count (CBC) to distinguish dengue infection from the other causes in acute febrile illness patients.
Methods: This was a retrospective study. All patients who presented with acute fever between September 2013 and July 2015 were included. The diagnosis with dengue infection must be confirmed by serology (Dengue NS1 antigen or IgM antibody). The control group included rickettsia, leptospirosis, malaria, and primary bacteremia patients who also presented with acute febrile illness without localizing signs. The sample size by calculation was 300 and divided in to 2 groups. Clinical data and CBC results from diagnosis until recovery were reviewed and compared between two groups.
Results: One hundred fifty-four dengue patients and 146 control patients were included. Headache, nausea, loss of appetite and bleeding diathesis were significantly presented in dengue patients compared to control group 47.4 *vs* 34.2, 33.8 *vs* 15.1, 34.4 *vs* 15.8, 5.8 *vs* 0% respectively (p=0.05). There were several hematologic parameters from CBC which were diverse in dengue patients compared to control group. Moreover, this study also identified the day of fever which these parameters were statistically significant. The dengue group had higher hemoglobin and hematocrit from day 3 to day 10 (highest on day 7; 14.3 *vs* 12.9 g/dl, 43.3 *vs* 39.2%, p <0.001), lower white blood cell (WBC) count from day 1 to day 10 (lowest on day 4; 3,333 *vs* 8,561 per cu.mm., p <0.001) and lower platelet count from day 3 to day 10 (lowest on day 6; 68,910 *vs* 196,137 per cu.mm., p <0.001). For the differential count of WBC, dengue infection showed higher monocytes on day 1-4 (highest on day 2; 11.7 *vs* 5.4%, p <0.001), higher atypical lymphocytes on day 5-9 (highest on day 7; 13 *vs* 0.4%, p <0.001) and higher eosinophils on day 9-10 (highest on day 9; 2.2 *vs* 0.7%, p 0.001). Furthermore, the neutrophil to lymphocyte ratio of dengue group was >1 on the first 5 days then reversed on day 6 to Day 9 (0.69, 0.89, 0.83, and 0.80 respectively) but in non-dengue group, the ratio was always >1.
Summary/Conclusions: We identified important clinical features and useful CBC parameters to differentiate dengue patients from other causes of acute febrile illness patients. This could be done in local hospital to give diagnosis, tailor to further investigation and early treatment.

Myelodysplastic syndromes - Biology

PB1903

DIFFERENT WHO SUBTYPES OF CHROMOSOMALLY NORMAL MDS SHARE COMMON AMPLIFIED AREAS ON ACGH/SNPA

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Background: Various studies have reported that aCGH and SNPa can reveal hidden prognostically relevant karyotype alterations in chromosomally normal MDS patients as compared to conventional cytogenetics: aCGH offers a superior resolution of unbalanced chromosomal defects and SNPa identifies areas of copy neutral loss of heterozygosity (LOH).
Aims: Thus, the principal aims of the present study were to test the power of aCGH/SNPa in chromosomally normal MDS patients, to establish whether the extension and the number of cryptic gains/losses and LOH areas correlated with the WHO MDS subtype and to check whether patients with the same WHO subtype shared common abnormal areas.
Methods: aCGH/SNPa were carried out on the DNA of mononuclear cells from fifteen patients examined between January 2013 and December 2015. There were four females and eleven males, whose median age was 66 years (range 24-78). According to WHO classification, 9 patients were diagnose as RA, one as RA with ringed sideroblasts, one as refractory cytopenia with multilineage dysplasia (RCMD), one as RAEB-1 and 3 as RAEB-2. According to R-IPSS, 11 patients were considered low-risk, 2 intermediate-1 risk and 2 intermediate-2 risk. Median follow-up was eight months (range 1-23). At the time of the analyses one patient who progressed to AML and was submitted to two allogeneic HSCT (allo-HSCT) has relapsed and died; the RAEB-1 patient progressed to RAEB-2 and is alive nine months after an allo-HSCT. Molecular karyotyping was performed using the SurePrint G3 Human Cancer CGH+SNP Microarray Kit 4X180 (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's enzimatic labeling protocol. The array was scanned through the Agilent scanner G26000DA and analysed with CytoGenomics software (v3.0.6). FISH was carried out to confirm aCGH/SNPa results; commercial probes were obtained from Abbot Molecular Inc. (Chicago, Il, USA) and Kreatech (Amsterdam, NL) and applied according to manufacturer's guidelines.
Results: aCGH/SNPa revealed an abnormal pattern in all MDS patients. In the three RAEB-2 patients aCGH showed the highest number of cryptic chromosomal lesions and many areas of LOH. Unexpectedly, two RAEB-2 patients presented a monosomy 7 together with additional defects including a p53 loss and a 5q deletion. The clinical value of these aCGH/SNPa results was strengthened by the fact that one patient progressed to AML that never achieved complete remission despite two allo-HSCT, whereas the other two patients experienced a quick AML progression. In the other twelve patients aCGH/SNPa detected small chromosomal lesions which size varied between 5kb and 1.8Mb and LOH areas of much longer size (range 1Mb-5.7Mb). Interestingly, by comparing the various chromosomal lesions, it was observed that different patients with different WHO MDS subtypes shared common amplified areas that included the NOTCH1 gene in five patients and the FGFR3 gene in three.
Summary/Conclusions: i) aCGH/SNPa can truly reveal cryptic chromosomal lesions in chromosomally normal MDS patients, a finding that determines a change in patients' R-IPSS score; ii) advanced MDS presented the highest number of lesions which size was longer than that of lesions present in early MDS; iii) patients with different WHO MDS subtypes shared common amplified areas that contained cell cycle genes with a potential role in MDS pathogenesis.

PB1904

THE ASXL1 GENE ALTERATIONS IN BONE MARROW CELLS OF PATIENTS WITH DELETION 20Q AND MYELOID DISORDERS

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Background: In recent years, identification of gene mutations together with chromosomal aberrations allows the better characterization and treatment stratification of patients with myeloid disorders. Deletion of 20q - del(20q) - is a recurrent abnormality observed in myelodysplastic syndromes (MDSs), myeloproliferative neoplasms (MPNs), or acute myeloid leukemia (AML). As a sole aberration this finding gives a good prognosis. In our previous study we proved a fusion of the *ASXL1* and *TSHZ2* genes resulting in an isodicentric chromosome of a deleted 20q in a patient with MDS. The *ASXL1* gene maps to chromosome region 20q11.21 and in contrast with del(20q) recurrent mutations in this gene are generally associated with poor prognosis.
Aims: The aim of this study was to determine the frequency of *ASXL1* gene alterations in bone marrow cells of patients with del(20q) and/or with ider(20q) as a sole aberration and to characterize the breakpoints with molecular cytogenetic techniques.

Methods: Fluorescence *in situ* hybridizations (FISH) with locus specific probes for 20q11 and 20q12 regions (Abbott, Kreatech) confirmed the cytogenetically observed deletions of 20q in a cohort of 21 patients (15 male, 6 female, median age 69 years) with myeloid disorders (MDS 14x, MPN 3x, myelofibrosis 2x, thrombocytopenia 2x). In nineteen patients deletion of 20q was a sole aberration, in two patients an isodicentric chromosome of deleted 20q was detected by FISH with a subtelomeric probe 20p/20q (Abbott). Metaphase FISH mapping with a set of four bacterial artificial chromosome (BAC) probes (BlueGnome) localized in 20q11.21 and 20q13.2 was used to determine the breakpoints. Array comparative genomic hybridization (aCGH, CytoChip Cancer 4x180K v2.0, BlueGnome/Illumina) was performed on DNA samples of bone marrow cells of 11 patients with suspected *ASXL1* gene deletion to find out the gene copy number variation.
Results: A weak signal of RP11-358N2 BAC probe (30.93-31.12 Mb from telomere on the short arm, 20q11.21) was observed in six patients (29%), suggesting the proximal breakpoint of the deletion in the *ASXL1* gene (30.95-31.03 Mb, 20q11.21). However, the distal breakpoint in the *TSHZ2* gene (51.80-52.11 Mb; 20q13.2) was found in one patient only. In seven patients (33%) the signal of RP11-358N2 BAC probe was not present on the derivative chromosome confirming the *ASXL1* gene deletion. In the remaining eight patients the proximal breakpoint of the deletion was determined distally to the *ASXL1* gene. In the group of six patients with proximal breakpoint in *ASXL1* gene aCGH determined the breakpoint: in one patient in exon 1, in one patient in exon 4, in three patients behind the exon 4, in one patient in exon 5-8 and in one patient in exon 12. Five patients died in a group of thirteen patients with *ASXL1* gene alteration.
Summary/Conclusions: Deletion 20q is assumed to play a key role in pathogenesis of myeloid malignancies. FISH with BAC probes used is a reliable fast technique that can point out patients with *ASXL1* alterations and therefore potentially worse prognosis than expected. In our cohort, the *ASXL1* gene was altered in thirteen out of twenty-one patients (62%). We suppose that the determination of the *ASXL1* gene alterations in del(20q) cases may have the prognostic impact, but the relations with clinical data should be studied in a larger cohort of patients.
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PB1905

ASSOCIATION BETWEEN POLYMORPHISM OF FOLATE AND METHIONINE METABOLISM RELATED GENES AND ABERRANT DNA METHYLATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME
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Background: Abnormal DNA methylation is often seen in patients with myelodysplastic syndrome (MDS). Polymorphism of folate and methionine metabolism (FMM) related genes can influence the methylation process and, therefore, modify the risk of MDS development.
Aims: The aim of this study was to assess the association between polymorphism of FMM related genes and aberrant methylation of promoter regions of several tumor supressor genes in MDS patients.
Methods: Fifty-one patients with MDS (24 men and 27 women, mean age 62.4 yrs) were genotyped for the MTHFR C677T, MTHFR A1298C, MTR A2756G, MTRR A66G and MTHFD G1958A polymorphisms by PCR-RFLP technique. The differences in allele and genotype frequencies were assessed by Fisher's exact test with computation of odds ratios (OR), their 95% confidence intervals (CI) and p-values. Methylation-specific PCR was used to study the methylation status of promoter regions of *SOX7*, *p15^{INK4b}*, SFRP1, SFRP4 and SFRP5 genes.
Results: Twenty-one patients (9 men and 12 women) had aberrant methylation of 0-2 genes (0-2 MG) and 30 patients (15 men and 15 women) had aberrant methylation of 3-5 genes (3-5 MG). The MTRR 66 GG genotype was more frequently seen in the group 0-2 MG than in the group 3-5 MG (47.6% *vs* 13.3%, respectively, OR=5.9, 95%CI: 1.5-22.9, p=0.011). The MTHFR 1298 CC variant was present in 6 patients from the 3-5 MG and in 1 patient from the group 0-2 MG and (20.8% *vs* 4.8%, respectively, OR=5.0, 95%CI: 0.6-45.1, p=0.2). The presence of the MTHFR 677T allele was increased in male patients from the group 0-2 MG when compared to male patients in the 3-5 MG group (100% *vs* 46.7%, respectively, OR=21.5, 95%CI: 1.2-437, p=0.01), and female patients from the 0-2 MG group (100% *vs* 33.4%, respectively, OR=35.9, 95%CI: 1.7-770, p=0.005). At the same time, positivity for the MTHFR 677T allele was not different between women from 0-2 MG and 3-5 MG groups (33.4% *vs* 46.7%, respectively, OR=1.8, 95%CI: 0.4-8.4, p=0.7). The simultaneous presence of the MTHFR 677T allele and MTRR 66GG genotype was more often detected in the 0-2 MG group than in the 3-5 MG group (33.3% *vs* 10.0%, OR=4.5, 95%CI: 1.0-20.1, p=0.07).
Summary/Conclusions: We conclude that polymorphism of the FMM related genes could have an important role in mechanism of epigenetic disturbances in MDS associated with aberrant methylation of CpG islands of tumor-supressor genes.

PB1906

DEPLETION OF PROINFLAMMATORY MONOCYTES AND INCREASED LEVELS OF CD56 EXPRESSION ON CLASSICAL MONOCYTES IN THE PERIPHERAL FROM PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES
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Background: Myelodysplastic syndromes (MDS) are hematologic neoplasms characterized by morphologic dysplasia and peripheral blood (PB) cytopenias. Flow cytometry (FC) studies have revealed that bone marrow monocytes (Mon) from MDS patients have abnormal maturation patterns and aberrant antigen expression. However, the immunophenotypic alterations in PB monocytes (PB-Mon) have been much less explored.
Aims: To evaluate the levels of CD11b, CD11c, CD13, CD14, CD15, CD16, CD64 and HLA-DR expression in PB-Mon from patients with lower risk MDS (LR-MDS), as compared to normal individuals (NI). To quantify the proinflammatory (CD14+loCD16+) and classical (CD14+hiCD16-) PB-Mon subsets, and to search for abnormal CD56 expression on PB-Mon from LR-MDS patients.
Methods: Fourteen patients with LR-MDS (8 males, median age 76 years), and an equal number of NI (blood donors, 8 males, median age 55 years) were studied. Patients who were being treated with myeloid growth factors were excluded, as did patients with active infections and other concomitant neoplasms. The median time from the diagnosis was 7.6 years, ranging from 0.5 to 12.6 years. Seven patients had refractory anemia with ringed sideroblasts (RARS), 4 patients had refractory cytopenias with unilineage dysplasia (RCUD), and 3 patients had refractory cytopenias with multilineage dysplasia (RCMD). Eight patients had low IPSS risk and 6 patients had intermediate 1 IPSS risk. The median PB-Mon count was of 426/mm3 (17 to 1132), in LR-MDS (>1000/mm3 in 1 case) and 482/mm3 (294 to 937), in NI (no cases with >1000/mm3). PB samples were collected into EDTA-K3 containing tubes. Cell immunophenotyping was performed by 8-color FC using fluorochrome conjugated monoclonal antibodies with different specificities (T1: CD15-FITC, CD13-PE, CD34-PerCPCy5.5, CD10-PC7, CD11b-APC, CD14-APC-H7, CD16-V450, CD45-KO; T2: HLA-DR-FITC, CD64-PE, CD34-PerCPCy5.5, CD56-PC7, CD11c-APC, CD14-APC-H7, CD16-V450, CD45-KO), and a whole blood stain-lyse-and-then wash method (FACSLysing, Becton Dickinson–BD). A normal PB sample was run in parallel with each patient. Sample acquisition was performed in a FACSCanto II flow cytometer (BD), calibrated according to the Euroflow SOP. Data analysis was done with Infinicyt (Cytognos). Results are expressed as median, minimum and maximum values of the median fluorescence intensity observed for each marker. P values <0.05 were considered statistically significant (Mann-Whitney U test).
Results: PB-Mon from patients with LR-MDS had FSC and SSC similar to the PB-Mon from NI (p>0.05). In the same way, the overall levels of CD13, CD14, CD15, CD45 and CD64 expression on PB-Mon from patients with LR-MDS did not differ significantly from those observed in NI (p>0.05). In contrast, PB-Mon from LR-MDS patients had significantly higher levels of CD56 (p=0.006), and lower levels of CD11c (p=0.004), CD16 (p=0.005) and HLA-DR (p=0.042), and showed a tendency for a lower CD11b expression (p=0.089), as compared to NI. Additionally, PB-Mon from LR-MDS patients had a marked decrease in the fraction of CD14+loCD16+ subset (3.1% ranging from 0.1 to 8.1% and 11.5% ranging from 4.1 to 27.7%, respectively; p<0.001). Moreover, CD14+hiCD16- PB-Mon from LR-MDS patients had a significantly higher% of CD56+ cells, as compared to NI (14.8% ranging from 0.1 to 98.7% and 7.1% ranging from 0.1 to 14.9%, respectively; p=0.026).
Summary/Conclusions: PB-Mon from patients with LR-MDS have abnormal levels of CD11b, CD11c, CD16 and CD56 expression and a high classical/proinflammatory Mon subset ratio.

PB1907

PRIMARY RESISTANCE TO AZACYTIDINE IN MDS: PRELIMINARY DATA ON THE POTENTIAL ROLE OF HIF-1A EXPRESSION
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Background: The hypomethylating agent azacytidine is the standard of care

for patients with high-risk myelodysplastic syndromes (MDS) (IPSS intermediate-2 or high) and patients with AML with 20-30% bone marrow blasts. Nevertheless, approximately 40% of patients fail to respond, whereas even responders will inevitably relapse. Currently, the exact mechanisms of azacytidine failure are largely unknown and there is no approach to circumvent azacytidine resistance. Hematopoietic and leukemia stem cells (HSCs and LSCs, respectively) reside in a particularly hypoxic niche and several reports have demonstrated the significance of hypoxia in the regulation of both physiological and malignant hematopoiesis. In addition, Hif-1α regulates the expression of human equilibrative nucleoside transporters (ENTs) and ribonucleotide reductase (RR), both of which are involved in the transport and intracellular metabolism of hypomethylating agents.
Aims: The scope of this study is to investigate the potential role of Hif-1α in azacytidine resistance in MDS patients.
Methods: Bone marrow samples from 26 *de novo* MDS patients and 2 CMML patients (21 males and 7 females) with a median age of 75 (60-89) and 10 healthy donors were collected. MDS patients were classified according to WHO as RCMD (1/28), RAEB I (6/28) and RAEB II (19/28) and received treatment with azacytidine at the dose of 75mg/m^2 x7 days SC. Before treatment, bone marrow mononuclear cells were isolated using the Ficol-paque method, followed by RNA extraction using TRIzol reagent and cDNA preparation using Superscript II reverse transcriptase. Hif-1α expression was estimated by real time PCR TaqMan gene expression assay, using the appropriate primers and probes. Relative gene expression was calculated by comparative threshold cycle (ΔΔCt) method. β-actin was used as a housekeeping gene.
Results: Out of the 28 patients used in our study, 17 responded to azacytidine-treatment (including CR, PR and HI) while 11 failed to respond. We found by real time PCR that the ΔΔCt ratio of Hif-1α/β-Actin expression for control samples was 0,72±0,13, for MDS patients who responded to azacytidine- treatment was 1,564±0,439, while for non-responders 0,839±0,17.
Summary/Conclusions: Our data suggest that increased pretreatment expression of Hif-1α might be associated with better response to azacytidine, indicating a potential role of hypoxia signaling in azacytidine resistance. These encouraging initial results need to be further confirmed in a larger number of patients. The identification of hypoxia related mechanisms in azacytidine resistance will provide novel insights regarding the links of hypoxia signaling with the epigenetic derangement in the pathobiology of MDS and can serve as a platform for the therapeutic targeting of the Hif-1α pathway.

PB1908

PU.1 EXPRESSION CORRELATES WITH PATIENT DISEASE STATUS IN THE MYELODYSPLASTIC SYNDROMES, A NEW PROGNOSTIC MARKER?
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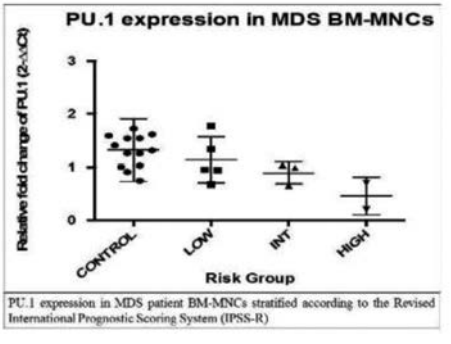
Background: Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell disorders characterised by ineffective haematopoiesis and dysplasia, manifesting as variable degrees and combinations of peripheral blood cytopenia. The result is often transfusion-dependent anaemia, increased risk of infection, bleeding complications, and an increased potential of progression to acute myelogenous leukaemia (AML). In recent years treatment with 5-azacytidine (AZA) has seen an increase in patient survival for intermediate and high-risk group MDS patients, although the precise mechanism of action is not yet fully understood. Previous studies have demonstrated down regulation of the PU.1 transcription factor in high-risk MDS patients, which can be reversed by administration of AZA. However, it is not currently known if PU.1 levels correlate with MDS disease severity and/or prognosis.
Aims: Assess if PU.1 expression levels in MDS patients correlate with disease prognosis as determined by risk group stratification using the Revised International Prognostic Scoring System (IPSS-R). In addition, we will use commercially available cell lines (SKM-1, MOLM-13, K562, HL60) to explore the potential of AZA in correcting down-regulated PU.1 expression that is seen in high-risk MDS.
Methods: BM specimens were collected from 10 patients diagnosed with MDS who were stratified according to IPSS-R guidelines (5-low, 3-int, 2-high risk) and from 13 haematological normal controls. Samples were enriched for the mononuclear fraction by Ficoll separation. Total RNA was extracted and analysed by Real Time PCR for PU.1 expression relative to the housekeeping gene GAPDH using the $2^{-\Delta\Delta CT}$ method. *In vitro* models of MDS, SKM-1 and MOLM-13 were treated with 1 μM AZA for 24, 48, or 72hr followed by analysis of PU.1 expression analysis by RT-qPCR.
Results: Analysis of patient samples revealed that PU.1 expression is significantly lower in high-risk patients compared controls. In addition, preliminary data suggests that PU.1 expression also correlates with disease severity and could provide insight as a prognostic marker. PU.1 expression was significantly increased upon treatment with 1 μM AZA in commercially available cell lines.
Summary/Conclusions: PU.1 is found to be down regulated in high-risk MDS and it appears that expression correlates well with the IPSS-R classification of prognosis group. As such, PU.1 provides a potential biomarker for MDS diagnosis and progression. In addition, PU.1 expression in MDS *in vitro* models is increased by treatment with AZA providing a potential mechanism for AZA function *in vivo*.



PU.1 expression in MDS patient BM-MNCs stratified according to the Revised International Prognostic Scoring System (IPSS-R)

Figure 1.

PB1909

MLPA® (MULTIPLEX LIGATION-DEPENDENTPROBEAMPLIFICATION) IS A FEASIBLE, COST-EFFECTIVE, TECHNIQUE FOR GENETIC CLASSIFICATION OF MYELODYSPLASTIC SYNDROME
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Background: Since their inclusion in IPSS-R in 2012, cytogenetic alterations play a decisive role in the prognosis of patients with myelodysplastic syndrome (MDS). These alterations may be identified through several methods; cytogenetic study, including karyotype and FISH, is the most widely used and proved. However, new molecular techniques for the detection of these alterations have appeared which may be equally effective and far less expensive.
Aims: This study evaluates the MLPA cost-effectiveness in detecting cytogenetic alterations comparing it directly to FISH as gold standard test and karyotype.
Methods: We prospectively collected 131 bone morrow mononuclear cells samples from 118 MDS patients from September 2011 to June 2015. DNA was isolated by salting out and Multiplex Ligation-dependent Probe Amplification (MLPA), MRC Holland, following manufacturer recommendations. We did cytogenetic studies (karyotype and FISH) by conventional techniques. Human material and clinical information was obtained with informed consent and approval of institutional ethics committee.
Results: We obtained MLPA data from 131 bone marrow samples from 118 patients with morphological diagnosis of MDS, examined in our Institution. Karyotype was performed in 104 samples, the technique failed in six and 21 were not sent. FISH was only performed in cases with with abnormal or dubious karyotype; MLPA was performed in 125 samples, in 6 cases we did not receive sample. Alterations were detected in 39.4%, 71.4% and 52% by kariotype, FISH and MLPA, respectively. Specificity of MLPA could be assessed by comparing it with karyotype. Instead we could analyze the sensitivity of MLPA comparing with the most sensitive standard technique, FISH. Forty results were included for sensibility analysis, 31 studies corresponded to new diagnosis and 9 to follow-up samples. Comparing MLPA with karyotype, MLPA showed a high specificity 95% ($p<0.05$) (S: 71% E 95%). We could compare fISH and MLPA in 40 cases (Figure 1), MLPA analysis showed sensitivity of 75% and specificity of 85.7% ($p<0.05$). The results were correlated in 74.2%. We observed a increase of sensitivity (85% and 82% ($p<0.05$) in the subgroup of new diagnosis. Moreover, MLPA detected several alterations which had gone unobserved by FISH, such as: –Y, SMAD4+ y 8pq+. MLPA also proved less expensive in both reagents and staff time.

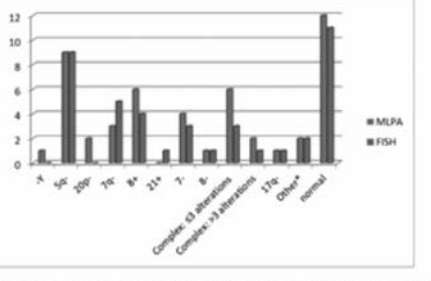

Figure. Detection comparison of the most frequent and clinically relevant abnormalities between MLPA and FISH.

Figure 1.

Summary/Conclusions: Our data indicate that MLPA is a useful tool, as valid as FISH and with a better cost-effectiveness. Although it has sensitivity limitations (in cases of follow-up), it can be used as first-line screening and also as a complementary test for the cytogenetic study. Furthermore, MLPA allows the simultaneous assessment of many loci and the detail study of the most important ones, adding more information to the analysis of copy number variations, which are the most frequent and relevant abnormalities in MDS.

PB1910

CHARACTERIZATION OF THE ERYTHROPOIETIC RESPONSE OF THE DYSPLASTIC MARROW

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Background: Myelodysplastic syndromes (MDS) are characterized by peripheral citopenias within the context of a dysplastic, and frequently hypercellular bone marrow. Anemia is one of the most frequent findings, with 20 to 40% of patients responding to exogenous erythropoietin (Epo), with or without the addition of G-CSF, despite normal to elevated levels of serum endogenous Epo, reflecting the complexity of erythropoietic control in these syndromes. Nevertheless, a different likelihood of response to exogenous Epo has been identified between patients with Normal (<=20 IU/L), Mildly Elevated (20-100 IU/L), Moderately Elevated (100-500 IU/L) and Markedly Elevated (>500 IU/L) endogenous Epo levels.
Aims: To further characterize the early erythropoietic response in MDS and its relation to serum Epo levels, and thereby analyze the medullary response to anemia, focusing on the percentage of erythroblasts in maturation stages I to IV, the expression of surface transferrin receptor (TfR, CD71) and the levels of hemoglobin (Hb) and ferritin.
Methods: We prospectively analyzed 57 newly-diagnosed MDS patients, focusing on MDS subtype; bone marrow morphology; and endogenous Epo levels, serum ferritin levels and Hb concentration at diagnosis. Flow cytometry was performed on all patients (CD45, CD34, CD117, HLA-DR, CD71, CD36, CD35, CD44 and CD105) and on 16 normal controls (samples collected during orthopedic surgery).
Results: Patients (63.2% female) had a mean age of 71.2±11.3 years (range: 22-89) and controls (68.8% female) had a mean age of 63±15; 70.2% of patients had RCMD, 8.8% had RCUD, 8.8% had RCRS, 7.0% had RAEB-1, 3.5% had RAEB-2 and 1.8% had 5q- syndrome; 35% of patients had Normal Epo, 53% had Mild elevations, 8% had Moderate and 4% had Marked elevations. There was no association between the percentage of erythroblasts (by flow or morphology) and the MDS subtype (p=NS), degree of dysplasia (p=NS), Epo levels (p=NS), ferritin levels (p=NS) or Hb levels (p=NS), nor between Epo levels and the MDS subtype (p=NS), although, as expected, there was an inverse correlation between Hb and Epo levels (r= -0.35, p=0.0014). There were no differences in the percentages of erythroblasts in stages I to IV between the normal marrow and MDS patients with Normal-to-Mildly elevated Epo levels (p=NS); however, there was a significant threefold increase in Stage I (2.2±1.0% *vs* 0.7±0.1%, p<0.001) and Stage II (4.8±2.9% *vs* 1.8±0.2%, p=0.02) erythroblasts in patients with Epo levels over 100 IU/L compared to patients with Epo<100 IU/L. There was a decrease in the expression of TfR in patients with MDS with Epo<100 IU/L compared to Epo>100 IU/L, for Stages I (p<0.001), II (p=0.019) and III (p<0.001), but not IV (p=NS), with patients with Epo>100 IU/L reaching a similar expression of transferrin receptor as normal marrows, across all stages (p=NS for I to IV).
Summary/Conclusions: There was an expected inverse correlation between Hb and Epo levels that, however, was not reflected in the percentage of erythroblasts in the bone marrow. The distribution of erythroblasts by maturation stage was identical in normal marrows and patients with MDS with normal to mildly elevated (<100 IU/L) Epo, as observed in the clinical model of response to exogenous Epo. The percentage of early stage erythroblasts increased in patients with Epo levels >100 IU/L, likely reflecting an increase in the commitment of CD34+ cells to the erythroid lineage. The expression of surface transferrin receptor decreased in all stages with Epo<100 IU/L compared to the normal marrow, with levels over 100 IU/L being necessary to obtain similar levels of expression of TfR to the normal marrow, possibly explaining the clinical model of increased likelihood of response to exogenous Epo in patients with levels <100 IU/L, and the fact that the expression of surface transferrin receptor is Epo-dependent both through transcriptional and post-transcriptional mechanisms.

PB1911

USEFULNESS OF MUTATIONAL ANALYSIS FOR DIAGNOSIS OF IDIOPATHIC CYTOPENIA OF UNDETERMINED SIGNIFICANCE AND MYELODYSPLASTIC SYNDROME

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Background: Myelodysplastic syndrome (MDS) is a heterogeneous group of myeloid neoplasms characterized by cytopenias that are associated with impaired hematopoietic differentiation, and have a risk of progression to acute myeloid leukemia (AML).
Aims: Aim of this study is to identify the frequency of clonality revealed by next generation targeted sequencing in patients with idiopathic cytopenia of unknown significance.
Methods: A total of 36 patients with idiopathic cytopenia of undetermined significance (ICUS), were enrolled in this study. Targeted sequencing of 87 selected genes was performed.
Results: The putative mutations were analyzed compared to a normal reference control population. 25. /36 (69.4%) ICUS patients harbored at least one mutation.

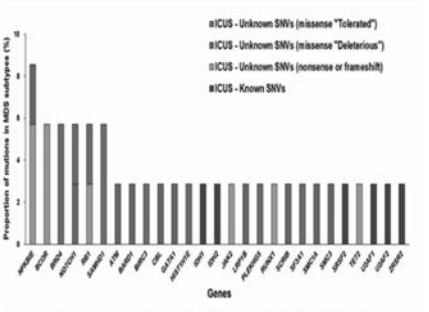

Figure. Frequencies of mutated genes in 36 ICUS patients

Figure 1.

Summary/Conclusions: The molecular profiling of target genes can improve diagnostic accuracy for ICUS and MDS and can assist better subclassification of prognostic groups of MDS patients.

PB1912

THE USAGE OF NEUT-X AND NEUT-Y PARAMETRES IN A ROUTINE HEMATOLOGY LABORATORY AS DIAGNOSTIC MARKERS OF MYELODYSPLASTIC SYNDROMES (MDS)

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Background: NEUT-X and NEUT-Y are structural parameters provided by a hematological analyzer, SYSMEX XE-5000. Notably, NEUT-X evaluates the neutrophil granularity and NEUT-Y the nuclear maturity, which are the average value side scatter diffraction and fluorence, respectively.
Aims: The aim of this study is to investigate the values of NEUT-X and NEUT-Y as diagnostic markers in a routine hematology laboratory in patients with Myelodysplastic Syndromes (MDS)
Methods: The investigation was conducted in AHEPA University hospital. A total of 134 whole-blood samples were collected in tubes with K3-EDTA and were analysed in approximately 3 h of collection, from outpatients and inpatients from different hospital units. 67 of them were from patients with MDS defined from clinical and laboratory findings. Also, 67 randomly selected samples from patients without MDS were included in this study, as a control group. These patients didn't have, perforce, normal values for the complete blood count (CBC). Some of them had anemia due to various causes. Patients with MDS revealed in their majority microscopically hypogranulation or agranulation (N=45) and patients in the other group showed in their blood films neutrophils with normal granulation. The two groups were similar in regard to sex (67 males - 67 females) and age (52 - 90 years avg=78 years). The values of the NEUT-X and NEUT-Y parameters were measured by SYSMEX XE-5000. Mean value comparison independent samples tests was used in order to examine the statistical significance between patients with MDS and control group. The significance level was defined as p <0.05 All statistical analysis was performed using SPSS 22 and Mc Excel 2010.
Results: The values of NEUT-X and NEUT-Y (mean+/-SD) for the control group were (137,49+/-3.17) and (41,02+/-2,70) respectively. The MDS group revealed lower mean values for NEUT-X and NEUT-Y (131,44+/-9,01) and (38,40+/-8,21). The values of NEYT-X and NEYT-Y of MDS were statistically significant lower than the NEYT-X and NEYT-Y values of control (p-values were less than 0,05).
Summary/Conclusions: There is a proportion between low NEUT-X and NEUT-Y values and neutrophil dysplasia. These structural parameters can contribute as auxiliary indicators to the differential diagnosis MDS from other

diseases especially in elderly people with anemia. Also, they can be used as an important part of the daily routine complete blood count.

PB1913

LOW LEVELS OF CD10, CD11B, CD13, AND CD16 EXPRESSION IN THE PERIPHERAL BLOOD NEUTROPHILS FROM PATIENTS WITH LOWER RISK MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic neoplasms characterized by morphologic dysplasia, aberrant hematopoiesis and peripheral blood (PB) cytopenias, and an increased probability of transformation to acute leukemia. Diagnosis of MDS relies on well-defined cytological and cytogenetic criteria but is challenging in a significant number of patients. The detection of abnormal maturation patterns and aberrant antigen expression in the bone marrow (BM) cells has been extensively studied by flow cytometry (FC) and is now considered a promising tool to improve MDS diagnostics. However, the value of immunophenotyping the PB cells from patients with MDS has been largely ignored. Having regard to accessibility of PB samples, it would be useful to establish FC criteria for the diagnosis of MDS in the PB.
Aims: To evaluate the levels of CD10, CD11b, CD13, CD15, CD16 and CD45 expression in PB neutrophils (PB-Neut) from patients with lower risk MDS (LR-MDS), as compared to normal individuals (NI).
Methods: Fourteen patients with previously diagnosed LR-MDS (8 males, median age 76 years), and an equal number of NI (blood donors, 8 males, median age 55 years) were studied. Patients who were being treated with myeloid growth factors were excluded, as did patients with active infections and other concomitant neoplasms. The median time from the diagnosis was 7.6 years, ranging from 0.5 to 12.6 years. Seven patients had refractory anemia with ringed sideroblasts (RARS), 4 patients had refractory cytopenias with unilineage dysplasia (RCUD), and 3 patients had refractory cytopenias with multilineage dysplasia (RCMD). Eight patients had low IPSS risk and 6 patients had intermediate 1 IPSS risk. The median PB-Neut count was of 2590/mm3 (365 to 6945), in LR-MDS (<1500/mm3 in 4 cases) and 4181/mm3 (3083 to 8633), in NI (no cases with <1500/mm3). PB samples were collected into EDTA-K3 containing tubes. Cell immunophenotyping was performed by 8-color FC using fluorochrome conjugated monoclonal antibodies with different specificities (CD15-FITC, CD13-PE, CD34-PerCPCy5.5, CD10-PC7, CD11b-APC, CD14-APC-H7, CD16-V450, CD45-KO), and a whole blood stain-lyse-and-then wash method (FACSLysing, Becton Dickinson–BD). A normal PB sample was run in parallel with each patient PB sample. Sample acquisition was performed in a FACSCanto II flow cytometer (BD), calibrated according to the Euroflow SOP. Data analysis was done with Infinicyt (Cytognos). Results are expressed as median, minimum and maximum values of the median fluorescence intensity observed for each marker. P values <0.05 were considered statistically significant (Mann-Whitney U test).
Results: PB-Neut from patients with LR-MDS had significantly lower FSC (p=0.008) and SSC (p<0.001), as compared to those of NI. In addition, the levels of CD10 and CD11b (p<0.001 in both cases), CD16 (p=0.002) and CD13 (p=0.022) expression in PB-Neut from patients with LR-MDS were significantly decreased in patients with LR-MDS, as compared with NI. No significant differences were observed for CD15 and CD45 expression (p>0.05).
Summary/Conclusions: PB-Neut immunophenotyping may provide useful information for the diagnosis of MDS, as a complement to cytomorphology. Each center should establish its own normal reference values, on the basis of monoclonal antibodies (clones, fluorochromes) and experimental conditions used. In addition, should be given special attention to the conditions of calibration and stability of the cytometer.

Myelodysplastic syndromes – Clinical

PB1914

MULTIDISCIPLINARY EVALUATION AT BASELINE AND DURING TREATMENT IMPROVES THE RATE OF COMPLIANCE AND EFFICACY OF DEFERASIROX IN ELDERLY MYELODYSPLASTIC PATIENTS

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Background: Myelodysplastic syndromes (MDS) are one of the most common hematologic malignancies, the median age at diagnosis is 75 years in most of the series; therefore it can be considered a disease of the elderly patients (pts). Red blood cell transfusion (RBCT) is the main stone of supportive care and a cardinal option to keep patients alive while waiting for effects of specific therapeutic strategies. Transfusion dependency and the consequence iron overload (IOL) has been identified as an independent factor associated with decreased survival. The most important guidelines recommend to start iron chelation therapy (ICT) in all MDS pts with low- and INT1 risk disease with life expectancy >1 year, who have elevated serum ferritin (SF) up to 1000 mcg/L or evidence of iron overload and/or received at least 20 RBCT.
Aims: The aim of the study was to assess the effectiveness of (ICT) in relation of dosing and right management of adverse events (AE) particularly the renal injury. We also evaluate hematological response.
Methods: The safety and the efficacy of DFX were examined in a retrospective multicenter observational study of transfusion MDS patients with International Prognostic Score System (IPSS) low- or INT-1 risk. We included all pts treated with DFX up to 12 months, divided into two groups: the first one (group A) not under multidisciplinary assessment and the second group (group B) with pts under multidisciplinary control by hematologist, Internist, Nephrologist and Immune-hematologist. All pts received DFX at starting dose of 10 mg/kg/day increased up to 30mg/kg/day according to transfusion regimen, SF, IOL, and tolerance.
Results: We evaluated 44 MDS pts (13 female, 31 male); 26 belonging to the first group and 18 to the second group. The mean age was respectively 74.3±9.0 and 77.9±5.5. The ECOG 0-1 was 84.6% and 83.3%, respectively. The median of RBCT prior starting DFX was 20 (range 3-60) in the first group and 13 (4-150) in second group. The median serum ferritin level at baseline was 1125.5 ng/mL (388-2099) and 1317.0 ng/mL (160-3018), respectively. Serum ferritin level decreased at least of 20% as follows: in 29% of pts of first group and in 31% of second group at 3 months, in 17% and 36%, respectively, at 6 months. At 12 months percentages were 22% and 58%, respectively (p=0.06). The drug related AE was evaluated by the Common Terminology Criteria for Adverse Events (CTCAE version 4.02). The SAE occurred in 11% at 3 months, 29% at 6 months and 16% at 12 months. The most Common AE were diarrhea and serum creatinine increase. The rate of drop out after renal AE was respectively 0% and 10%. The positive hematological response in overall pts was observed in 16% at 6 months and 20% at 6 months.
Summary/Conclusions: Un appropriate multidisciplinary assessment of the pre-existing or concomitant comorbidities, the evaluation of the home therapy and of the possible interaction with DFX, a vigilance in co-administration with nephrotoxic drugs may represent strategies to improve the safety and the adherence to ICT and thus the effectiveness. Early starting therapy with DFX at lower doses, maintaining the same dose for the first months avoiding rapid iron depletion, regular clinical and laboratory monitoring appears essential to identify early treatable renal and potentially renal injury avoiding serious adverse effects without necessarily interrupting DFX therapy.

PB1915

PEPTIDE VACCINATION AGAINST CANCER TESTIS ANTIGENS IN COMBINATION WITH AZACITIDINE FOR PATIENTS WITH MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA: AN ONGOING PHASE I STUDY

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Background: Myelodysplastic Syndrome (MDS) is a clonal disorder and characterized by increasing bone marrow failure due to accumulation of genetic and epigenetic changes in hematopoietic stem cells. Patients with high-risk

disease have a poor prognosis and a high risk of progression to Acute Myeloid Leukemia (AML). The dysplastic cells harbor chromosomal breakage, point mutations and promoter hyper-methylation of tumor suppressor genes. For most patients, who are not eligible for bone marrow transplantation, hypomethylating agents, such as azacitidine (AZA), are currently the only treatment option. The demand for more effective therapies in this patient group is huge. Though the mechanism of AZA is not fully elucidated re-expression of tumor suppressor genes can serve as a mechanism for growth arrest. In addition, there is accumulating evidence for an up-regulation of cancer testis antigens (CTA), which could lead to increased immune recognition of tumor cells and immune-mediated tumor cell killing. CTA's are known to be immunogenic and are only expressed at immunoprivileged sites and on malignant cells, making them attractive as targets for therapeutic cancer vaccination.

Aims: We have set up a phase I trial where we combine the treatment of hypomethylating agents with a peptide vaccine, to boost an immune response against four selected tumor associated antigens which are known to be regulated by methylation, in patients with high-risk MDS and AML.

Methods: For this vaccine specifically three CTA's were chosen where abundant re-expression has been shown following AZA treatment, including NY-ESO-1, MAGE-A3 and PRAME. WT-1 is additionally included as this protein has proven to be an important antigen in hematological malignancies and is likewise upregulated in response to AZA treatment. The peptides are between 25-29 mer and include a broad selection of HLA class I and II epitopes. Each vaccine contains ~50 µg of each peptide and is mixed as a suspension with Montanide ISA-51. The use of synthetic long peptides has shown superior effect in contrast to minimal peptide sequences. They contain several CD4 and CD8 T- cell epitopes for a broad range of HLA types, and thus allowing inclusion of participants without prior selection based on HLA expression. Inclusion commence after six courses of AZA and following a treatment evaluation. If there is continued indication of AZA treatment, a set of three vaccinations is given together with the following three courses of AZA. An additional vaccination is then given every six months for two years or until there is unfavorable disease progression.

Results: At the time of writing we are planning for the inclusion of our first patient which is expected to take place in late March 2016. Preliminary results from the first vaccinations will be available to show at the conference.

Summary/Conclusions: 15 patients from the department of Hematology at Herlev Hospital, Copenhagen, Denmark, will be included starting March 2016. The primary endpoint is to elucidate whether the combination of AZA and peptide vaccination is a safe and tolerable treatment, but specific immune responses and clinical efficacy will also be evaluated.

PB1916

PROGNOSTIC FACTORS IN CHRONIC MYELOMONOCYTIC LEUKEMIA FROM SOUTH AMERICA

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Background: Chronic myelomonocytic leukemia (CMML) is a clonal hematologicdisorder sharing features of myelodysplastic syndromes (MDS)and chronic myeloproliferative (MP) disorders.The International ScoringPrognostic System (IPSS) for MDS and its recent revision (IPSS-R)excluded CMML-MP cases. Thus, the CPSS (CMML Prognostic Scoring System) was designed specifically for patients with CMML in 2013. Also, the German Group proposed to divide CMML-1 into: CMML-0 and CMML-1 according to the limit of <5% bone marrow blasts in 2014.However, both proposalshave not been widely reproduced.

Aims: To evaluate different prognostic factors for survival and evolution to AML, to validate the CPSS and to test the new proposed cut-point for BM blast in South American population with CMML *de novo.*

Methods: This is a multicenter analysis of 234 patients with *de novo* CMML from Argentina (152) and Brazil (82) diagnosed between March 1985 to December 2015. Clinical and hematologic data of patients was retrospectively collected and diagnosis of *de novo* CMML wasperformed according to WHO 2008 criteria.

Results: The CMML population showed a median age of 71 (range 15-95) years being 83% older than 60 years old, with a sex ratio M/F: 2.1. With a median overall survival of 31 months, 56 (24%) evolved to AML and 122 (52%) died. Table 1 shows the distribution of patients according to CMML-MD and CMML-MP subtypes; to WHO classification and to German proposal, karyotypes according to CPSS. Also, the different variants for the CPSS were calculated, including transfusion requirement, hemoglobin level limit of 10g/dL and the adjusted by gender. As it is shown, most of the prognostic parameters and all CPSS proposals were useful to predict outcome in our population (Kaplan Meier and log-rank, p<0.05). Since transfusion data was not available for most patients, only models including both hemoglobin cut-points were analyzed in multivariate analysis (Cox Regression, Backward-Stepwise method). The CPSS (hemoglobin threshold of 10 g/dL) sustained its independence both for survival [p<0.001, exp(B) 1.912] and evolution to AML [p<0.001, exp(B) 2.352]. Also LDH level and the German Proposal, not included in the original CPSS system, were analyzed in a multivariate model. This last cut-off for BM blast showed an additive value to predict survival and evolution to LMA [p=0.053 and p=0.003, exp(B) 1.335 and 2.057] when compared with the CPSS-Hb variant [p<0.001 and p=0.052, exp(B) 1.654 and 1.537]. Our results showed that the new proposed classification for CMML dividing them into CMML-0, -1 and -2 may add prognostic value to the CPSS.

Table 1.

Variable		N (%)	OS (50%, months)	*P*	Evolution to AML (months, 25%)	*p*
Age	<60 yrs	38 (17)	28		12	
	≥60 yrs	191 (83)	35		31	
LDH	Normal	98 (54)	42		93	
	Elevated	83 (46)	26		13	
Hb	≥10g/dL	126 (56)	55	*<0.001*	66	*<0.001*
	<10g/dL	101 (44)	25		11	
Hb	M ≥9 y F ≥8 g/dL	169 (75)	40	*<0.001*	31	
	M <9 y F <8 g/dL	58 (26)	16		9	
Plt	≥100000/µL	100 (45)	54		53	
	<100000/µL	125 (56)	25,1		15	
Karyotypes-CPSS	Good	140 (73)	50	*<0.001*	35	
	Intermediate	20 (10)	38		19	
	Poor	33 (17)	18		9	
CMML	- MDS	132 (58)	45	*<0.001*	27	
	-MP	95 (42)	20		14	
CMML	-0	124 (59)	54		93	*<0.001*
	-1	37 (18)	24	*<0.001*	22	
	-2	48 (23)	11		7	
Transfusion dependency	Yes	90 (69)	24		12	
	No	40(31)	103		*NR*	
CPSS (transfusion dependency)	Low	17 (12)	105	*<0.001*	NR	*<0.001*
	Int1	45 (33)	57		31	
	Int2	61 (44)	23		13	
	High	15 (11)	12		5	
CPSS (Hb limit 10g/dL)	Low	53 (28)	99	*<0.001*	NR	*<0.001*
	Int1	59 (31)	36		26.3	
	Int2	63 (33)	23		13	
	High	17 (9)	12		7	
CPSS (Hb according to gender)	Low	64 (34)	99	*<0.001*	NR	*<0.001*
	Int1	60 (31)	33		26	
	Int2	56 (29)	15		12	
	High	11 (6)	18		3	

Summary/Conclusions: Our results showed that the new proposed classification for CMML dividing them into CMML-0, -1 and -2 may add prognostic value to the CPSS.

PB1917

SERUM ERYTHROPOIETIN (SEPO) TESTING, LEVELS, AND SUBSEQUENT TREATMENT PATTERNS FOR TRANSFUSION-DEPENDENT PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

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Background: The European Society for Medical Oncology and the National Comprehensive Cancer Network have developed guidelines for the treatment of MDS, describing sEPO level as a useful measure at initial evaluation and for supporting decisions about whether to treat with erythropoietin-stimulating agents (ESAs). A high pre-treatment sEPO level is associated with a lack of response to ESAs, and therefore patients with high sEPO levels should be considered for other therapies.

Aims: To evaluate rates of sEPO testing and sEPO levels, and the subsequent use of ESAs among patients who became transfusion-dependent (TD) at or after MDS diagnosis.

Methods: A retrospective chart review of patients with MDS was conducted in the US. 26 oncologists and hematologists provided data from sequential patient charts. Patient inclusion criteria were: age ≥18 years, diagnosis of International Prognostic Scoring System Low/Intermediate-risk MDS within the last 1–5 years, and TD onset ≥12 months previously. Patients who had high-risk disease prior to TD onset, had a concurrent malignancy, or were in an MDS clinical trial were excluded. Demographics, disease history, treatment history, and lab values were collected and reported descriptively. Results were compared using chi-square tests.

Results: Information was collected on 133 TD MDS patients. 57.9% were male and median age was 67 years (range 37–89), with a median of 25 months since diagnosis (range 12–58). 66 (49.6%) patients had an sEPO test at diagnosis; of these, 39.4% had levels ≥200 mU/mL, including 13.6% ≥500 mU/mL. For patients without sEPO testing, reasons included practice dynamics (28.4%), testing not considered worthwhile (28.4%), and no intent for ESA use (17.9%). Excluding this latter group, 21.8% of untested patients were treated with ESA *vs* 36.4% of those tested (*P*=0.08).

Summary/Conclusions: Of US patients with TD MDS, only half had undergone sEPO testing at diagnosis. Many had sEPO ≥200mU/mL, including a proportion with ≥500 mU/mL; alternative treatments to ESAs should be considered for these patients. Nevertheless, ESA use occurred in nearly a fifth of patients not tested at diagnosis, suggesting that expanded sEPO testing may identify additional patients not appropriate for ESAs.

PB1918

IS A 1ST LINE ERYTHROPOIETIN APPROACH IN MYELODYSPLASTIC PATIENTS WITH DEL 5Q- USEFUL?

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Background: Efficacy and safety of Erythropoietin (EPO) in patients with myelodysplastic syndromes (MDS) have been demonstrated by various studies, with response rates ≥60%. On the other hand, MDS patients with 5q deletion (del5q) and transfusion requirement can achieve high response rates with Lenalidomide, but this drug has shown severe hematologic and extra-hematologic toxicities.
Aims: Thus, it could be advisable to evaluate the efficacy of EPO treatment as 1st therapeutic choice in this subset of patients.
Methods: To address this issue, 65 consecutive patients with MDS and del5q- [M/F 18/47, median age at onset 72.9 yrs, interquartile range (IR) 65.2-81.1] who received EPO treatment at our Institution from 6/2000 to 7/2015 were retrospectively evaluated.
Results: According to WHO 2008, 30 patients (46.2%) were classified as del5q- syndrome, 1 (1.5%) as refractory anemia, 20 (30.8%) as refractory citopenia with multilineage dysplasia, 9 (13.8%) as refractory anemia with excess of blasts-1 and 3 (4.6%) as refractory anemia with excess of blasts-2: according to IPSS risk assessment, 30 patients (46.2%) were low-risk, 23 (35.4%) intermediate-1, 9 (13.8%) intermediate-2 and 3 (4.6%) high-risk. As to karyotype, 42 patients (64.6%) had an isolate del5q-, while in the remaining 23 patients (35.4%) there were other co-existing cytogenetic abnormalities. Median age at EPO start was 73.3 yrs (IR 65.9 - 81.7), with a median interval from diagnosis of 6.2 months (IR 1.5-14.3). Median Hb level at baseline was 9.0 g/dl (IR 8.2-9.5) and 25 patients (38.5%) were transfusion dependent. Median EPO level at baseline was 120 mU/ml (IR 68.8-270). Thirty-one patients (47.7%) received standard dose EPO (30,000 - 40,000 UI weekly) and 34 (52.3%) high-dose (60,000 - 80,000 UI weekly). On the whole, 22/65 patients (33.8%) [5/25 (20%) with transfusional requirement and 17/40 (42.5%) without transfusional requirement at baseline] achieved an erythroid response according to IWG 2006 criteria, after a median EPO treatment period of 1.9 months (IR 1.0-2.7): among these 22 responding patients, 14 (63.6%) lost the response after a median response period of 14.6 months (IR 7.6-29.6), while 8 patients (36.4%) are still in response continuing EPO treatment. In the remaining 43 non responding patients, EPO treatment was discontinued after a median period of 4.0 months (IR 2.0-5.9) due to resistance. The median overall survival of the entire cohort from EPO initiation was 47.5 months (95% CI 33.7-61.2), without differences between responding and non responding patients (p=0.1).
Summary/Conclusions: This retrospective evaluation showed a substantial reduced efficacy of EPO treatment in patients with del5q- compared to other MDS low-risk subgroups, thus supporting the choice of lenalidomide as 1st line approach in such subset of MDS, particularly in patients with del5q- and transfusional requirement.

PB1919

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) SCREENING IN PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) IN CLINICAL PRACTICE: FREQUENCY AND INDICATIONS

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Background: MDS is a group of bone marrow disorders characterized by ineffective hematopoiesis leading to peripheral blood cytopenias. Most MDS patients (pts) develop significant anemia and red blood cell (RBC) transfusion dependence (TD). In PNH, mutations in the phosphatidyl inositol glycan (PIGA) gene lead to lack of the glycosylphosphatidyl inositol (GPI) anchor on the cell surface allowing complement-mediated lysis to occur. The PNH phenotype includes direct antiglobulin test (DAT) negative hemolysis and cytopenias including TD anemia, hemoglobinuria (resulting in iron deficiency in some pts), and thrombosis. PNH clones are detected in up to 50% of MDS pts, might confound the reason for RBC TD and PNH+ MDS pts may have better response to immunosuppressive therapy (IST). Eculizumab is the first specific treatment for PNH approved in Canada (2009). It reduces hemolysis and RBC transfusion requirements, prevents thrombosis, improves renal function, quality of life and overall survival (OS).We wanted to determine whether PNH as a contributor to anemia is considered in MDS pts.
Aims: We wanted to determine whether PNH as a contributor to anemia is considered in MDS pts.
Methods: All pts with MDS seen at St. Paul's Hospital were reviewed. Only pts with a bone marrow biopsy confirmed MDS diagnosis (dx) since 2009 were included. Data extracted were baseline clinical and laboratory features, clinical course, treatment and outcome. Indicators of hemolysis including increased levels of lactate dehydrogenase (LDH), bilirubin (BILI) andreticulocyte count (RETICS), decreased haptogobin (HAPTO), and results of DAT testing were recorded. High resolution PNH testing was done by flow cytometry for expression of FLAER, CD24, CD14, and CD59 on neutrophils, monocytes and RBC.
Results: Of 395 MDS pts, 152 were diagnosed in 2009 or later. Median age at MDS dx was 73.5 (range 38-91) years and 66% were male. MDS dx was: RA, n=7; RARS, n=17; RCMD±RS, n=40; RAEB, n=23; hypoplastic, n=4; other, n=61. IPSS scores were: low, n=53; int-1, n=59; int-2, n=32; and high, n=8. MDS treatment was supportive care only in 53 pts. The erythropoietin (EPO) level was ≥500mIU/mL in 4 pts. LDH, BILI and RETICS were measured (and elevated) in 96 (23), 109 (10) and 142 (14) pts, respectively and HAPTO was decreased in 2 of 7 measured. DAT was negative in 9 of 13 pts and serum ferritin level <100ng/mL in 14 of 116. No pts had hemoglobinuria or thrombosis. The hemoglobin was <100G/L in 86 at MDS dx. 79 (52%) pts were RBC TD, with a median transfusion requirement of 4 (1-8) units/8 weeks. PNH testing was positive in 1 of 11 pts tested. Reasons for PNH testing were: anemia, n=3 (with abdominal symptoms and pre-MDS dx, n=1 each); new MDS dx, n=2;hypoplastic MDS, n=2; decreased HAPTO; increased RBC transfusion requirements; and iron deficiency, n=1 each; see Figure and Table. Of patients with an RBC transfusion requirement >4 units/8 weeks none underwent PNH testing. At a median follow up of 21.1 (0.7-69.9) months for all patients, 113 were alive and the median OS was not reached.

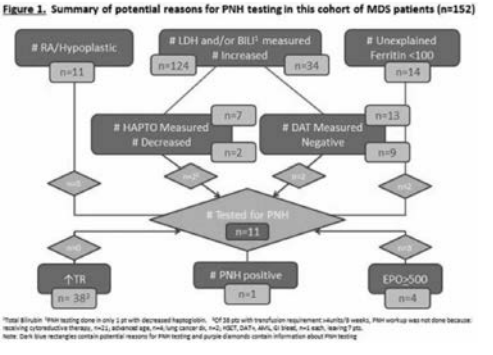

Figure 1.

Summary/Conclusions: PNH was tested for infrequently in MDS patients in clinical practice. Only 11 (7%) of MDS pts since 2009 had PNH testing done despite potential indicators of hemolysis in 27%. Clinical rather than laboratory indicators prompted PNH testing in 6 of 11 pts. Although the clinical significance of PNH clones in MDS is not yet fully defined, complement mediated hemolysis could exacerbate anemia. As there is now an effective treatment available, and PNH+ MDS pts may respond to IST, screening for PNH in MDS should be considered.

PB1920

COMPASIVE USE OF 5-AZACITIDINE IN PATIENTS WITH LOW/INT-1 RISK MYELODYSPLASTIC SYNDROMES

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Background: 5-azacitidine (AZA) significantly prolonged overall survival in higher-risk patients with myelodysplastic syndromes (MDS) in international phase III trial (AZA-001). However, data about efficacy of AZA in lower risk MDS are less consistent and only few studies have addressed this topic.
Aims: Evaluate efficacy and security of AZA in low/int-1 risk MDS patients.
Methods: we evaluate the efficacy and safety in low and intermediate-1 risk MDS patients.
Results: In our institution, a total of 59 MDS patients were treated with AZA between 2006 to September 2015. We evaluated 40 patients diagnosed according to WHO criteria as low/intermediate-1 International Prognostic Scoring System (IPSS) risk MDS. At baseline, median age was 75 year old (range 46-90), male/female ratio 26/14. Median time from diagnosis to Aza treatment was 19,8 months (range 1-185). 80,9% patients were transfusion-dependent, 85,7% had received a prior treatment (rhu-EPO+G-CSF 44,4%, only rhu-EPO 55,5%). Low/Int-1 risk patients received AZA dose of 75mg/sqm/d subcutaneously during days 1-7 in a 28-day cycle. The median number of monthly cycles was 11 (range 1-50), and 58% completed at least 6 treatment cycles, respectively.86% patients had a overall response (53% transfusional independence, 23% PR, 10% CR). OS was 44 months. Overall adverse events documented in these

patients were neutropenia (33%), anaemia (22%) and thrombocytopenia (17%). Non-hematological adverse events: injection site reaction 39%, 50% constipation, 22% diarrhea and 10% fever. Response duration ranged from 4 to 43 months (median 12 months). 25% patients lost response. There were no significant differences in response rate according to age, previous treatment, transfusion requirements, basal EPO and Hb pre-AZA. 8 patients (13%) were transformed to AML after median 23 months (7-36), Time since AZA treatment 14 months (1-24m).
Summary/Conclusions: 1.- 86% patients achieved a overall response. 2.- Time to response is early (3 months), although some patients response later (5 cycles or more). 3.- Efficacy and safety of AZA treatment is a valid alternative in low/int-1 risk MDS patients.

PB1921

A REAPPRAISAL OF THE AZA TREATMENT OUTCOME: ASSESSING THE VALUE OF DISEASE STABILISATION AND EFFECT OF TREATMENT SCHEDULE MODIFICATION
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Background: 5-Azacytidine is a chemical analog of cytidine used in the treatment of myelodysplastic syndrome. We considered two unanswered questions in the treatment of myelodisplastic syndrome: the significance of stable disease and the impact of dose reduction or treatment delay on the outcome of patients.
Aims: In this study we considered a population of patients affected by myelodysplastic syndrome with IPPS intermediate 2 or high risk treated with 5-Azacytidine. We analysed the prognostic impact of stable disease and treatment schedule modification.
Methods: We retrospectively analysed 57 patients treated with 5-Azacytidine from 2008 to 2015 at San Gerardo Hospital, Hematological Unit (Monza, Italy). We defined the response to treatment according to International Working Group 2006. All patients continued the treatment until loss of response, even if they obtained stable disease. The median number of cycles of drug was 7, from a minimum of 1 to a maximum of 27.
Results: The median time between the start of treatment and the loss of response is 24 months. In the subgroup of patients who did not receive bone marrow transplantation the median time is 20 months. The median survival after loss of response is 4,8 months. We established 3 classes: 20 patients obtained a response to treatment, defined as complete response, partial response, marrow CR, cytogenetical response, hematologic improvement; 15 patients obtained stable disease; 22 patients failed the treatment. The median survival from the diagnosis in responding patients, patients with stable disease, or treatment failure is respectively 27 months, 30 months and 10 months. Similarly the median survival from start of treatment of these 3 classes is respectively 23 months, 27 months and 3,7 months. Fourteen out of 57 patients required, during the treatment, a dose reduction of 5-Azacytidine or treatment delay because of toxicity. Considering the treatment efficacy we deduced that 53% of patients who did not need a treatment schedule modification obtained a response or stable disease (23 of 43). Indeed 85% of patients who required a dose reduction or a treatment delay obtained a response or stable disease (12 of 14). This difference, despite the few cases, appears statistically significant with p value 0,02. In the subgroup of patients who required a dose reduction the median survival from diagnosis is 31 months *versus* 25 months in patients who do not have dose reduction. Similarly the median survival from start of treatment is respectively 23 months and 16,7 months. The median survival of all patients who required a treatment schedule modification, as dose reduction or treatment delay, is 31 months from the diagnosis and 23 months from the start of treatment, *versus* 21 months and 16 months in patients who respected the schedule.
Summary/Conclusions: This study shows that in patients affected by myelodysplastic syndrome with IPSS intermediate 2 or high risk treated with 5-Azacytidine, the stable disease should be considered response to treatment. The only patients who have low median survival are treatment failures, in fact outcome is similar in responders and in patients with stable disease. Furthermore dose reduction or treatment delay does not seem to adversely affect the therapy efficacy and survival, but they reduce the treatment toxicity.

PB1922

DIAGNOSTIC VALUE OF PERIODIC ACID-SCHIFF POSITIVITY OF BONE MARROW ERYTHROBLASTS IN MYELODYSPLASTIC SYNDROMES
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Background: The revised 2008 WHO classification confirmed minimal morphological criteria of myelodysplastic syndrome (MDS) diagnosis: at least 10% of bone marrow (BM) cells of at least one hematopoietic cell lineage must show unequivocal dysplasia to be considered as dysplastic. Morphological abnormalities of erythroid cells include cytoplasmic Periodic acid-Schiff (PAS) positivity, but the PAS positivity diagnostic power is not yet fully clear. Neither was this morphological aspect taken into account by the "Rete Ematologica Lombarda (REL)" clinical network that proposed a structured and reproducible approach for the precise recognition of BM dysplasia (Della Porta *et al*. Leukemia 2015; 29:66-75).
Aims: Since dysplastic alterations of BM precursors and of peripheral blood cells are still fundamental for diagnostic classifications, the aims of our study were to evaluate the diagnostic value of erythroblast PAS positivity in MDS and to investigate a possible correlation between PAS positivity levels and other morphological and clinical features.
Methods: We retrospectively examined the results of the cytochemical PAS staining for glycogen in BM smears from 120 MDS patients, 105 patients with non-clonal cytopenia and 49 healthy subjects. By counting 100 nucleated cells for the erythroid lineage and classifying them for their degree of PAS reactivity, we developed a PAS score for MDS identification on the basis of a ROC curve analysis.
Results: PAS positive erythroblasts were observed in 74 (62%) MDS patients, 43 (41%) patients with non-clonal cytopenia and 12 (24%) healthy subjects ($p<0.0001$). In MDS, both positivity rates (mean 3.9%, range 0-31%) and scores (mean 5.8, range 0-49) were significantly higher than those in normal and pathologic controls ($p=0.0001$ and $p<0.0001$, respectively). Positive erythroblasts showed diffuse or granular cytoplasmic reactivity. In MDS, no significant relationship was detected between erythroblast PAS positivity rate or score and dyserythropoiesis grading, multilineage dysplasia or excess blasts, whereas there was a significant inverse correlation between PAS score values and percentages of BM erythroblasts ($p=0.041$) and between PAS score values and percentages of ring sideroblasts ($p=0.012$). Anemic patients showed higher score values than non-anemic subjects ($p=0.016$). PAS positivity was unrelated to karyotype abnormalities. A ROC curve analysis allowed us to identify a PAS score value >0 (AUC=0.642, $p=0.0001$) and a PAS positive erythroblast percentage >0 (AUC=0.629, $p=0.0002$) as optimal cut-off to discriminate MDS patients from controls with sensitivity and specificity ranging from 62% to 66%. Positive and negative predictive values of PAS positivity were 74% and 58%, respectively. Considering the most discriminant morphological features for dyserythropoiesis, the weight of both PAS positivity rate and score in the recognition of BM dysplasia was lower than that of ring sideroblasts and megaloblastosis, but higher than that of defective hemoglobinisation, nuclear lobulation, multinuclearity, cytoplasmic fraying, pyknosis, and internuclear bridges.
Summary/Conclusions: The evaluation of BM erythroblast PAS positivity may be useful in the work-up of patients with suspected MDS, especially if there is only unilineage dysplasia without excess blasts or ring sideroblasts. This parameter should be included in the diagnostic morphological panel to be used for a correct application of the WHO classification.

PB1923

NFE2L2,KEAP1AND NQO1GENE EXPRESSION IN MYELODYSPLASTIC SYNDROME AND MONOCLONAL GAMMOPATHIES-CLINICAL IMPLICATIONS
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Background: Oxidative stress (OS) deregulation has been associated with almost all neoplastic diseases, including leukemia, where it contributes to disease development and progression. NRF2 (*Nuclear factor erythroid-2-related factor 2*) is a transcription factor codified by *NFE2L2* gene (*Nuclear factor erythroid2-like 2*) that activate many antioxidant and detoxification genes, like the NQO1 (NAD(P)H:quinone oxidoreductase 1) gene, through its binding to antioxidant response element (ARE) and regulate ARE-mediated gene expression and induction. Moreover, NRF2 is strongly regulated by KEAP1 (*Kelch-like ECH-associated protein 1*). NRF2-KEAP1 system seems to have an extremely important role in OS regulation, contributing for disease development and/or influence therapy response.
Aims: Our aim was to evaluate the expression levels of *NFE2L2*, *KEAP1* and *NQO1* genes in Myelodysplastic Syndrome (MDS) and Monoclonal Gammopathies (MG) patients, and its correlation with clinical and laboratorial data, in order to identify potential biomarkers of diagnosis and/or prognosis.
Methods: We evaluated 135 samples, 53 MDS (26 refractory cytopenia with multilineage dysplasia; 8 chronic myelomonocytic leukemia; 7 refractory cytopenia with unilineage dysplasia; 5 refractory anemia with excess blasts-1; 4 refractory anemia with ringed sideroblasts; 2 refractory anemia with excess blasts-2; 1 5q-), 40 MG (15 monoclonal gammopathies of undetermined significance; 25 multiple myeloma) and 42 controls (Ctl). Samples were collected after informed consent obtained in accordance with the Helsinki Declaration. Real-time PCR was used to evaluate the gene expression level of *NFE2L2*, *KEAP1*,

NQO1 and *GUS* (control gene). Results were considered statistically significant when $p<0,05$.
Results: Our results showed that patient's group presents higher *KEAP1* expression levels comparing to controls (Patients: 0,175; CTL: 0,097; $p=0,04$), while *NFE2L2*did not presented differences between any groups. When we analyzed by pathology, it was observed that *KEAP1* gene expression levels was higher in GM patients compared with controls (GM:0,2059; CTL: 0,09717; $p=0,009$). No association was observed between MDS and CTL; however RCDM patients have lower levels of *NFE2L2* (RCMD: 1,754; CTL: 5,033; $p<0,05$) and higher levels of *KEAP1* (RCMD: 0,185; CTL: 0,097; $p=0,005$). Furthermore, our preliminary results for *NQO1* gene expression showed that MDS patients present lower levels than controls (MDS: 0,1884; CTL: 01969; $p=0,0058$). No associations were found between clinical and laboratory data (erythropoietin and ferritin levels) and the analyzed genes. Through ROC analysis we observed that *NFE2L2* (cut off <2,044; sensitivity: 75%; specificity: 83,3%) and *KEAP1* levels (cut off >0,1627; sensitivity: 54,17%; specificity: 86,67%) might be diagnostic biomarkers for RCMD patients, as well as, *NQO1* levels for MDS patients (cut off >0,00654; sensitivity: 74,29%; specificity: 78,26%). Also, *KEAP1* levels might be biomarkers for MM patients (cut off >0,1645; sensitivity: 52,38%; specificity: 86,67%). Survival analysis showed that RCMD patients with *NFE2L2* expression levels over 2,044 and *KEAP1* levels under 0,1645 present a tendency for lower survival. The same is observed in MM patients with *KEAP1* levels under 0,1645.
Summary/Conclusions: Our results suggest that the expression pattern of *NFE2L2*, *KEAP1* and *NQO1* genes might be associated with the development of hematological malignancies, and may have a great potential as diagnostic and prognostic biomarkers for Myelodysplastic Syndrome and Multiple Myeloma.
This study is supported by Center of Investigation in Environment Genetics and Oncobiology (CIMAGO), Jorge J. by LPCC-NRC/CIMAGO 2015 Grant, Pires A. by LPCC-Pfizer 2015 Grant andAlves R. is supported by Portuguese Foundation to Science (FCT) grant (SFRH/BD/51994/2012).

PB1924

BIOSIMILAR ERYTHROPOIETIN ALFA FOR THE THERAPY OF ANEMIA IN "LOWER RISK" MYELODYSPLASTIC SYNDROMES. INTERIM RESULTS FROM A PROSPECTIVE OBSERVATIONAL STUDY OF THE "RETE EMATOLOGICA LOMBARDA" (EPOREL)

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Background: Biosimilar EPO use for the treatment of anemia of MDS is based on therapeutic equivalence extrapolated from originator drug employed in other clinical settings; specific prospective evaluation of their activity in MDS are scarce. From November 2014 "lower risk" pts with newly-diagnosed MDS referred to the haematologic centres of REL and symptomatic anaemia participate to an observational multicenter prospective study on efficacy and safety of biosimilar EPO (EPOREL1 protocol).
Aims: 1) *Primary endpoint*: to prospectively assess the response rate of "lower risk" MDS anaemic pts treated with biosimilar EPO alfa; 2) *Secondary endpoint*: to validate the prognostic power of Scandinavian Myelodysplasia Group score (SMG) (Hellstrom-Lindberg E et al, Br J Haematol 1997; 99:344) in this setting.
Methods: The study was in accordance with the ethical standards of the Committee of Human Experimentation of the coordinating center. MDS were diagnosed according to WHO 2008 criteria and classified according to IPSS and IPSS-R. EPO alfa was administered subcutaneously at the starting dose of 40.000 U/w. EPO was doubled in not responsive pts after 2 ms, reduced at mainteinance dose if hgb ≥12 g/dl and stopped in case of refractoriness, relapse, AML evolution, death or major toxicity. Responses were defined by IWG criteria 2006.

Table 1.

"LOWER RISK" MDS 21 pts			
WHO 2008	N (%)	IPSS	N (%)
RCUD-RA +/- RS	9 (43)	LOW	12 (57)
RCMD +/- RS	9 (43)	INTERMEDIATE 1	9(43)
RAEB I	2(9.5)		
MDS/MPN	1(4.7)	IPSS-R	N (%)
		VERY LOW	2 (9.5)
		LOW	8(38)
		INTERMEDIATE	11 (35)

Results: Data were collected from the charts of 26 consecutive pts treated with biosimilar EPO alfa; 21 were evaluable (five too early). Median age was 79 yrs (71-90); F/M 0,3. Their diagnostic and prognostic characteristics are described in Table 1. Twelve (57%) pts were transfusion dependent with median transfusion need of 2 units/ms (1-3). Median haemoglobin was 8,7 (7,5-9,8) and median endogeneous serum EPO, evaluable in 19 pts (90%), was 50 U/L (11-1410). Fifteen pts (71%) responded after a median time of 1 mo (1-10; D.S. 4,1, C.I.95%), 5/12 (42%) transfusion dependent pts reached transfusion independency. EPO dose was incremented to 80.000U/w in 13 pts (62%) after a median of 2 ms (1-8). After a median f.up of 6 ms (2-15), all responsive pts maintained the response and are still on treatment. Ten of 11 (91%) pts with SMG score good and 1/6 (17%) pts with SMG score intermediate achieved an erythroid response (p=0.005). No adverse events were reported up to now. AML evolution was documented in three non responsive cases after a median follow-up of 3 ms (2-6): two pts were off therapy; one case IPSS int1, IPSS-R int, normal karyotype, evolved during treatment. Two pts died: one off-therapy after AML transformation for septic shock, one responsive with previous cardiopathy for congestive hearth failure.
Summary/Conclusions: To our knowledge this is the first prospective study on MDS pts treatment with biosimilar EPO alfa (HX575). The response rate and safety profile of biosimilar EPO alfa is comparable with published data on originator EPO, even in this older MDS cohort. The study confirms the prognostic power of SMG score in this setting. Further evaluation after prolonged follow-up on larger series of pts is warranted.

PB1925

PROGNOSTIC FACTORS IN MYELODYSPLASTIC SYNDROMES AND THEIR INFLUENCE ON OVERALL SURVIVAL

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Background: Prognostic factors have been evaluated so far in different combinations in order to detect those that would influence overall survival (OS) in patients with MDS and would be incorporated in future revisions of prognostic scoring systems.
Aims: 1.Analysis of chromosomal abnormalities with 'Multiplex ligation-dependent probe amplification' (MLPA), 2.Evaluation of the prognostic significance of age, sex, bone marrow (BM) blast percentage, cytopenias, MCV, transfusion dependency, serum ferritin, LDH, serum albumin, comorbidities and chromosomal abnormalities and 3.Their implication on OS and transformation in acute myeloid leukemia (AML).
Methods: 215 adult patients with 'de novo' and t-MDS were included in the study, diagnosed at the University Clinic of Hematology in Skopje, from January 2011 till April 2015, with follow-up period of 52 months. Patients were divided into two groups: 70 patients as an investigational group (IG) and 145 patients as a control group (CG). Detection of the chromosomal abnormalities was performed with the method MLPA. Informed consent was obtained.
Results: Patients were classified according to the FAB and WHO classifications. Risk stratification was made in accordance with IPSS and R-IPSS. OS and transformation in AML were followed. We evaluated the association among 17 factors and OS in the IG. The univariate analysis revealed only 4 from 17 factors associated with OS: platelet number, blast percentage in the BM, IPSS and R-IPSS. Multivariate analysis revealed that predictors of the event were platelets <100x109/L, IPSS - low risk and R-IPSS-intermediate risk. We also evaluated the association among 14 factors and OS in the CG. The univariate analysis revealed only 5 from 14 factors associated with OS. The OS was associated only with age, platelet number, blast percentage in the BM, WHO subtypes and comorbidities. Multivariate analysis revealed that predictors of the event were age <50 years and platelets <100x109/L. Transformation in AML was detected in 26 (12.1%) patients.
Summary/Conclusions: MLPA proved to be a reliable method for detection of chromosomal abnormalities in MDS. Platelets <100x109/L, IPSS - low risk and R-IPSS-intermediate risk in the IG and age <50 years and platelets <100x109/L in the CG influenced OS in our cohort. Only age was not incorporated in the IPSS and R-IPSS. Probably, it should be taken in consideration in further refinement of the prognostic scoring systems.

PB1926

CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF MYELODYSPLASTIC SYNDROMES (MDS) IN ADULTS ACCORDING TO THE DATA OF MUNICIPAL HEMATOLOGY DEPARTMENTS IN MOSCOW, RUSSIA

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Background: The myelodysplastic syndromes (MDS) are a distinct group of clonal disorders of hematopoietic stem or progenitor cells characterized by ineffective hematopoiesis, peripheral cytopenias, abnormal dysplastic cell mor-

phology, and potential for clonal evolution to secondary acute myeloid leukemia (AML). AML is eventually diagnosed in up to 30-40% of MDS cases. Population-based registry data in the USA and Europe indicate an incidence of MDS in western countries of around 3-5 cases per 100.000 person-years with male predominance. The epidemiology of MDS has been carefully investigated in western countries while exact data on the epidemiology of MDS in Russia are absent.

Aims: To study the registered incidence of MDS in adults in the city of Moscow, to evaluate methods of diagnosis confirmation and choice of therapy in the system of Health Care.

Methods: The observational study included adult patients with newly diagnosed MDS in 2010, who were residents of Moscow. Evaluation of overall survival (OS) conducted as of September 2014.

Results: A total of 201 (male-110, female-118) adult patients were reported to the system of Moscow Health Care with newly diagnosed MDS in 2010. Median age at diagnosis was 71.5 years (range, 23.9-93.7). The incidence rate of MDS was 2.0 cases per 100.000 persons per year in the general adult population. Incidence accurately increased with age (Fig. 1). The maximum incidence 18.1 cases per 100.000 men was registered in the age over ≥85 years and 9.8 cases per 100.000 women in age 75-79 years. Most frequent morphological types of MDS distributed as follows: refractory anemia (RA)-33.8%, refractory anemia with ring sideroblasts (RARS)-8.4%, refractory cytopenia with multilineage dysplasia (RCMD)-12.0%, refractory anemia with excess blasts-1 (RAEB-1)-12.9% и RAEB-2-21.9%. Cytogenetic variants of MDS assessed only in 36 (17.9%) patients. All patients divided into 5 groups depending on the type of first-line therapy: 69 patients treated with epoetin alfa or beta (rHuEpo) as monotherapy; 20-low-dose ara-C (LDAC); 12-hypomethylating agents (azacitidine, decitabine); 60-symptomatic (red cell transfusion for low-risk MDS) and 38 - palliative care (elderly and weakened high-risk patients). Two patients with 5q- syndrome treated with lenalidomide. With a median follow-up for survivors 46 months 4-year overall survival (OS) for all patients was 34.8±13.4% (median 24.3 months). Early mortality over the first 60 days was 5.0%. rHuEpo used primarily in low-risk patients: 4-year OS 60.7±5.9%. Hypomethylation agents and LDAC applied in advanced MDS morphological variants: 4-year OS 25.0±12.5% (median 21.1 months) and 15.0±8.0% (15.3 months) respectively. Depending on the morphological variant of the 4-year OS was the following: 5q- syndrome-57.1±18.7% (median › 46 months); RA-50.8±6.1% (35.7); RARS-35.3±11.6% (32.8); RAEB-1 - 32.8±9.4% (24.7) and RAEB-2-5.7±3.7% (15.3).

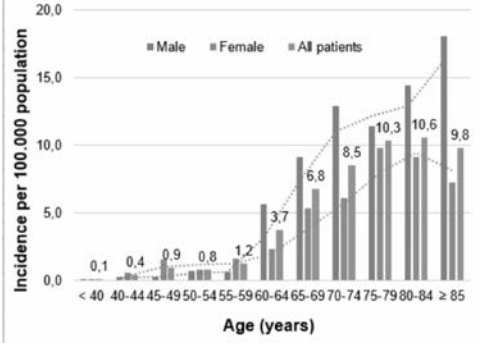

Figure 1.

Summary/Conclusions: The incidence of MDS in Moscow, Russia is 1.5-2 times lower than in Europe and the United States. Current diagnostic standards under the mandatory health insurance does not include genetic and molecular studies. Implementation a cytogenetic study and FISH in MDS typical chromosomal abnormalities in all patients with unexplained cytopenias will improve the diagnostic level.

PB1927

HIPERFERRITINEMIA IN MYELODISPLASTIC SYNDROME (MSD) PATIENTS CORELATION WITH EVOLUTION AND SURVIVAL

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Background: Most myelodysplastic (MDS) patients have anemia and many of them require red blood cells (RBC) transfusions leading to iron overload. Hematological improvement during iron chelation therapy was first pointed out more than twenty years ago. This phenomenon seems to be more frequent after introduction of Deferasirox. The most simple test assessing iron overload is serum ferritin concentration.

Aims: Assessment of hyperferritinemia incidence in MDS patients at the moment of MDS diagnosis, and correlation between ferritin level and evolution an survival in patients diagnosed with MDS.

Methods: The retrospective data collection from a single center experience (Department of Hematology County Hospital, Timisoara, Romania) between January 2005 and December 2014 included 121 patients (73men and 48 women) with MDS. All the patients had complete blood count and serum ferritin level, and complete follow-up data.

Results: Ferritin level above1000 ng/mL was found in 45 patients (31%) (Group 1) and ferritin level ≤1000ng/mL in 76 patients (69%) (Group 2). Most patients with significant hiperferritinemia, were RBC transfusion dependent (78% of patients). Among patients with ferritin level ≤1000 ng/mL, 48% were RBC transfusion dependent. Serum hemoglobin concentration was lower in Group 1 patients in comparison with Group 2 patients (6.2 g/dL *vs* 9,3 g/dL, p<0,001). The most frequent MDS subtype in Group 1, were patients with refractory anemia (RA) (32%), compared with patients with ferritin ≤1000 ng/mL - 15% (p<0,04). According to IPSS score, there were no differences between studied groups. Median follow up was 15 months. There was an improved overall survival (OS) in RBC transfusion independent patients compared to RBC transfusion dependent patients, but mean OS was not significantly statistically different in studied groups. No correlation was found between ferritin level and time to acute myeloid leukemia (AML) transformation.

Summary/Conclusions: Hiperferritinemia >1000 ng/mL does not influence survival and time to AML transformation in MDS patients. The most frequent MDS subtype in patients with ferritin level >1000 ng/mL was MDS RA. Among patients with ferritin level >1000 ng/mL 76% were RBC dependent.

PB1928

EFFICACY AND SAFETY OF XIAOAIPING TABLETS IN THE TREATMENT OF ELDERLY PATIENTS WITH INTERMEDIATE-RISK/HIGH-RISK MYELODYSPLASTIC SYNDROME: A PILOT TRIAL

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Background: Myelodysplastic syndromes (MDS) represent a group of biologically and clinically heterogeneous clonal hematopoietic neoplasms with very poor treatment efficacy and clinical outcomes and patients have been recommended enrolled in clinical trials. Traditional Chinese medicine has also made some progress in the treatment of MDS in resent years. Xiaoaiping tablet, an herbal medicine widely used as an anticancer wonder drug, is the extract of *Marsdenia tenacissima* (*Tong-guan-teng* in Chinese folk medicine),which is officially recorded in the Chinese Pharmacopoeia *Marsdenia tenacissima.* Since 1990s,Xiaoaiping preparations including tablet,injection and syrup which are all extractions of the plants, demonstrate anti-tumor effect on gastric, hepatic carcinoma and leukemia.

Aims: To observe the efficacy and safety of Xiaoaiping tablets in the treatment of elderly patients(over 60 years-old) with intermediate-risk/high-risk myelodysplastic syndrome.

Methods: Twenty-four patients were enrolled in the study. They received Xiaoaiping tablets (2.4-3.0g,oral,3 times daily) combined with supportive care therapy. The primary endpoint of efficacy was measured by the number of days between blood transfusions as well as haematolgical indicies. The changes in ECOG performance status were observed as well as adverse events.

Results: Twenty-one patients of the twenty-four completed therapy. Two patients (9.5%) had a complete remission (CR) 3 patients (14.3%) had a partial response (PR), the overall response rate was 23.8%. Eleven patients had haematological improvement (HI), with the HI rate being 52.4%. Haematologic indices of hemoglobin, total neutrophil count and platelet post Xiaoaiping-treatment were significantly higher than those before treatment (P<0.05).After treatment, the average interval of transfusion of red blood cells (RBC) and platelets was extended (P<0.05). The ECOG scores post-treatment was lower than those before treatment (P<0.05). Toxicities were limited. There were no grade 3 or 4 adverse events.

Summary/Conclusions: Xiaoaiping tablet was an efficacious intervention as measured by hematological indices, the number and frequency transfusions and patients performance status. It was an effective and safe agent in the treatment of elderly patients with intermediate-risk/high-risk MDS, and th**e** tablets might be a promising therapy for this group of patients.

PB1929

MYELOTOXICITY OF PEPTIDE RECEPTOR RADIONUCLIDE THERAPY OF NEUROENDOCRINE TUMORS: A DECADE OF EXPERIENCE

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Background: Myelodysplastic syndrome (MDS) and acute leukemia (AL) have been associated with peptide receptor radionuclide therapy (PRRT) in heavily pre-treated patients with a prior history of exposure to alkylating agents. Commenced 15 years ago PRRT is now becoming established as first and second line therapy for gastrointestinal and pancreatic neuroendocrine tumors (GEP-

NETS). Current therapies gain only marginal, albeit statistically significant, increase in progression free survival (PFS) without meaningful objective responses and are accompanied by significant toxicity. With the incidence of GEPNETS rising in the Western world, myelotoxicity is now the most significant potential adverse event following PRRT.
Aims: This review of the literature, and our own decade of experience with lutetium-177-octreotate-capecitabine +/- temozolamide PRRT-chemotherapy of GEPNETS analyses the risk of both short and long-term hematotoxicity.
Methods: All of the literature on myelotoxicity in associated with PRRT of NETS reported in papers published over the past 15 years has been reviewed and compared with our own experience of lutetium-177 PRRT combined with octreotate-capecitabine-temozolomide radiosensitising chemotherapy. Relevant primary research concerning myelotoxicity and PRRT was identified using medical databases. Key articles published from 2000 onwards were obtained primarily from PubMed, Ovid and Cochrane databases. Specific focus was given to articles published in *Cancer Biotherapy and Radiopharmaceuticals, European Journal of Nuclear Medicine and Molecular imaging, Neuroendocrinology, Journal of Clinical Oncology* and *Endocrine Related Cancer*.
Results: Sixteen key articles involving primary research were identified; 12 articles relating to PRRT as monotherapy and 4 articles relating to PRRT in combination with chemotherapy and/or novel agents. A total of 2225 patients were treated (2104 treated with PRRT monotherapy and 121 with PRRT combination) all of whom had been exposed to prior therapies, including somatostatin analogs, alkylating agents, radiotherapy, prior PRRT and surgery with the majority having had exposure to greater than two prior lines of treatment. The average age of patients in these studies ranged from 53 to 64 years with median duration of follow up ranging from 6 to 62 months. Short-term myelotoxicity was observed in 221 patients (10%), occuring in 213 of 2104 patients treated with PRRT monotherapy and 8 of 121 patients treated with PRRT combination. Acute toxicity manifested as modest self-limited grade 3/4 toxicity (CTCAE or WHO) most often affecting platelets then WBC and lastly hemoglobin, and was commonly observed during the first cycle of treatment, with the lowest nadir predictive of time taken for recovery. Toxicity manifesting early was easily managed with dose modification or therapy cessation, and was ameliorated by appropriate patient selection based on age, prior therapies, comorbidities and adequate baseline myeloid function. Long-term toxicity in the form of MDS and AL was a rare stochastic event occurring in only 32 (1.4%) of all the patients treated with PRRT (24 cases of MDS, 6 cases of MDS with subsequent transformation to AL and 2 cases of AL alone). Where bone marrow biopsy was performed the majority of cases of MDS displayed complex cytogenetic abnormalities, consistent with secondary MDS following exposure to chemotherapy with alkylating agents or irradiation. The development of MDS was a risk factor for AL. No significant difference in relative risk of MDS/AL was noted between patients salvaged with PRRT monotherapy or PRRT combination (1.4% and 1.6% respectively). Factors associated with development of significant short and long-term myelotoxicity included age >70 years, impaired renal function, baseline cytopenias, prior number of therapies, prior chemotherapy (especially alkylating agents) and prior radiation therapy. The latter two factors were associated with a significant of development of MDS and AL.
Summary/Conclusions: Early therapy with PRRT-containing regimens improves outcomes, minimizes myelotoxicity and renders the risk of MDS and AL negligible.

PB1930

ASSESSMENT OF B AND T LYMPHOCYTES FUNCTION IN PATIENTS WITHMYELODYSPLASTIC SYNDROMES
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Background: An activated immune system has been observed in patients with myelodysplastic syndrome but its exact contribution to disease development and control is not fully clarified. The successful use of immunosuppressive therapies, the potentially curative role of allogeneic stem cell transplant, and the more recent data showing improved peripheral cytopenias and elimination of certain common cytogenetic abnormalities with immunomodulatory agents; highlight the role immune dysregulation in the development of MDS.
Aims: The aim of this study is to assess B and T cell function in patients with MDS and correlate them to the risk status of MDS.
Methods: The study included 30 adult Egyptian patients diagnosed with MDS based on blood picture, bone marrow examination and cytogenetic studies. Patients were classified according to IPSS risk scoring system. Immune system assessment was done by performing: antinuclear antibodies, coombs' test, serum protein electrophoresis and CD4/CD8 ratio in peripheral blood.
Results: We've found an association between inverted CD4/CD8 ratio and higher risk strata, presence of neutropenia and transfusion dependence. Regarding Humoral immune system involvement, positive coombs test was related significantly to younger age, higher bone marrow blasts count. Polyclonal gammopathy-as well-was found to be associated with higher bone marrow blasts count and presence of cytogenetic abnormalities.
Summary/Conclusions: Immune dysregulation-in the form of inverted CD4/CD8 ratio, positive ANA, positive coombs' test and polyclonal gammopathy-is well documented in myelodysplastic syndrome, and it is probably related to younger age, higher IPPS risk, higher bone marrow blasts count and the presence of cytogenetic abnormalities.

PB1931

DOMICILIARY AZACITIDINE: A NEW EXPERIENCE IN OUR CENTER
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Background: Azacitidine is an antineoplastic agent used in adults with no indication of bone marrow transplantation and with a diagnosis of intermediated-2 or high risk myelodisplastic syndrome (MDS), high risk chronic myelomonocytic leukemia (CMML) or myeloid acute leukemia (AML).
Aims: Show the experience of using domiciliary Azacitidine and to evaluate its effectiveness in a third level hospital. Evaluate the patient's satisfaction. Estimate the efficiency of the treatment.
Methods: Retrospective observational study including patients who have received treatment with domiciliary Azacitidine from June 2015 to January 2016. The following variables were chosen: gender, age, base pathology, treatment cycles, secondary effects, readmissions, *Exitus* and satisfaction. Domiciliary hospitalization unit (DH), trains patients and their families and monitors vital signs during the treatment. We have revised the official cost for one-day stay in an adult day hospital (ADH) *versus* domiciliary administration according to the July 2014 issue of "BOIB". Telephone surveys were conducted (table 1), asking patients and their families (with a score from 0 to 10), to evaluate the quality of the service.
Results: We have nine patients, six male and three female, between the ages 54-84. The treatment cycles at home vary between 1 and 8. We had no readmissions during the treatment. Three adverse effects appeared: itching, cutaneous reaction and asthenia. Of the nine patients, two died due to disease progression. We compared the costs for one treatment cycle: at the ADH the cost was 3,850 Euros (550 Euros a day for seven days) with the costs of the same treatment at home, which was 2,037 Euros (291 Euros a day for 7 days). If we calculate all the 34 cycles received, the costs would be 130,900 Euros for receiving the drug at the hospital, *versus* 69,258 Euros at home. In 7 months that we conducted the domiciliary program, the hospital saved 61,642 Euros. The telephone survey showed that 100% of the questions were answered with the maximum score.

Table 1.

1. Did you receive a more personalised medical care with the DH in comparison to ADH?
2. Did you perceive the treatment at home as more comfortable and did it have less interferences in your daily routines?
3. Did you notice any different secondary effects at home compared to the ADH?
Questions for the family:
4. Did you have less interferences in your daily routine?
5. Do you think that your family member (patient) has more privacy and feels more comfortable receiving the treatment at home?

Summary/Conclusions: There were no readmissions during the treatment and the secondary effects were minor, so we conclude that home administration is safe. All patients had a high level of satisfaction and experienced an improvement in their quality of life. The treatment with Azacitidine in DH has the same effectiveness and more efficiency than in ADH.

PB1932

A RETROSPECTIVE REVIEW OF AZACITIDINE THERAPY IN AN IRISH UNIVERSITY HOSPITAL SETTING
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Background: Azacitidine (AZA) is a a hypomethylating agent and nucleoside analogue of cytidine. Hypomethylating agents have revolutionised the therapy of intermediate-2 and high-risk myelodysplastic syndrome (MDS), chronic myelomonocytic leukaemia (CMML) and acute myeloid leukaemia (AML) in patients fit for neither intensive therapy nor haematopoietic stem cell transplantation (HSCT).
Aims: This retrospective review aimed to examine the usage of AZA commenced for any indication during a three year period in a university teaching hospital in Ireland. Data collected pertained to patient and disease characteristics, safety, efficacy, overall and progression free survival and the effect of therapy on transfusion dependence.

Methods: A retrospective analysis of patients receiving AZA at University Hospital Limerick in a three year period from October 2012 until October 2015 was conducted. Data was collected from patients' paper and electronic records and the electronic laboratory system. Included patients received at least one cycle of AZA. The World Health Organization Classification was used to classify disease status. Overall (OS) and progression-free survival (PFS) were calculated from the date of commencement of AZA therapy and were calculated using Kaplan-Meier estimates.
Results: Twenty-eight (28) patients commenced AZA between October 2012 and October 2015. 64% of patients were male and the cohort had a median age of 70 years (range 42-80 years). These patients comprised 3 patients with IPSS intermediate risk (level 2) MDS, 5 patients with IPSS high-risk MDS, 6 patients with CMML, 2 patients with MDS/MPN overlap, 1 patient with hypoplastic MDS, 1 patient with refractory anaemia with ring sideroblasts (RARS-MDS) and 10 patients with AML. Of the 10 patients with AML, 4 patients had 20-30% bone marrow myeloblasts confirmed morphologically and by flow cytometry and 6 patients had >30% blasts. 26 patients (93%) were transplant-ineligible due to age or comorbidity and 2 had received previous reduced intensity haemopoietic stem cell transplantation. An additional 2 patients had received prior intensive induction chemotherapy for AML. 55% of patients responded to therapy including achievement of stable disease. 66% of patients required red cell support initially, of whom 37% achieved red cell independence. CTCAE grade 3 or 4 toxicities were mostly haematological, with 66% of patients experiencing same. 52% of patients required at least one admission during AZA therapy, predominantly for severe infections (88%). Median OS and PFS were as follows: AML 9.4 months and 8.7 months, high-risk MDS 3 months and 2.6 months and CMML 8 months and 4.5 months.
Summary/Conclusions: AZA improved OS for patients with AML who were ineligible for intensive therapy or HSCT in our institution. AZA was well tolerated in general and brought about transfusion independence in 37% of transfusion-dependent patients. The most significant toxicities were haematological and relating to severe infection, often requiring hospital admission. Hypomethylating agents continue to provide an excellent therapeutic option for these difficult-to-treat patients.

Myeloma and other monoclonal gammopathies - Biology

PB1933

CLINICAL CHARACTERISTICS, OUTCOME AND PROGNOSTIC FACTORS OF SURVIVAL OF MULTIPLE MYELOMA PATIENTS ACHIEVING AT LEAST VERY GOOD PARTIAL RESPONSE AFTER FIRST LINE TREATMENT IN THE ERA OF NOVEL AGENTS

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Background: Depth of response defined by conventional methods *i.e.* the achievement of at least very good partial response (≥vgPR) or complete response is correlated with OS in both eligible and non eligible for transplantation Multiple Myeloma (MM) patients. However, there are limited data regarding the prognostic factors for OS in patients who manage to achieve ≥vgPR.
Aims: Our aim was to study the clinical characteristics and outcome of MM patients who achieve ≥vgPR after first line treatment and seek for prognostic factors for OS.
Methods: We reviewed the records of 464 consecutive symptomatic MM patients diagnosed and treated in a single center between 2000-2015 (median age: 68 years, range: 29-90 years; M/F: 247/217; IgG: 261, IgA: 118, light chain: 65, non-secretory: 15, IgD: 4, IgM:1; ISS1: 142, ISS2: 146, ISS3: 176).
Results: Two hundred and sixty-eight patients (58%) achieved ≥vgPR after first line treatment (group A) and 196 (42%) had <vgPR (group B). With regard to clinical characteristics, patients in group A were younger, they had lower β2-microglobulin levels and higher levels of serum calcium (p<0.05 for all parameters). Molecular cytogenetics revealed that high-risk features *i.e.* del17p and/or t(4;14) and/or t(14;16) were present in 33% of patients. Revised International Staging System (R-ISS) was evaluated in 404/464 patients (87%). The number of patients with high risk features or the distribution of R-ISS did not differ between the two groups. Overall, the number of patients treated with novel agents (NA) in first line did not differ between groups; however, in group A, the number of patients who received thalidomide-based regimens was marginally higher (p=0.01). High dose therapy and autologous transplantation (ASCT) was offered in 20% of patients in group A *vs* 7% in group B (p<0.001). The median progression-free survival (PFS) of patients in group A *vs* group B was 26 months (95% CI: 23-29) *vs* 11 (95% CI: 8-13) (p<0.001). The median PFS2 did not differ between groups. After a median follow up of 8 years (95% CI: 80-114), 69/268 patients of group A are alive (26%) *vs* 61/196 patients of group B (31%). The median OS for patients in group A *vs* those in group B was 44 months (95% CI: 39-48) *vs* 27 months (95% CI: 18-36) (p<0.001). With regard to group A, in the univariate analysis, age, creatinine, LDH, ISS, R-ISS, the presence or not of high-risk molecular cytogenetics, the type of 2nd line treatment and ASCT predicted for overall survival (OS). In the multivariate analysis, the presence of high risk molecular cytogenetics was the only independent negative predictor for OS (p=0.01, HzR: 0.57 95% CI: 0.37-0.8). Median OS for patients with high risk cytogenetics *vs* standard risk patients, was 68 months (95% CI: 45-91) *vs* 46 months (95% CI: 40-52) (p=0.01); 5-year OS was 60% in group A *vs* 37% in group B. In addition, the presence of high risk molecular cytogenetics strongly predicted for PFS (HzR: 0.57 95% CI: 0.4-0.8, p=0.01).
Summary/Conclusions: Despite the established prognostic significance of the achievement of ≥ vgPR, OS within this group is not uniform. High-risk molecular cytogenetics independently predict for PFS and OS suggesting that high-risk patients display gene instability, which compromises sustained response and eventually patients' survival. Despite depth of response, close monitoring, including minimal residual disease is warranted in patients with high-risk molecular cytogenetics in order to optimize treatment strategy, after first line therapy.

PB1934

Abstract withdrawn.

PB1935

IDENTIFICATION OF PROGNOSTIC RELEVANT GENETIC ABNORMALITIES IN MULTIPLE MYELOMA USING MICROARRAY-BASED GENOMIC PROFILING IN ROUTINE DIAGNOSTICS

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Background: Multiple myeloma (MM) is a neoplasm that exhibits a broad

heterogeneity in both biological behavior and clinical presentation. Specific copy number abnormalities (CNAs) such as hyperdiploidy, 1p loss, 1q gain, 13q loss and 17p (*TP53*) loss, and IGH translocations, such as t(4;14)(p16;q32) and t(14;16)(q32;q23), play key roles in the pathogenesis of MM and, in addition, provide important information on its prognosis and treatment response. In routine diagnostics such prognostic relevant chromosomal abnormalities are detected by interphase fluorescence *in situ* hybridization (iFISH) on enriched plasma cells. However, iFISH analysis of multiple loci is laborious and provides only genetic information of the probe targets. Microarray-based whole genome profiling, on the other hand, will provide genome-wide genetic information, allowing the detection of small (cryptic) copy number alterations (CNAs) and copy neutral loss of heterozygosity (CNLOH), that are nor identified by targeted iFISH.
Aims: In this study we wanted to validate the detection of prognostic relevant copy number aberrations in MM by micro-array based genomic profiling.
Methods: We subjected 37 MM samples to iFISH with the diagnostic probe for the detection of prognostic relevant CNA, *i.e.* D5S23/D5S721/CEP9/CEP15 for ploidy assessment, LSI 13 (13q14) for the identification of loss of 13q, LSI TP53 (17p13.1) for the identification of loss of 17p, and CDKN2C/CKS1B for the identification of loss of 1p32 and gain of 1q21. Next, we performed microarray-based genomic profiling using the high density SNP-based CytoScan HD array platform.
Results: All prognostic relevant CNAs as detected by iFISH were also observed by microarray-based whole genome profiling. Micro-array was able to detect small clones with CNA, present in only 15% of the cells, which was lower than the EMN-proposed detection limit of 20% for CNA. In addition, in four cases micro-array revealed a copy neutral loss of heterozygosity (CNLOH), and in four other cases a 1p21 or 1p16 loss, which was outside the 1p32 iFISH target region.
Summary/Conclusions: In routine diagnostics the micro-array approach is a good and fast alternative for iFISH for the detection of prognostic relevant CNAs in multiple myeloma.

PB1936

CYR61/CCN1 STIMULATED THE PROLIFERATION AND DIFFERENTIATION OF OSTEOBLASTS IN MYELOMA BONE DISEASE IN VITRO
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Background: Myeloma bone disease (MBD) is a most common complication of multiple myeloma, which can cause high mortality. Cysteine-rich 61 (Cyr61 or CCN1), one secreted protein in bone marrow(BM) microenvironment, has diverse effects on many cellular activities like growth and differentiation. However, the effect of CCN1 on osteoblasts (OBs) in MBD is unclear.
Aims: To explore the effect of CCN1 on osteroblasts in MBD.
Methods: The levels of CCN1 in MBD patients were detected by ELISA and RT-PCR. The OBs from MBD patients were cultured with CCN1 in vitro, and proliferation and differentiation of OBs were observed. The transcription factors, runt-related transcription factor 2 (Runx2), β-Catenin and bone morphogenetic protein-2 (BMP2), were investigated by RT-PCR.
Results: The results showed that CCN1 level elevated in BM supernatant and CYR61 overexpressed in OBs in newly diagnosed MBD patients. After 30ng/L CCN1 stimulation for 24 hours in vitro, the OBs quantity increased to (3.39±1.21) × 10^5/mL, significantly higher than the blank (*P*=0.046). Meanwhile, the amount of mineralized nodules (14.33± 5.72/HPF) was also significantly increased than the blank group (9.11±0.97/HPF) (*P*=0.048). Futhermore, Runx2 and β-Catenin upregulated in OBs after CCN1 stimulation.

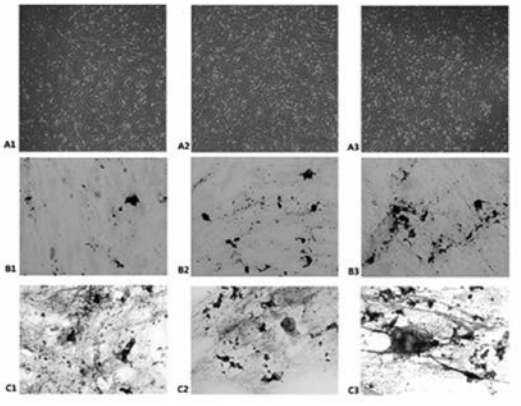

Figure 1.

Summary/Conclusions: In conclusion, CCN1 can stimulate the proliferation and differentiation of OBs via Wnt pathway in MBD.

PB1937

STABLE IN VITRO BORTEZOMIB RESISTANCE OF MYELOMA CELLS IS CHARACTERIZED BY CHROMOSOME 1Q21 COPY NUMBER GAIN
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Background: Bortezomib (Bz), the first agent of a new class of drugs in Multiple Myeloma (MM) -proteasome inhibitors (PI)-, has shown remarkable activity in MM and forms an important part of modern MM treatment. Nevertheless in the clinical setting refractoriness to the agent eventually develops in most cases with detrimental effects in their subsequent survival. Numerous publications have addressed this issue through *in vitro* developed models of acquired Bz resistance (BzR), however the results were quite different in each one and no common factor between them could be identified.
Aims: In order to address these issues a native effort was made for the development of an *in vitro* model of BzR that would resemble the clinical reality in the most accurate way.
Methods: Two MM cell lines were used, one resembling a multisensitive (JJN3) and the other a multiresistant (U266) drug behavior, that were both sensitive to Bz.Instead of a continuous increasing exposure to the agent, an intermittent one was chosen to resemble in a more accurate way the clinical pattern of drug exposure and subsequently the mechanisms of BzR.
Results: As a result of this method a 20 fold increase in the 48h Bortezomib IC50 was noted for both cell lines. The Bz resistant cell lines proved to be stable in the phenotype of resistance. The increase in the IC50 was able to be verified a year after the cell lines were cultured in normal medium. Bz resistant cell lines were cross-resistant with other PI, even with the PI Carfilzomib which belongs to a different drug category. A stable ratio of 3 fold increase in carfilzomib IC50 for every 10 fold increase in Bz IC50 was found in various different levels of BzR. Both basal activity and IC50 for the Bz specific β5-subunit of the proteasome was not statistical different in both Bz resistant and naive cell lines implying that the proteasome was not directly implemented in BzR and no pump flow mechanism was implicated. Interestingly enough the two MM cell lines through the various stages of their acquired BzR followed different phenotypical pathways. JJN3 through their evolution of their BzR and in accordance with various publications developed a more immature phenotype with loss of percentage and intensity of CD138 by flow cytometry, decreasing levels of the plasma cell differentiation factor XBP-1 and decreased immunoglobulin production both in terms of cell line specific immunoglobulin gene probe expression and cytoplasmic immunoglobulin content. On the contrary U266 retained percentage and intensity of CD138, while exhibiting increasing levels of XBP-1 and increased immunoglobulin production both in terms of cell line specific immunoglobulin gene probe expression and cytoplasmic immunoglobulin content. Driven from the stable nature of BzR of the cell lines, we applied metaphase cytogenetics, spectral karyotyping (SKY) and Fluoresence *in situ* Hybridization (FISH) to detect any structural genetic abnormalities. Both metaphase cytogenetics and SKY did not reveal any differences, while FISH was able to detect an increase in chromosome 1q21 copy number evident in both Bz resistant cell lines all in the form of jumping translocations.
Summary/Conclusions: In conclusion this work provides the *in vitro* verification of the linkage of 1q21 amplification and BzR. The fact that 1q21 amplification has been found to correlate with prognosis and a relapsed/refractory phenotype not only in MM but also in a multitude of solid tumors implies that at least part of BzR is linked with universal drug resistance mechanisms in cancer.

PB1938

ROLE OF EPO IN THE ANGIOGENIC ACTIVITY OF BONE MARROW ENDOTHELIAL CELLS OF MGUS AND MULTIPLE MYELOMA PATIENTS
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Background: Increasing evidences suggest several biological roles for erythropoietin and its receptor (Epo and EpoR), unrelated to erythropoiesis, including angiogenesis.
Aims: Here, we detected the expression of EpoR in bone marrow-derived endothelial cells from monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) patients (MGECs and MMECs, respectively) and assessed whether Epo plays a role in MGECs- and MMECs-mediated angiogenesis.
Methods: This study was designed to determine the effects of Epo on ECs from monoclonal gammopathy of undetermined significance [MGUS, (MGECs)] and MMECs in *in vitro* and *in vivo* experimental assays.
Results: We show that EpoR is expressed by both MGECs and MMECs even though at a higher level in the first ones. Both EC types respond to rHuEpo in terms of cell proliferation, whereas other responses, including activation of JAK2/STAT5 and PI3K/Akt pathways, cell migration and capillarogenesis are enhanced by Epo in MGECs, but not in MMECs. In addition, the conditioned

media of both Epo-treated cells induce a strong angiogenic response *in vivo* in the chorioallantoic membrane assay, comparable to that of vascular endothelial growth factor (VEGF).
Summary/Conclusions: Overall, these data highlight the effect of Epo on MGECs- and MMECs-mediated angiogenesis: MGECs are more responsive to Epo treatment than MMECs, probably because over-angiogenic phenotype of MMECs is already activated by their autocrine/paracrine loops occurring in the "angiogenic switch" from MGUS.
Acknowledgments. This work was supported by European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n.278570 to DR.

PB1939

POLYMORPHISM OF IL-10 RECEPTOR B AFFECTS THE PROGNOSIS OF MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE AND/OR BORTEZOMIB

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Background: Interleukin-10 (IL-10) and IL-10 receptor (IL-10R) single nucleotide polymorphisms (SNPs) have been implicated in the pathogenesis of many cancers, including hematologic malignancy. However, no previous studies have examined the possible association between IL-10/IL-10R SNPs and MM, especially prognosis in MM patients.
Aims: We investigated the influence of IL-10 -592C/A, IL-10RA I224V and IL-10RB K47E on the risk to develop multiple myeloma (MM) and clinical features of MM.
Methods: We extracted the genomic DNA from 128 MM patients and 202 healthy controls, and determined IL-10 promoter-592C/A (rs1800872), IL-10RA (rs2228055) and IL-10RB K47E (rs2834167) genotypes by using the PCR-restriction fragment length polymorphism method. Overall survival (OS) was defined as the interval from the date of diagnosis to the date of death or last clinical appointment.
Results: No statistically significant differences were observed in the genotype and allele frequencies of IL-10 -592C/A, IL-10RA I224V and IL-10RB K47E between MM patients and healthy control. IL-10RA II genotype was significantly associated with lower hemoglobin level than IV and VV genotypes (mean±standard deviation, 9.21±2.46 *vs* 10.3±2.33 g/dl, P=0.021). IL-10 -592 AA genotype was significantly associated with better OS than CA and CC genotypes (median OS, 74.5 *vs* 46.3 months, P=0.047). We observed significant differences in survival between patients treated with thalidomide and/or bortezomib and with conventional treatment (median OS, 74.5 *vs* 38.2 months, P=0.021). Therefore, we also examined the effect of IL-10 and IL-10R polymorphisms on clinical variables and OS in patients treated with thalidomide and/or bortezomib. IL-10-592 AA genotype was significantly associated with low albumin level than CA and CC genotypes (mean±standard deviation, 9.21±2.46 *vs* 10.3±2 .33 g/dl, P=0.021). In addition, IL-10RB EE genotype was significantly associated with poor survival than KK and KE genotypes (median OS, 46.3 *vs* 78.8 months, P=0.015) (Figure 1).

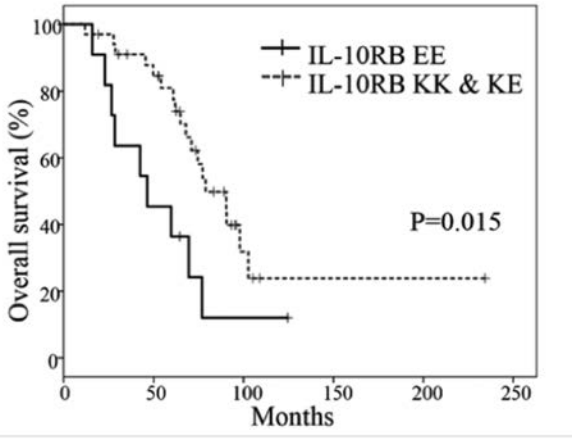

Figure 1. OS of patients treated with thalidomide and/or bortezomib according to the IL-10RB K74E genotype.

Summary/Conclusions: Our findings indicate that IL-10 and IL-10R gene polymorphisms may not contribute to susceptibility to MM, but they may be associated with the severity and prognosis of MM. Especially, IL-10RB K47E polymorphism influence the poor prognosis of patients treated with thalidomide and/or bortezomib.

PB1940

DIETARY HABITS ACROSS THE LIFESPAN AND RISK OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: A POPULATION BASED SCREENING STUDY

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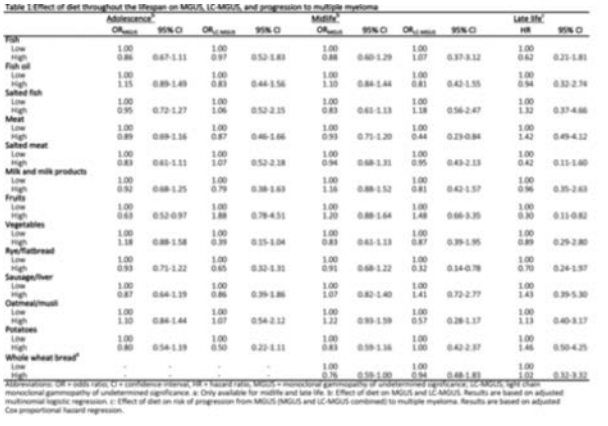
Background: All multiple myeloma (MM) cases are preceded by the premalignant state, monoclonal gammopathy of undetermined significance (MGUS). The etiology of MGUS and MM is to a large extent unknown. However, researchers have found an elevated risk of MM to be associated with low occupation-based socioeconomic status, income, education, and high body mass index (BMI), indicating that lifestyle related factors, such as diet, may be important risk factors. Few studies on the effect of diet on MM have been conducted and the results have been inconclusive. No studies have been conducted on the effect of diet on MGUS.
Aims: The aim of this study was to explore the effect of high *vs* low intake of fish, salted/smoked fish, fish oil, meat, salted/smoked meat, milk and milk products, fruits, vegetables, potatoes, rye bread, whole wheat bread, and oatmeal/muesli on MGUS and progression to MM.
Methods: This study was based on participants from the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-RS; N=5,764; mean age=77 years [range=66-98]; 58% females), which is a continuation of the population-based Reykjavik Study (N=30,795). AGES-RS was initiated in 2002 and ended in 2006. Participants in the AGES-RS provided retrospective information on dietary habits in adolescence (14-19 years), midlife (40-50 years), as well as at study baseline using a short food frequency questionnaire. Serum protein electrophoresis and free light chain analyses was performed on all subjects to identify MGUS and light chain-MGUS (LC-MGUS). They were followed prospectively until 2014 and information on MM diagnosis was collected through the Icelandic Cancer Registry. Logistic regression and Cox regression models were used to analyze risk of MGUS and MM, adjustments were made for age and sex.
Results: A total of 300 MGUS (5.2%) and 52 LC-MGUS (0.9%) cases were identified. We found that high consumption of fruits in adolescence and high consumption of whole wheat bread in midlife were inversely associated with MGUS (odds ratio (OR)=0.63, 95% confidence interval (CI) 0.52-0.97 and OR=0.76, 95% CI 0.59-1.00, respectively), and that high consumption of meat and rye bread in midlife were inversely associated with LC-MGUS (OR=0.44, 95% CI 0.23-0.84 and OR=0.32, 95% CI 0.14-0.78, respectively). Other food items did not have an effect on MGUS or LC-MGUS risk (Table 1). Additionally, in an analysis were MGUS and LC-MGUS were combined (cMGUS) we found that high consumption of rye bread and potatoes in both adolescence and midlife were inversely associated with cMGUS (OR=0.70, 95% CI 0.55-0.95 and OR=0.63, 95% CI 0.45-0.96, respectively) when compared to individuals with low intake at both time points. A total of 18 individuals were diagnosed with MM during a mean follow-up of 8.2 years. We found an inverse association between high fruit intake in late life and progression to MM (hazard ratio (HR)=0.30, 95% CI 0.11-0.82). Other food items did not have an effect on progression (Table 1).

Table 1.



Summary/Conclusions: Our findings suggest that certain dietary factors during adolescence and/or midlife might reduce risk of MGUS and LC-MGUS.

Results regarding LC-MGUS should be interpreted with caution due to low statistical power. The findings also suggest that high late life intake of fruits might reduce the risk of progression to MM. The exact biological mechanism for these associations is not clear and more studies are needed to clarify the underlying mechanism for this finding.

PB1941

HEAVY-LIGHT CHAIN IMMUNOASSAYS FOR DIAGNOSIS AND MONITORING IGA MULTIPLE MYELOMA: SAVING PITFALLS ON PROTEIN QUANTIFICATION

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Background: Diagnosis and follow-up of Multiple Myeloma (MM) include the assessment of the involved protein named monoclonal component (MC) by serum protein electrophoresis (SPEP) with M-spike densitometry as the gold standard. MC quantification is usually not problematic for patients with IgG MM due to protein characteristics (molar weight, protein charge and migration); however, for IgA MM there are often some difficulties to impossibility their quantification. About 25% of monoclonal gammopathies are IgA subtype; due to the co-migration in alpha or beta area and the presence of IgA dimers, the diagnosis and follow-up is difficult based in the quantification of M-spike using conventional SPEP. The separate determination of intact immunoproteins of monoclonal and polyclonal isotype (heavy/light IgAk/IgAL, HLC) allows quantifying the monoclonal IgA and also assesses immunoparesis status.
Aims: To evaluate the correlation between M-spike concentration by SPE and the quantification by HLC and ratio, and their usefulness as biomarker for early biological relapse detection in IgA MM.
Methods: Every patient with an IgA monoclonal gammopathy, diagnosed of secretory MM according to the criteria for MM diagnosis by the IMWG, and treated in our hospital, was taken in account. The diagnosis and follow-up of monoclonal gammopathies were performed in our Hematology-immunoproteins lab. A chart review was performed collecting: demographic data, clinical stage at diagnosis and protein data: (SPEP, UPE of 24h), quantification of total immunoproteins, inmunofixation (IFX), quantification of FLC, HLC and their ratios (Freelite™, Hevylite™, The Binding Site, Birmingham, UK). Time of study: May 2008-Agosto 2015.
Results: Forty-seven patients were included. Females/males: 23/24; mean age: 67.43 (32-88 years); IgAk: 26 (55.3%); IgAL: 21 (44.7%); 34% of patients had free light chains detectable in urine. A total of 182 protein assessments were registered, 51 present a value of M-spike below 10 g/L, in 62 (34.0%) there were no M-spike by SPEP, but in 39 an abnormal HLCr demonstrated monoclonality confirmed by IFX in 30 samples. The isotype analysis at diagnosis for IgAk patients reveals: mean M-spike concentration 9.61 g/L (0-37.2), HLC-IgAk 24.71 g/L (0.02-63.94), HLC-IgAk minus HLC-IgAL: 24.52 g/L (0-63.94); for IgAL patients shows: mean M-spike concentration 11.4 g/L (0-25.6), HLC-IgAL concentration 17.61 g/L (0.6-41.1), and HLC-IgAL minus HLC-IgAk 16.95 g/L (0.06-41.08). Immunoparesis at diagnosis (abnormal HLC ratio) was present in all patients. In 144 samples with positive MC by SPEP, the mean concentration of M-spike for IgAk samples was 18.43 g/L (1-64.22), HLC-IgAk 22.54 g/L (0.2-109.20), and HLC-IgAk minus HLC-IgAL: 22.51 g/L (0-109.19); and for IgAL samples: 16.2 g/L (1-49.50), HLC-IgAL 20.87 g/L (0.56-55.65), and HLC-IgAL minus HLC-IgAk 20.35 g/L (0.10-55.27). The correlation between both techniques for patients with measurable M-spike for SPEP was excellent, remarking the sensitivity of the HLC (p=0.003 for IgA-k and p<0.001 for IgA-L); the HLCr is a good biomarker for monitoring response.
Summary/Conclusions: In this study, 34.0% of SPEP studies in IgA MM do not identify MC, nevertheless more than 50% of them showed monoclonality quantify by HLC-IgA, improving the quantification in cases with low concentrations. The incorporation of this tool for diagnosis and monitoring in IgA MM patients improve the accuracy of follow up. An updated analysis including the value of HLCr in relapse detection will be included in case of acceptance.

PB1942

IL-1R AND IL-1RA IN THE DEVELOPMENT OF MULTIPLE MYELOMA

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Background: Various cytokines are involved in the pathogenesis of Multiple myeloma (MM). They play a significant role in the development of tumor clone. It is known that single nucleotide polymorphisms (SNP) in the gene regulatory regions can influence on cytokines production which can effect on the development of the disease.
Aims: The aim of this study was to identify SNP genes of IL-1R (pst11970C/T) and IL-1RA (mspa111100T/C) associated with the development of MM among the residents of the North-West region of Russia as well as determining the severity of bone tissue damage.
Methods: We analyzed 50 MM patients which were divided into two groups depending on the detected changes in the bones (median age: 69.7±8.6): 1st group (22 patients) - with severe osteolytic bone lesions (III stage of the *Durie-Salmon staging* system); 2nd group (28 patients)-with manifestations of osteoporosis and isolated pockets of lysis (II stage of of the *Durie-Salmon staging* system). The diagnosis of symptomatic MM was verified based on the criteria of the International Working Group on myeloma. The most frequent type of myeloma that was observed in patients was G type (80%). The frequencies of myeloma A, as well as disease of light chains were about 20%. The control group consisted of 50 healthy unrelated Caucasoid blood donors (median age: 51.2±6.9). All analyzed people were from St.-Petersburg, Russia. Genomic DNA was extracted from the peripheral blood and gene genotyping was performed by use of PCR-SSP (Cytokine genotyping Kit, Invitrogen). Allele frequencies and expected Hardy Weinberg equilibrium (HWE) for each SNP were determined. P values less than 0.05 were considered statistically significant.
Results: Based on the SNP analyses we found that in general cohort of patients with MM some genotype frequencies differed from the control group. For instance, genotypes of IL-1RTT and IL-1RATT were varied in control group and group of patients 0.33 *vs* 0.16 and 0.28 *vs* 0.46 respectively (p≥0.05). However, genotype IL-1RTT in MM patients with severe bone lesions (1st gr.) occurs more often than in group of patients with symptoms of osteoporosis (2nd gr.): 0.33 *vs* 0.06 respectively (p≤0.05). Although, genotype IL-1RCT in the 2nd gr. was registered frequently compare to 1st gr. and control group- 0.50 *vs* 0.33 and 0.33 respectively (p≥0.05). However, IL-1RATT genotype frequency was less common in healthy people (0.28) compare to patients with MM (0.45 in 1st gr., 0.47 in 2nd gr.; p≤0.05). Finally, IL-1RACT was higher in control group (0.50) compare to patients with severe osteolytic bone lesions (0.33) and patients with symptoms of osteoporosis (0.40), p≥0.05.
Summary/Conclusions: Thus, our results allow to describe some genotypes as markers associated with the development of MM (IL-1RATT). In addition, it can be assumed that the genotype IL-1RTT associated with the development of severe osteolytic bone lesions in multiple myeloma in turn genotype IL-1RCT as an immunogenetic marker of a lighter form of bone disease manifested with osteoporosis and isolated pockets of lysis.

PB1943

ZOLEDRONIC ACID INHIBITS CELL GROWTH OF MULTIPLE MYELOMA CELLS AND SHOWS SYNERGISTIC ANTIMYELOMA EFFECTS WITH BORTEZOMIB VIA DOWNREGULATION OF PIM-2 THROUGH NF-KB PATHWAY

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Background: Recent studies showed that the third-generation biphosphonate Zoledronic acid (ZOL) can exhibit direct antitumour activity. However, its possible machnism remains unknown.
Aims: To explore the mechanism of Zol's antitumor activity.
Methods: In this study, RPMI-8226 cell line were treated with ZOL alone at various concentrations and combined with Bortezomib in vitro, then Cell proliferation, cell apoptosis, downsteam signaling pathway were detected by qRT-PCR and Western blot.

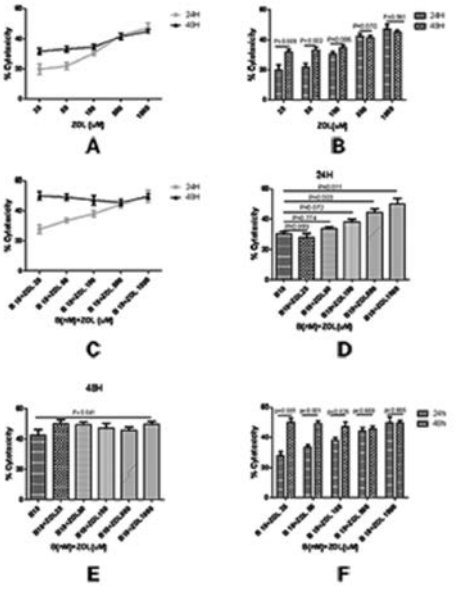

Figure 1.

Results: We found that ZOL alone strongly inhibited proliferation of myeloma cells *in vitro* and induced their apoptosis. The same results were obtained in ZOL combined with Bortezomib after 24h. Futhermore, we found that Ras, pAKT and NF-kB were suppressed by ZOL alone and combined with Bortezomib at 24h. We demonstrated NF-Kb/pim-2 pathway which were also inhibited at 24h. However, it did not show the same results at 48h. additionally,NF-kB plays a pivotal role in regulating of pim-2 which proved by the result of downregulation of pim-2 was appered when the NF-kB inhibitor(Ro 106-9920) was used in RPMI-8226 cell line.
Summary/Conclusions: In conclusion, ZOL and the combination of ZOL with Bortezomib inhibited proliferation of myeloma cell via inhibiting NF-Kb/pim-2 pathway. Ras and pAkt were also inhibited. Combination of Zol with Bortezomib within 24 hours maybe more benefitial to alleviate tumour load in MM patients.

PB1944

VALIDATION OF REVISED INTERNATIONAL STAGING SYSTEM (R-ISS) IN THE ERA OF NOVEL AGENTS: REAL-WORLD DATA ON 481 MYELOMA PATIENTS FROM A GREEK MYELOMA CENTER

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Background: Risk stratification has a crucial role in the management of Multiple Myeloma (MM). International staging system (ISS) has been a powerful tool for risk stratification and prognosis in MM for many years. Recently, a revised ISS (R-ISS) has been validated in a large study that included patients enrolled in international randomized studies. This staging system combined ISS with two additional prognostic factors of overall survival (OS), that is abnormal lactate dehydrogenase (LDH) and high risk molecular cytogenetics *i.e.* del17p and/or t(4;14) and/or t(14;16). According to the aforementioned study, R-ISS represents a single powerful prognostic system that stratifies MM patients more effectively than conventional ISS with respect to the relative risk of their survival. Data regarding the prognostic value of R-ISS outside clinical trials, are limited.
Aims: Herein, we have presented real-world data regarding the prognostic value of R-ISS, in a large number of MM patients from a single Greek Myeloma Center.
Methods: We have reviewed the records of 481 consecutive symptomatic MM patients diagnosed and treated in our center between 2000-2015 (median age: 68 years, range: 29-90 years; M/F: 258/:223; IgG: 271, IgA: 124 Light chain: 66, non-secretory: 15, IgD: 4, IgM:1 ISS1: 153, ISS2: 148, ISS3: 180).
Results: R-ISS was available in 411 patients (M/F: 221/190; median age: 68 years, range: 29-90 years; IgG: 237, IgA: 103 Light chain: 53, non-secretory: 13, IgD: 4, IgM: 1; ISS1: 101, ISS2: 144 and ISS3: 165). Fifty-seven (13.8%) patients had R-ISS1, 286 patients had R-ISS2 (69.5%) and 68 patients had R-ISS3 (16%). High-risk molecular cytogenetics were present in 27% of patients while high LDH was present in 27% of patients. Overall, 280/481 patients (58%) were treated with novel agents (NA) combinations in first line (IMiD-based: 168, Vel-based: 110, conventional therapy: 194, missing data: 9). Second line therapy was administered in 230/481 patients; 78% was treated with NA (Lenalidomide-dexamethasone: 67, Vel-based: 44, Thal-based: 70, chemotherapy: 49). Autologous transplantation was offered in 68/190 (36%) eligible patients. After 1st line therapy, the objective response rate was 82%, while at least very good partial response (≥vgPR) was achieved by 58% of patients. After a median follow up of 8 years (95% CI: 80-114), 135/481 (28%) patients are alive. In the univariate analysis, age, creatinine, ISS, R-ISS, molecular cytogenetics, type of 1st and 2nd line treatment, achievement of ≥vgPR and autologous transplantation (ASCT) predicted for overall survival (OS). In the multivariate analysis, R-ISS and ≥vgPR were independent predictors for survival (p<0.001, HzR:0.4, 95% CI: 0.2-0.6 and p=0.03, HzR: 0.6, 95% CI: 0.4-0.9, respectively). Median OS for patients with R-ISS1, R-ISS2 and R-ISS3, was 92 months (95% CI: 55-129), 36 months (95% CI: 32-40) and 20 months (95% CI: 13-27), respectively (p<0.001). A subgroup analysis confirmed the prognostic role of R-ISS irrespective of age (<65 or ≥65), period of diagnosis (<2006 or ≥2006), type of treatment (bortezomib-based, IMiD-based or chemotherapy) and administration or not of ASCT.
Summary/Conclusions: Our results confirm that R-ISS is the most powerful prognostic factor for OS in an unselected MM population. Its predictive value is maintained across different groups of patients providing a simple and available tool for risk stratification of MM patients, which is essential for therapeutic decisions.

PB1945

THE EFFECT OF HYPOXIA ON NOTCH PATHWAY IN MULTIPLE MYELOMA NICHE

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Background: Multiple myeloma (MM) is an incurable hematological tumor representing 13% of all hematological malignancies and tumor cells accumulate in the bone marrow (BM) of patients. Several evidences demonstrate that Notch signaling mediates critical events in MM progression. The Notch pathway is highly conserved and consists of 4 receptors and two different ligand families (Delta and Serrate). Recently Notch receptors and ligands have been shown to be upregulated during MM progression and their signaling positively regulates cell proliferation, drug resistance and BM infiltration. Hypoxia is defined as a low oxygen condition and can induce angiogenesis; HIF-1α is the key mediator of hypoxic response and a transcription factor that can switch on different types of genes, which, in turn, regulate angiogenesis (VEGF) and proliferation of various stem cell populations. Recent reports indicate that HIF-1α may positively regulate Notch signaling and enhance the expression of Notch downstream genes. In MM, hypoxia can sustain stem cell-like population in MM and HIF-1α derived from bone marrow endothelial cells can have a role in promoting drug resistance in patients with MM.
Aims: The aim of this work is to evaluate if and how hypoxia can affect the activation of Notch signaling in MM microenvironment and the underlying mechanism.
Methods: OPM2, U266, H929 and RPMI8226 cell lines were cultured in complete RPMI-1640 medium. To induce an hypoxic-like condition, cells were treated with Cobalt Chloride ($CoCl_2$) 100 μM for 24 h. To analyze the modulation of the expression of HIF-1α, the MM cells were treated with $CoCl_2$ 100 μM for 2h, 6h, 12h and 24h. We evaluated the effect of hypoxia on cells proliferation, apoptosis and cell cycle; Western Blot and qPCR were used to analyze how hypoxia can affect the activation of the Notch pathway. Cell proliferation: absolute cell counts were determined using the volumetric count tool of the BD FACSVerse™ System (BD Biosciences, USA). Apoptosis: MM cell lines were treated with $CoCl_2$ 100 μM for 24h and stained with Annexin V-FITC and Propidium Iodide, then processed using the BD FACSVerse™ System (BD Biosciences). Western Blot: 100 μg of proteins were loaded on a 8% polyacrilamide gel and transfer onto a nitrocellulose membrane. The membrane was incubated with the following antibodies: Actin (Santa Cruz Biotechnology), HIF-1α (Santa Cruz Biotechnology) and Notch2-cleaved (Abcam). Quantitative PCR reactions were carried out on a 7500 Fast Real-time PCR system (Applied Biosystems) using the MaximaTM SYBR Green/ROX qPCR Master Mix (ThermoScientific).
Results: The MM cell lines OPM2, U266, H929 were treated with $CoCl_2$ 100 μM for 24 to mimic hypoxic condition and we evaluated the effect on cell proliferation and apoptosis: hypoxia inhibits proliferation in all MM cell lines used (H929, U266 and OPM2) and induces apoptosis only in H929 cell lines. To analyze the molecular effects of hypoxia, we treated MM cell lines with $CoCl_2$ 100 μM for 2h, 6h, 12h and 24h and performed a Western Blot. HIF-1α is highly expressed in treated cells if compared to normal controls; the hypoxic condition induced the expression of pro-angiogenic factors *i.e.* VEGF and SDF-1α. Interestingly these have been reported to be controlled by Notch. Consistently, hypoxia induced the activation of Notch2 receptor.
Summary/Conclusions: Our results, although still preliminary, indicate that hypoxia can affect MM cell lines survival and proliferation and finally and that it is able to induce the activation of the oncogenic Notch pathway.

PB1946

A NOVEL TOOL TO MONITOR ANGIOGENESIS IN BONE MARROW SMEARS: DETECTION AND QUANTIFICATION OF ENDOTHELIAL PROGENITOR CELLS BY MULTIPLE-LABELING IMMUNOFLUORESCENCE ANALYSIS

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Background: Angiogenesis is a crucial event in cancer, as it is present in tumor growth, invasion and metastasis. It is considered to have a key role in several haematological malignancies that affect the bone marrow, such as multiple myeloma. Tumor neovascularization and angiogenesis involve the recruitment of endothelial progenitor cells (EPCs), which are bone-marrow derived circulating progenitors with the potential to differentiate into cells of the endothelial lineage. Two types of EPCs have been described: early EPCs, which can also be called angiogenic cells, and mature EPCs or endothelial outgrowth cells. These two types of cells differ in markers expression, morphology, proliferative potential and *in vitro* function, like vascular tube formation. Identification of EPCs is still a challenging task, since these cells display no specific cell-surface antigen. Even so, it is widely accepted that early EPCs are positive for the following markers: cluster of differentiation (CD)133, which is expressed by haematopoietic and progenitor cells; CD34, characteristic of hematopoietic stem cells and activated endothelium of small vessels; and vascular endothelial growth factor receptor (VEGFR)-2, present in endothelial lineage. Regarding mature EPCs, they are considered $CD133^-/CD34^+/VEGFR\text{-}2^+$.
Aims: To overcome the difficulty in the assessment of EPCs, usually by flow

cytometry and cell culture, we decided to develop a new triple-labelling immunofluorescence analysis protocol to assess EPCs in bone marrow smears as a tool to monitor the angiogenesis process and disease progression in pathologies like multiple myeloma.
Methods: Bone marrow smears archived at the Hematology Service of Instituto Português de Oncologia Dr. Francisco Gentil, Lisbon, from multiple myeloma patients were used. Smears were fixed and permeabilized with a 75% methanol/25% acetone/0.01% Triton X-100 solution and blocked with 10% goat serum/1% bovine serum albumin in PBS or 10% rabbit serum serum/1% bovine serum albumin in PBS, according to the host species of the secondary antibodies. Triple labelling was performed using antibodies against CD133, CD34 and VEGFR-2, followed by species-specific secondary antibodies, and nuclei labelling with DAPI. Smears were analysed under a confocal laser microscope and/or a widefield epifluorescence microscope with appropriate excitation and emission filters.
Results: With this protocol, it was possible to identify cells that were triple ($CD133^+$, $CD34^+$ and $VEGFR\text{-}2^+$) and double labelled ($CD133^-$, $CD34^+$ and $VEGFR\text{-}2^+$), which correspond to early EPCs and mature EPCs, respectively. Furthermore, quantification of the total number of labelled nuclei allowed the establishment of the percentage of early EPCs and mature EPCs relatively to the cellular population of the bone marrow smear. Moreover, analysis of samples of the same patient collected at different time points allowed the establishment of differences in EPCs subsets along the course of the disease.
Summary/Conclusions: Our protocol is an easy and accessible method to monitor angiogenesis based on analysis of EPCs, which can be implemented in any laboratory equipped with a suitable microscope. This protocol may be used to study multiple myeloma, as well as other pathologies involving enhanced angiogenesis in the bone marrow, therefore constituting a novel tool for early detection of angiogenesis and efficient monitoring of neovascularization along disease progression and treatment.

PB1947

THE EFFECT OF MIR-146A OVEREXPRESSION ON THE PROLIFERATION AND APOPTOSIS OF MULTIPLE MYELOMA CELL LINE

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Background: After lymphoma, multiple myeloma (MM) is the second most common hematological malignancy worldwide contributing 1% of all cancers and nearly 2% of cancer mortalities. Deregulation of microRNAs has been implicated in the pathogenesis of multiple myeloma. miR-146a is one of the most important microRNAs in tumor initiation and progression which has a dual effect and act as a tumor promoter or a tumor suppressor.
Aims: The present study aimed to evaluate the functional effect of miR-146a in multiple myeloma cells.
Methods: The expression of miR-146a was examined in myeloma cell line, L363 by qRT-PCR. The effect of overexpression of miR-146a on proliferation and apoptosis of myeloma cells was further evaluated by lentiviral-based delivery method.
Results: According to our results, the expression of miR-146a significantly increased in cells transducted with transfer vector compered to non-transducted cells (P-value=0.001). Transfection of miR-146a resulted in significant decrease in tumor cell proliferation (P-value=0.001) and significant increase in apoptosis of multiple myeloma cells (P-value=0.004).
Summary/Conclusions: According to previous studies, miR-146a has a dual effect in cancers. Our result suggested that miR-146a acts as a tumor suppressor in MM. It seems that miR-146a regulates an miR-146-NF-κB negative feedback regulation loop in myeloma cells by inhibiting the expression of IRAK1 and TRAF6 and consequently prevents tumor cell proliferation and enhanced apoptosis.

PB1948

MAST CELLS INDUCE MYELOMA PLASMA CELLS' PROLIFERATION

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Background: Mast cells (MCs) are inflammatory cells that participate actively in multiple myeloma (MM) progression. They mainly enhance angiogenesis through production of various pro-angiogenic molecules, whereas they may also participate per se in the vascular plexus.
Aims: The aim of the study is to explore whether MCs participate in other aspects of MM progression, such as proliferation of plasma cells.
Methods: We studied 57 newly diagnosed patients with active MM, 26 of them in at least partial remission after receiving bortezomib-containing regimens, and 20 age and sex-matched healthy controls. We studied in bone marrow MCs density (MCD) and plasma cells' proliferation rate (using immunohistochemical expression of tryptase and PCNA, respectively) and in sera levels of angiopoietin-2 (Ang-2) end endoglin (sCD105) (using ELISA).
Results: All variables were higher in active MM patients compared to both healthy subjects and responders to conventional treatment ($p<0.001$ in all cases). Moreover, in active MM patients, MCD correlated positively with PCNA expression ($r=0.490$), Ang-2 ($r=0.554$) and sCD105 ($r=0.566$) levels ($p<0.001$ for all cases).
Summary/Conclusions: MCs enhance angiogenesis and probably through this procedure they also induce proliferation of plasma cells. Although the correlation seems to be rather indirect, MCs are major participants in MM progression and therefore could be considered possible targets for therapeutic interventions.

PB1949

EARLY DETECTION OF PLASMA CELL DYSCRASIA IN SERUM PROTEIN ELECTROPHORESIS

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Background: Monoclonal gammopathies are characterized by an excessive production of a single, or rarely more, types of immunoglobulins. They arise from the proliferation of one specific B cells malignant clone (plasma cell dyscrasia), which in turn generates an homogeneous population of monoclonal immunoglobulins. Early detection of these clones can be made by serum protein electrophoresis, which shows a different plot curve or a small narrow peak.
Aims: To early identify plasma cell dyscrasias by small changes in the electrophoretic curve profile, enabling a sooner diagnosis, effective follow-up and treatment.
Methods: A retrospective analysis was made. A total of 7265 electrophoretic curves from 4987 patients (no replicates) were analyzed - 3568 men and 1419 women with average ages of 51.0 and 57.8 years respectively. Patients with an electrophoretic changed pattern were divided into 2 groups. Group-I: alterations of the curve pattern between beta and gamma regions, including distortions and small peaks; Group-II: evident monoclonal peak. Electrophoresis was performed in agar gel and the chains were identified by immunofixation. Blood cells counts were studied in both groups. Statistical analysis was performed by SPSS and the critical level of significance was set at $p\leq0.05$.
Results: Monoclonal gammopathy was identified in 3.5% of the patients (n=176). The average age of this group was 70.0 years old and they were mostly male. To characterize these monoclonal gammopathies by immunofixation, a second harvest was performed in 34 of these patients-54.8% was associated with heavy chain IgG (n=17), 22.6% associated with IgA (n=7) and 19.4% with IgM (n=6) - kappa light chains 58% and lambda light chains 42%. 1 patient had biclonal gammopathy IgG and IgM (lambda light chain) and 3 patients did not have any band alteration. In Group-I (18 patients -58.1%) the average age was 69.6 years. The mean quantification of beta region was 15.4g/L (reference range 7-13 g/L). The mean quantification of gamma region was 13.3 g/L (reference range 6-16 g/L). The mean quantification of distortions and small peaks from this group by densitometry was 4.7g/L. This population had an average of $4.4x10^{12}$/L red blood cells and 13.6 g/L of hemoglobin. Monoclonal gammopathy of undetermined significance (MGUS) was the most common diagnosis (6 patients- 33.3%). This population of patients contrasts and differs significantly from Group-II (13 patients - 41.9%). Group II average age was 74 years old. Electrophoretic curve displayed a well-defined monoclonal peak of 14.7g/L ($p<0.001$), with an average of $3.83x10^{12}$/L red blood cells ($p=0.048$) and 11.2g/L of hemoglobin ($p=0.010$). Multiple myeloma (4 patients-33.3%) and Waldenström macroglobulinemia (2 patients-15.4%) were the most common diagnosis.
Summary/Conclusions: The characterization of monoclonal gammopathies by immunofixation allowed an early detection of situations that can lead to a plama cell dyscrasia. Initially, the protein levels in electrophoresis can be normal or slightly higher so, to detect these diseases in a timely effective manner is extremely important to analyse the electrophoretic curve pattern.

PB1950

SERUM LEVELS OF E-SELECTIN AND VCAM-1 IN ACTIVE MYELOMA

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Background: Adhesion of myeloma plasma cells in bone marrow is an important hallmark for their homing. Various adhesion molecules participate in this process, such as E-selectin and VCAM-1. These molecules seem to participate in both cancer and inflammation.
Aims: The study, for the first time, of serum levels of E-selectin and VCAM-1 in active myeloma patients and correlation with bone marrow mast cell's density (MCD), serum levels of soluble endoglin (sCD105) (marker of endothelial activation) and disease progression.

Methods: We studied 54 newly diagnosed active myeloma patients and 20 healthy controls. We estimated bone marrow MCD using the immunohistochemical expression of tryptase and measured serum levels of E-selectin, VCAM-1 and sCD105 with ELISA.
Results: All parameters were higher in active myeloma patients compared to controls ($p<0.001$ for all cases) and were also increasing in parallel with ISS stages ($p<0.001$ for all cases). Significant positive correlations between MCD and levels of E-selectin ($r=0.448$), VCAM-1 ($r=0.597$) and sCD105 ($r=0.667$) ($p<0.001$ for all cases) were noted.
Summary/Conclusions: We found increased serum levels of the adhesion molecules, correlating with MCD, sCD105 and mainly with disease progression. These increased levels may represent increased bone marrow expression, which in turn may be the result of both enhanced angiogenesis and inflammation. By these means, E-selectin and VCAM-1 may be regarded as markers of disease activity.

PB1951

RISK FACTORS ASSOCIATED WITH EARLY MORTALITY IN PATIENTS WITH MULTIPLE MYELOMA IN THE NOVEL-AGENTS ERA
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Background: Although the introduction of novel agents improved the survival outcomes in patients with multiple myeloma (MM), some patients died within less than one year (early mortality, EM) following the diagnosis.
Aims: In this study, we evaluated the EM rate, and investigated the risk factors associated with EM in MM patients.
Methods: Retrospective data from 542 patients who were initially treated with a novel agent-containing regimen were analyzed.
Results: The median overall survival (OS) for the entire cohort was 56.5 months. The median OS in the 2010-2014 group was longer than in the 2002-2009 group (59.2 months *vs* 49.1 months, P=0.054). The rate of EM was 13.8%, and the most common cause of EM was infection and comorbidity. In multivariate analysis, the age-adjusted Charlson comorbidity index (ACCI ≥4), low body mass index (BMI <20 kg/m^2), thrombocytopenia, and renal failure were significantly associated with EM. The presence of none, 1, or ≥2 factors was associated with a 4.1%, 14.3%, or 27.4% risk of EM (P <0.001), respectively. The median OS times was significantly different depending on the presence of factors associated with EM (P <0.001).
Summary/Conclusions: In conclusion, the ACCI (≥4), low BMI, thrombocytopenia and renal failure were strong predictors for EM in the novel agent era. These data may help manage MM patients and improve survival.

Myeloma and other monoclonal gammopathies - Clinical

PB1952

EXPECTANCY OF LIFE AND INCIDENCE RATE OF NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS. 15 YEARS RETROSPECTIVE ANALYSIS
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Background: In the last decade we have assisted to an amazing improvement in the management and expectancy of life of Multiple Myeloma (MM) patients. Past decade also shows us a new demographic data in our society: the increment of expectancy of life and an excellent performance status. New expensive but very effective antimyeloma (antiMM) agents are in the center of attention of Hematologic and Public Healthcare Systems.
Aims: We have analysed our data base and calculate incidence by sex, age and three 5-years periods of time at diagnosis and obtain tendencies to get ready for next decade of ageing people with best antimyeloma agents.
Methods: We retrospectively analysed the incidence of patients with new diagnostic of Multiple Myeloma (NDMM) from 1998 to 2012. Then we divide the cohort in several groups: sex and age at diagnosis (3 groups) and three 5-year period of time (1998-2002, 2003-07 and 2008-12). Characteristics of patients: n= 273. Male/female: 170/103. Median age at diagnosis: 74 years (Range: 39-100). We don't see differences in median age between that three 5-years periods. We have calculated the incidence per 100000 inhab/year using census data of our Local Registry of Tumours of our Public Health Area. *(Table 1)*
Results: There were a constant increase of Annual Average Incidence of more than 10% in the 3 periods: 4.57 *vs* 5,12 (+12%) *vs* 6,15 (+34,6%). This increment was due to older patients more than younger one. This young group accounts for a constantly near one quarter of all NDMM. There were a constant incidence of about 16-18 per 100000/year NDMM in population over 65 y. We don't observe large differences between groups over and under 75 years.
Summary/Conclusions: We have observed a more than expected incidence of NDMM in our Public Health Area. Demographics of other Public Health Areas Average Annual Incidence were quite different to ours, because of ageing of population in our Area. A preliminary analysis of incidence of period of 2013-14 are similar to last 2008-2012 period.

PB1953

IMPACT OF TIME SPENT WAITING FOR AUTOLOGOUS TRANSPLANTATION ON THE OUTCOME OF PATIENTS WITH MULTIPLE MYELOMA
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Background: Autologous stem cell transplantation (Tx) is still the standard of care for symptomatic myeloma patients (pts) eligible for high-dose procedure. Whether time lasting between stem cell collection and Tx (Wait-Tx) has an impact on Tx outcome itself is matter of debate.
Aims: The aim of this study is to evaluate whether Wait-Tx impacts on the outcome of transplanted myeloma pts.
Methods: We reviewed data of 233 myeloma pts treated with HD Melphalan between January 2000 and December 2013; 20 pts with primary refractory disease and 26 pts treated with a double transplant program were excluded. One hundred eighty seven (187) pts entered the analysis: Male/Female 99(53%)/88(47%), median age 55 years (28-69), Myeloma Type IgG/IgA/light chain/non secretory 111(59%)/38(20%)/33(18%)/5(3%), Durie&Salmon stage II/III 26(14%)/161(86%). Induction therapy consisted of 4 VAD cycles in 83 pts (44%) treated from 2000 to 2005 or 4 Bortezomib-based cycles in 104 pts (56%) treated from 2006 to 2013. Stem cell collection was primed with a single course of DCEP or HD-EDX followed by G-CSF at 10 mcg/kg until completing stem cell collection (PBSC). Tx conditioning regimen consisted of single HD-Melphalan infusion 200 mg/m^2 in 137 pts (73%); HD Melphalan was given at the reduced dose of 160 mg/m2 in 50 pts (27%) due to comorbidity. Response was defined according to International Myeloma Working Group criteria.
Results: Median time between stem cell collection and transplantation (Wait-Tx) was 4 months (range 0.9-13.8 months). After a median follow up after Tx of 49 months (range 24-75 months), 140 patients (74%) relapsed or progressed, median progression free survival (PFS) after Tx was 23 months (range 12-54 months), with a median OS after Tx of 70 months (range 34 months -not reached). PFS was not different between tertiles of Wait-Tx (T1= 0.9-3.2 months, T2= 3.3- 4.6 months, T3=4.7-13.8 months; logrank test p=0.45, cfr Figure 1). When multivariate analysis was performed including Wait-Tx, age at Tx, disease status at Tx (≥VGPR *vs* PR *vs* <PR), type of induction (bortezomib based *vs* VAD) as covariates, disease status at transplant ≥PR (HR 2.88, CI

1.65-5.03, p<0.05) and bortezomib based induction (HR 0.63, CI 0.42-0.94, p=0.024) significantly correlated with longer PFS, while Wait-Tx was not associated with an increased risk of progression (HR 0.98, 95%CI 0.86-1.12, p=0.76). Similar results were found as far as OS was concerned (Wait-Tx HR 1.12, 95% CI 0.94-1.32, p=0.180; bortezomib based induction *vs* VAD, HR 0.7, 95% CI 0.4-1.2, p=0.223; ≥PR, HR 2.04, 95% CI 1.05-3.9, p=0.034).

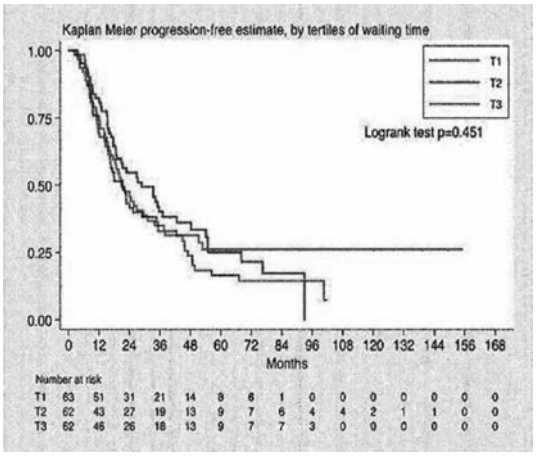

Figure 1.

Summary/Conclusions: Time frame between PBSC collection and stem cell infusion had no significant impact on the outcome of transplanted myeloma patients. Response to induction treatment and bortezomib based induction therapy are both predictive of better outcome ensuring a longer PFS after transplantation.

PB1954

SINGLE CENTRE OUTCOMES OF ALLOGENEIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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Background: The introduction of novel drugs and autologous stem-cell transplantation (SCT) have improved outcomes in multiple myeloma (MM) but most patients relapse and remain uncured. Allogeneic SCT is a potentially curative therapy but high transplant-related mortality (TRM) limits use of this strategy. Reduced-intensity transplants have reduced TRM but consequently higher relapse rates occur.
Aims: Our primary aim was to evaluate progression free survival (PFS) and overall survival (OS) in patients with MM who underwent allogeneic SCT at our centre. We also examined the effect of several factors on PFS and OS; pre-allogenic SCT disease status, prior autologous SCT and number of lines of treatment, in addition to conditioning regimen, age, and 6 month chimerism levels.
Methods: A retrospective analysis of medical records for all consecutive MM patients that underwent allogeneic transplantation at our centre between May 1999 and December 2015.
Results: 29 MM patients underwent allogeneic SCT at a median age of 51 with the median year of transplant being 2005. Prior to allogeneic SCT a median of 3.6 treatment lines (range 2-9) were administered, including an autologous SCT which 79.3% of the cohort received. The subtype of MM was; IgG 48.3%, IgA 20.7%, plasma cell leukaemia 17.2%, kappa light chain myeloma 10.4% and lambda light chain disease 3.4%. Prior to allogeneic SCT 41.2% were in complete remission, 14.8% in very good partial remission (VGPR), 29.2% in partial remission and 14.8% had stable disease. A sibling was the stem cell donor in 57.1% of cases, the remainder had matched unrelated donors. 51.7% of patients received fludarabine, melphalan and alemtuzumab conditioning, 13.8% received fludarabine, busulfan and alemtuzumab, other regimens used included those utilising anti-thymocyte globulin ATG or total body irradiation. PFS and OS were 35 and 56 months respectively. The 10 year PFS was 23% and OS was 49%. 14 deaths occurred during a median follow-up of 58.1 months. Age, pre-allogeneic SCT disease status, 6 month chimerisms and type of conditioning regimen had no significant effect on PFS or OS. Those with fewer than 4 lines of treatment prior to allogeneic SCT had longer OS (P=0.4) and PFS (P=0.015). IMiD therapy was given to 51.7% of patients pre-allogeneic SCT and 27.6% received velcade. Those that received 1 autologous SCT (n=16) had longer OS (P=0.026) compared to those that received 2 (n=7) or no autologous SCT (n=6), but PFS was not affected. Neutrophil engraftment occurred after a median of 13 days. Graft *versus* host disease in the first 100 days occurred in 4 patients and in 5 patients after 100 days. 4 patients had pre-planned maintenance therapy post allogeneic SCT. Donor lymphocyte infusion was given to 9 patients post allogeneic SCT at a median of 9 months post allogenic SCT, a median of 3 infusions were administered. 2 patients subsequently received an autologous SCT post allogeneic SCT at a median of 36.5 months. A median of 1 line of treatment (range 0-5) were given to the 13 patients who relapsed following allogeneic SCT.
Summary/Conclusions: Our data indicates that performing an allogeneic SCT after less than 4 lines of treatment and after 1 autologous SCT yields better outcomes. Low disease burden as demonstrated by achieving at least a VGPR is also optimal, although not significant in our data. The role of immunomodulation including maintenance therapy post allogeneic SCT needs to be fully examined in prospective clinical trials to optimise the chance of allogeneic SCT being curative.

PB1955

OUTPATIENT STEM CELL MOBILIZATION WITH INTERMEDIATE-DOSE CYCLOPHOSPHAMIDE IS A SAFE AND EFFECTIVE PROCEDURE

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Background: Autologous stem cell transplantation (ASCT), even in the era of new drugs, is the treatment of choice in younger and fit Multiple Myeloma (MM) patients. Cyclophosphamide (CY) at different doses has been shown to be an effective regimen for collecting peripheral blood stem cells (PBSC) in MM. Clinical trials have demonstrated that intermediate dose CY (3 and 4 g/m2, ID-CY) combined with G-CSF, is an efficient mobilizing regimen with less toxicity compared with high dose CY (7 g/m2, HD-CY) in term of neutrophil recovery, thrombocytopenia, need of transfusions and IV antibiotics. (Goldschmidt, BMT 1996; Fitoussi, BMT 2001). The method used for PBSC mobilization has not been shown to affect overall transplantation outcomes (Kumar, Blood 2009). Mobilization therapy is usually administered in an inpatient regimen, for the concern of toxicity.
Aims: Our purpose is to evaluate the safety of mobilization therapy administered in an outpatient regimen, with the prospect to lower costs and minimize patient inconvenience, maintaining an optimal yield.
Methods: One hundred patients with newly diagnosed MM underwent outpatient stem cell mobilization with CY 3 g/m2 (80%) or 4 g/m2 (20%) + G-CSF after induction therapy with VEL-based (81%) or VAD (19%) regimens. G-CSF 10 mcg/Kg was started by day +5 and continued until completion of apheresis. No antibiotics prophylaxis was routinely used. Day 0 was defined as the CY infusion day. CY was administered in 2-4 consecutive 1h infusions (depending on total dose). Hyper-hydration (3.5/4 l), antiemetics and the uroprotectant Uromitexan were began IV 1 hour before CY infusion. Subsequently, Uromitexan was continued at home orally in the next 12h. Furthermore, the patient was advised to drink 2.5/3 l of water in the next 24h. Blood count was monitored at day + 4 and daily from day +7. CD34+ cells were counted on peripheral blood by day 7; apheresis was started at leukocyte rise and with a value of at least 20 CD34+/µl. Number of apheresis depended on the number of CD34+ cells collected to obtain al least $4x10^6$ CD34+/Kg. Stem cells were then manipulated and cryopreserved with standard techniques.
Results: Results are actually available for 88 of these patients. Median age at mobilization therapy was 57y (range 34-68). Response prior of mobilization was CR/sCR in 18%, VGPR in 52%, PR in 27%, and PD in 3%. Chemotherapy was very well tolerated. Most frequently observed adverse events (AEs) were nausea and vomiting of grade 1-2. Two patients experienced cystitis (one grade 1, one grade 2), 2 patients fever and infections. Only one patient required hospitalization for AEs for fever without microbiological findings, rapidly regressed with IV antibiotics. There were no other significant AEs related to chemotherapy. All patients proceeded to stem cell harvest and reached CD34+ target, but 7 patients required administration of Plerixafor on demand. After mobilization, 85 patients proceeded to ASCT.
Summary/Conclusions: In conclusion, outpatient mobilization with ID-CY appears to be an efficient and safe procedure, with minimal and manageable side effects and low rate of hospitalization. Outpatient mobilization could ameliorate the quality of life of patients and reduce costs, avoiding or minimizing the hospitalization rate, without compromising the safety profile and the success of PBSC collect.

PB1956

CLINICAL CHARACTERISTICS OF PATIENTS WITH MULTIPLE MYELOMA PRELIMINARY RESULTS OF A PROSPECTIVE MULTICENTER REGISTRY OF THE COMUNIDAD VALENCIANA (SPAIN)

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Background: Multiple myeloma (MM) is the second most common malignant B-cell disorder. It accounts for 1% of all malignant diseases and for slightly more than 10% of all hematological malignancies. Based on international data, the incidence of MM in Spain has been estimated to be 0.056/1000 of population. However, most of this information is collected from secondary sources as press releases, industry, associations, analyst reports and others but real life epidemiological data based on national or local registries is lacking.
Aims: In order to have a better knowledge of the clinical characteristics at diagnosis of MM patients and to know the real incidence and prevalence of patients with newly diagnosed MM in our environment, the Grupo de Estudios de Mieloma (GREMI) developed in 2013 a prospective, multicenter registry including every patient undergoing a first diagnosis of MM in every public or private sanitary center of the Comunidad Valenciana, Spain.
Methods: The CV involves three regions in Spain (Alicante, Castellón, and Valencia) with a global population of 5,034,665 habitants. A total of 2,578,719 people live in Valencia, 1,868,438 in Alicante and 587,508 in Castellón. Diagnosis of MM is done according to the International Myeloma Working Group criteria. Primary data collection for the registry is done in every center by the local investigators. Immediately after a new diagnosis of MM is performed, the patient is registered on-line by means of an electronic database. Clinical and epidemiological data at presentation, the type of therapy administered, and survival status are included. We present the preliminary epidemiological, clinical, and laboratory results of the patients included in the registry in the period 2013-2014.
Results: From January 2013 to December 2014, a total of 332 patients have been registered in 23 centers. The overall incidence of patients with newly diagnosed MM (NDMM) in the Comunidad Valenciana in the study period was 0.066/1000 of population (0.046 in Alicante, 0.082 in Castellón, and 0.077 in Valencia). Three hundred and twenty-seven (98.5%) patients were of Caucasian descent. Median (range) age of the series was 70.76 (43.07-92.15) years and 95 (28.61%) of the patients were younger than 65 years. One hundred and seventy-seven (53.3%) patients were male. Overall, IgG myeloma was the most commonly observed type of myeloma (174 patients, 52.4%). Eighty-eight (26.5%), 52 (15.7%), and 82 (24.7%) patients presented with an International Staging System of I, II, and III, respectively. Clinical and laboratory data at time of diagnosis are shown in the Table 1.

Table 1.

	N=332
Characteristic (range)	N (%)
Median age – yr	70.76 (43.07-92.15)
Distribution – no. of patients	
18-64 yr	95 (28.60)
≥65 yr	237 (71.40)
Year of diagnosis	
2013	194
2014	138
Race	
Cucasian	327 (98.5)
Latin	4 (1.2)
African	1 (0.3)
Male sex	155 (46.68)
ECOG performance status	
0	123 (37)
1/2	97 (29.2) / 64 (19.3)
3/4	32 (9.6) / 16 (4.8)
Previous known MGUS	33 (9.9)
International Staging System Stage	
Distribution – no. of patients	
I	88 (26.5)
II	52 (15.7)
III	82 (24.7)
Lytic bone lesions	191 (57.6)
Hemoglobin	
Median (g/dL)	10.60 (3.70-15.70)
<10 mg/dL	122 (37.88)
Serum creatinine, mg/dL	
Median	0.96 (1-5)
Distribution – no. of patients	
<2 mg/dL	297 (89.46)
≥2 mg/dL	35 (10.54)
Serum calcium, mg/dL	
Median	9.43 (6-48)
>11 mg/dL	28 (8.43)
Serum LDH, UI/L	
Distribution – no. of patients	
<250 UI/L	280 (84.3)
≥250 UI/L	52 (15.7)
Extramedullary plasmacytomas	15 (4.5)
Type of myeloma	
IgG	174 (52.4)
IgA	84 (25.3)
IgD	6 (1.8)
Light chain	55 (16.6)
Non secretory	13 (3.9)
Serum M-component, g/dL	
Median	4.47
>3 g/dL	296 (89.15)
Urinary M-component, g/day	1.36
Median	

Summary/Conclusions: This is the first epidemiological registry for NDMM patients available in Spain. Our preliminary results indicate that the incidence of MM in the Comunidad Valenciana is similar to that generally reported. Cytogenetic data were routinely collected in the great majority of patients 248 (74.7%). Survival data during the study period will be presented.

PB1957

PATIENT REPORTED OUTCOMES (PROS) OF MULTIPLE MYELOMA (MM) PATIENTS FROM THE PANORAMA-1 STUDY WHO HAVE RECEIVED AT LEAST TWO PRIOR REGIMENS INCLUDING BORTEZOMIB AND AN IMMUNOMODULATORY AGENT

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Background: Panobinostat (PAN), in combination with bortezomib (BTZ) and dexamethasone (DEX), is approved in the EU for the treatment of adult patients with relapsed and/or refractory multiple myeloma (MM) who have received ≥2 prior regimens, including BTZ and an immunomodulatory drug, based on significant clinical benefit of PAN+BTZ+DEX PANORAMA-1.
Aims: This analysis focuses on the impact of PAN+BTZ+DEX on patient experience, including measures of symptoms, function, and health-related quality of life (HRQoL), measured by FACT/GOG-Ntx, QLQ-MY20, and EORTC-QLQ-30.
Methods: In PANORAMA-1, patients who had received between 1 and 3 previous treatment regimens were randomized 1:1 to receive up to 12 cycles of PAN or placebo (PBO), in combination with BTZ+DEX. PRO measures were administered at screening, cycle 1 day 1, and every 6 weeks thereafter until end of treatment. We present here the results at the end of 24 weeks, after completion of the first 8 cycles of the study treatment (treatment phase 1).
Results: In the approved EU population, PRO baseline data were available for 71 and 69 PAN+BTZ+DEX and PBO+BTZ+DEX treated patients, respectively, for the EORTC QLQ-C30, Global Health Status/QoL scale and for 70 patients in each treatment arm for the QLQMY20, Disease Symptoms (DS) subscale and the FACT/GOG-Ntx Neurotoxicity subscale. At Week 24, FACT-GOG-Ntx scores were similar across treatment arms, suggesting no difference in neurotoxicity symptoms (31.75 *vs* 33.57 for PAN+BTZ+DEX *vs* PBO+BTZ+DEX treated patients, respectively). In both treatment arms, symptoms scores were generally low on the scale of 0-100, with lower scores in the QLQ-MY20 DS subscale observed following treatment initiation and no difference between arms observed at Week 24 (23.84 *vs* 16.55 for PAN+BTZ+DEX *vs* PBO+BTZ+DEX treated patients, respectively). EORTC QLQ-C30 Global Health Status/QoL scores were generally stable after treatment initiation, and comparable in the 2 arms at Week 24 (53.82 *vs* 58.05 for PAN+BTZ+DEX *vs* PBO+BTZ+DEX treated patients, respectively).
Summary/Conclusions: These findings support the addition of panobinostat to the well-established BTZ+DEX regimen as an efficacious treatment option with limited symptomatology and impact on patients' HRQoL. With the increasing importance of patient-relevant humanistic benefits in determining the overall value of oncology products findings such as these are important for treatment decision making.

PB1958

RETROSPECTIVE COHORT ANALYSIS EXAMINING THE EFFICACY AND SAFETY OF (V)DTPACE IN NEWLY DIAGNOSED AND RELAPSED/ REFRACTORY MYELOMA PATIENTS-THE UK EXPERIENCE

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Background: Multiple myeloma remains incurable in spite of advances in treatment. In the UK, the combination of dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide and etoposide (DTPACE) continues to play a

role in patients with relapsed/refractory multiple myeloma (RRMM), either as a bridge to stem cell transplant, or for rapid tumor debulking. Recently, bortezomib has also been included (VDTPACE) - this combination was introduced by the Arkansas group in the Total Therapy 3 (TT3) study, where MM patients received this as induction prior to tandem autologous stem cell transplant (ASCT), resulting in enhanced response rates and prolonged survival. However, due to the intensity of both VDTPACE and DTPACE, these regimes have not been adopted by many centers and results outside the Arkansas group have therefore been rarely reported. At the Royal Marsden Hospital (RMH) we have used (V)DTPACE in selected cases where standard therapies are deemed inadequate, including newly diagnosed patients with specific high risk features, salvage treatment for those who were primary refractory and in patients with RR disease.

Aims: To assess response rates, impact on stem cell harvest and tolerability in myeloma patients treated with (V)DTPACE at RMH.

Methods: We retrospectively reviewed the medical notes for all myeloma patients treated with (V)DTPACE at RMH between 2010 and 2015. Our primary objective was ORR defined as PR or better. Secondary objectives included number of stem cells harvested (if applicable) and toxicities (as per CTCAE v4.0 criteria). Patient demographics including age, gender and prior treatment exposure were also analyzed.

Results: Between 2010 and 2015, 53 patients received DTPACE and 26 received VDTPACE. The median age was 55 years with a male predominance (61%). 7/79 (9%) patients received treatment upfront due to high-risk disease features, including 2 patients with plasma cell leukemia and 2 with extensive extramedullary disease at presentation. 8/79 (10%) were primary refractory (<PR) to first-line therapy while the rest (64/79, 81%) had RRMM receiving median 2 (range 2-8) prior lines of therapy including thalidomide (72%), bortezomib (75%), lenalidomide (46%) and autologous stem cell transplant (47%). All patients had EDTA clearance scan as well as cardiac assessment prior to treatment. 15/79 (19%) received 1 cycle (V)DTPACE, 58/79 (73%) received 2 and 6/79 (8%) received 3. 49/79 (62%) proceeded to transplant of which only 4 failed to mobilize stem cells. Mean stem cell harvest was $2.64x10^6$/kg. The ORR (≥PR) observed following DTPACE was 100% (20% CR) in newly diagnosed, 100% in primary salvage and 51% (13% CR) in RR patients. Following VDTPACE, ORR was 100%, 71% and 82% (6% CR) respectively. Commonly reported toxicities included Grade 3≥ neutropenia (94%) and Grade 3≥ thrombocytopenia (48%). No thrombo-embolic events or grade 3≥ neuropathy were reported. At analysis, 39/79 (49%) patients were alive. There were no treatment-related deaths. Survival data is currently analysed and will be presented at meeting.

Summary/Conclusions: Our results demonstrate the efficacy of VDTPACE in newly diagnosed and relapsed/refractory patients, in spite of prior exposure to thalidomide or bortezomib. Importantly this regimen can safely be used to salvage patients with an insufficient response to conventional first-line therapy. VDTPACE had no impact on stem cell mobilization, and was well tolerated with no treatment-related deaths reported.

PB1959

THE ROLE OF COMORBIDITY ON EARLY MORTALITY IN MULTIPLE MYELOMA. A SINGLE INSTITUTION POPULATION-BASED STUDY EMPHASIZING THE NEED FOR STANDARDIZATION

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Background: Multiple myeloma (MM) is a very heterogeneous and complex disease with variable survival. The variability in the outcome cannot be fully explained by the current systems of stratification. Early mortality (EM) remains a serious obstacle to further improve the recent trend towards increased survival demonstrated in recent years (Ríos-Tamayo et al, 2015). However, the definition of EM is not standardized, precluding a proper comparison between studies. Furthermore, no study has systematically focused on the impact of comorbidity on EM in MM to date.

Aims: The aim of this study is to assess the impact comorbidity on the outcome of MM patients, in terms of EM, in a large cohort of real-life patients. On the other hand, all relevant studies on this topic to date have been critically analyzed.

Methods: All newly diagnosed symptomatic MM patients recorded in our population-based registry from January 1985 to December 2015 were analyzed. The study was divided into six periods of five years. Twenty baseline comorbidities were studied, along with common prognostic factors. EM was measured at three key cutoff points: two (EM2), six (EM6) and twelve (EM12) months. Univariate and multivariate binary logistic regression models were used to test independent variables as risk factors for EM.

Results: Six hundred and thirty-one MM patients were recruited in our MM clinical registry during the period of study. A complete assessment of comorbidity was available in 426 patients (68.6%) at the moment of diagnosis. Excluding patients not fit for MM-directed therapy, the percentage for EM2, EM6 and EM 12 was 10.6%, 20% and 28.6%, respectively. For the whole cohort, only age and serum creatinine were independent risk factors for EM in all the cutoff points analyzed. The presence of respiratory disease and light chain MM (borderline) were associated with EM2, whereas the ISS III and liver disease were predictors for EM6, and finally, the lactate dehydrogenase level, the hepatitis virus C infection and the presence of respiratory disease were significantly associated to EM12. Table I highlights recent studies on this topic. The differences in the type of study as well as in the cutoff point used preclude appropriate comparisons.

Table 1. Results of recent studies on NDMM early mortality.

Authors (references)	Type of study	Study period	Year of publication	N.of patients	EM2	EM 6	EM12
Augustson et al	Multicenter (UK)	1980-2002	2005	3107	10	-	-
Kastritis et al (abstract)	Single institution	1994-2012	2013	509	6	13	18
Terebelo et al (abstract)	Multicenter (USA)	2009-2013	2013	1494	-	7	-
Kumar et al.	Single institution	2001-2010	2014	1038	-	-	13
Dimopoulos et al	Multicenter (Greece)	1990-2011	2014	1773	12/7/3[a]	-	-
Holmström et al	Multicenter (Denmark)	2005-2012	2015	1497	-	22	-
O'Donnell et al (abstract)	Single institution	2005-2015	2015	836	-	-	32[b]
Hsu et al	Single institution	2002-2015	2015	451	12.6	-	-
Chen et al	Single institution	2007-2013	2016	122	-	22.95	-
Ríos-Tamayo et al	Single institution (population-based)	1985-2015	-	621	10.6	20	28.6

Abbreviations: NDMM= Newly diagnosed multiple myeloma, EM2=Early Mortality at two months, EM6=Early Mortality at six months, EM12=Early Mortality at twelve months, a,% EM according to estimated glomerular filtration rate (<30, 30-59 or >30 ml/min), b% EM within 24 months.

Summary/Conclusions: The role of comorbidities in EM of MM patients remains to be determined. However, our study confirms the crucial role of renal failure and provides some evidence about the time-dependent impact of specific comorbidities in detailed cutoff points for EM. We suggest that all three cutoff points should be analyzed in the future, in order to allow comparisons between studies.

PB1960

CARFILZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN JAPANESE PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background: Carfilzomib (CFZ) is an epoxyketone proteasome inhibitor that binds selectively and irreversibly to the constitutive proteasome and immunoproteasome. The combination of CFZ with lenalidomide (Len) and dexamethasone (Dex) (CRd) has shown efficacy in a phase 3 study (ASPIRE) in relapsed multiple myeloma (Stewart et al, 2014).

Aims: This phase 1 study was designed to evaluate the safety, tolerability, efficacy and pharmacokinetics of CRd regimens in Japanese patients with relapsed or refractory multiple myeloma (RRMM). This was a company sponsored trial (Ono Pharmaceutical Co., Ltd.).

Methods: Adults with RRMM who had received at least 1 prior treatment were eligible. CFZ was administered as a 10-minute infusion on days 1, 2, 8, 9, 15, and 16 of 28-day treatment cycles (20 mg per square meter on days 1 and 2 of cycle 1 and 27 mg per square meter thereafter) during cycles 1 through 12 and on days 1, 2, 15, and 16 during cycles 13 through 18. Len (25 mg) was given on days 1 through 21 during cycles 1 through 18. Dex (40 mg) was administered on days 1, 8, 15, and 22 during cycles 1 through 18. The efficacy endpoint included the rate of overall response (ORR). Treatment responses and disease progression were assessed by investigators based on the central laboratory results with the IMWG Uniform Response Criteria. All patients provided written informed consent.

Results: Twenty six Japanese RRMM patients were enrolled. The median number of previous regimens was 4 (range, 1-10). The median number of cycles dosed was 4 (range, 1-8 cycles). The proportion of patients with previous therapies of bortezomib and lenalidomide was 88.5% and 61.5%, respectively. A total of 53.8% of patients had high risk abnormal cytogenetics [t (4; 14), t (14; 16), del (17p) or hypodiploid]. In this study, CRd regimen used in ASPIRE study was well tolerated in Japanese population. The ORR was 88.5% (90%CI, 72.8 to 96.8). Subgroup analysis demonstrated that the ORR was not affected by previous therapies and abnormal cytogenetics. The median PFS and OS were not estimated because of the short follow-up period. The most common AEs included lymphocyte count decreased (53.8%), platelet count decreased

(53.8%), hyperglycemia (38.5%), hypophosphatemia (38.5%), constipation (30.8%), white blood cell count decreased (30.8%), and rash (30.8%). The most common Grade 3 or higher AE were lymphocyte count decreased (42.3%), platelet count decreased (23.1%), hypophosphatemia (19.2%), anemia (11.5%), neutrophil count decreased (11.5%), white blood cell count decreased (11.5%) and hyperglycemia (11.5%). Peripheral Neuropathy was observed in 15.4% but no Grade ≥3 or peripheral neuropathy with pain was reported. None of the patients experienced interstitial lung disease. The plasma CFZ concentration showed a rapid decrease after intravenous administration with T1/2 of 0.580–0.740h. The exposure of CFZ increased in a dose-dependent manner with the AUCinf of 326-445 ng*h/mL, Cmax of 1540-2030 ng/mL.

Table 1.

	CRd (N=26)
Overall response rate, no. of patients (%)	23 (88.5)
CR	1 (3.8)
VGPR	5 (19.2)
PR	17 (65.4)
MR	1 (3.8)
SD	2 (7.7)
Subgroup, ≧PR/patients (%)	
Previous regimens	
1 – 3	12/12 (100.0)
≧4	11/14 (78.6)
Prior therapies	
Bortezomib	
Naive	3/3 (100.0)
1 regimen	12/12 (100.0)
≧2 regimens	8/11 (72.7)
Lenalidomide	
Yes	14/16 (87.5)
No	9/10 (90.0)
Cytogenetic risk	
High risk	11/14 (78.6)
Standard risk/Unknown	12/12 (100.0)

Summary/Conclusions: This is the first study that CRd regimen was evaluated in heavily pre-treated MM patients (median 4 prior regimens). CRd regimen was well tolerated and showed a compelling efficacy in Japanese RRMM patients. A comparison of this study with CRd arm in ASPIRE phase 3 study showed that the results of the ORR were similar (88.5% and 87.1%), despite the more heavily pretreated patient population in this study (median 4 *vs* 2 prior regimens). The safety and efficacy of the CRd regimen in Japanese patients seems consistent with that reported in ASPIRE phase 3 study.

PB1961

Abstract withdrawn.

PB1962

PROGNOSTIC SIGNIFICANCE OF EXTRAMEDULLARY DISEASE DETECTED BY PET/CT ON OUTCOMES OF AUTOLOGOUS TRANSPLANT IN MULTIPLE MYELOMA PATIENTS

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Background: Multiple myeloma is a heterogenous disorder with varied responses to transplantation. In the past investigators have reported numerous staging systems, risk stratification models based on laboratory/clinical parameters and genetic information to predict these heterogenous responses. But the disease mostly negative by biochemical tests before transplantation making it a poor choice to predict responses. ^{18}F-FDG-PET/CT has numerous advantages in terms of ability to assess extra medullary disease in addition to the extent of active disease. The relevance of extramedullary disease in predicting response is scarce and evolving.
Aims: To study the role of pre-transplant EMD in the prognostication of multiple myeloma patients post autologous transplant.
Methods: All autologous transplant patients underwent ^{18}F-FDG-PET/CT as part of the pre-transplant workup. The conditioning and treatment protocols were not modified based on PET/CT findings. Extramedullary disease (EMD) on PET/CT was defined as FDG uptake more than Liver SUVmax at any extramedullary site (including soft tissues or lymphoreticular structures including spleen) of at least 5mm in size. We aimed at identifying the prognostic value of the pre-transplant type of lesions on PET/CT in predicting biochemical/clinical progression-free survival (PFS) and overall survival (OS). We also correlated the EMD status with pre-transplant biochemical markers (SPEP, UPEP, SIFE, SFLC, β2M and LDH). The revised IMWG criteria were used for defining progression.
Results: A total of 43 patients underwent pre-transplant PET/CT evaluation of which seven patients had EMD. On Cox proportional regression hazard model EMD had a hazard for post-transplant all-cause mortality of 5.46 times in patients than the medullary disease (p-0.045) (Fig 1A). The 6-year median OS in patients with medullary and extramedullary PET lesions were 80.6%, and 57.1% respectively as shown Fig. 1B. Kaplan-Meier analysis showed poorer OS in patients with EMD χ2 (1-0.496, p-0.481). There was no significant difference in clinical or biochemical EFS among patients with EMD. The median PFS (Clinical) in patients with medullary and extramedullary PET lesions were 77.8%, and 71.4% respectively (p-0.997). The median PFS (Biochemical) in patients with medullary and extramedullary PET lesions were 55.6%, and 57.1% respectively (p-0.352). Engraftment was delayed in patients with EMD (Neutrophil-11.57 *vs* 10.83 days, platelet engraftment - 13.0 *vs* 12.63 days) though the results were not significant (p-0.274, 0.85). Pre-transplant β2M and LDH were significantly higher in patients with EMD (p-0.036). A chi-square test was performed and no relationship was found between the PET positivity and pre-transplant UPEP, SPEP and SIFE with χ2 (1, 42)-0.737, p-0.391, χ2 (1, 43)-0.632, p-0.580 and χ2 (1, 42)-0.305, p-0.580 respectively.

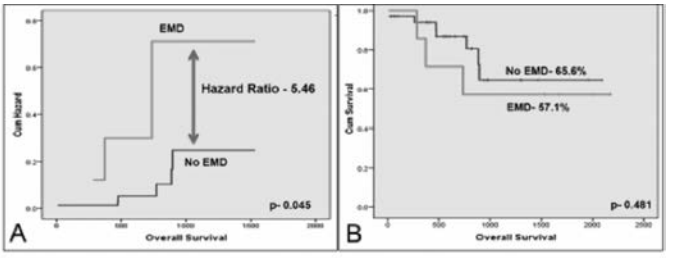

Figure 1.

Summary/Conclusions: EMD detected on ^{18}F-FDG-PET/CT has a higher hazard for mortality and significantly correlated with pre-transplant higher β2M and LDH levels. The Smaller sample size was a major limitation of the study and was responsible for certain differences in measured variables not being statistically significant. To conclude, detection of EMD by pre-transplant ^{18}F-FDG-PET/CT has a significant prognostic role in multiple myeloma patients undergoing autologous peripheral blood stem cell transplant.

PB1963

ASSOSIATION BETWEEN ENDOTHELIAL AND PLATELET DERIVED MICROPARTICLES AND VENOUS THROMBOSIS IN NEWLY DIAGNOSED PATIENTS WITH MULTIPLE MYELOMA (MM) AND NON HODGKIN LYMPHOMA (NHL) - PRELIMINARY REPORT

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Background: Multiple myeloma and non-Hodgkin lymphoma and its treatment are frequently complicated by development of venous thromboembolism (VTE). The incidence of thrombotic complications dramatically raise after highly prothrombotic therapeutic regimens e.g. with thalidomide and lenalidomide in MM.
Aims: The aim of this study was to examine whether procoagulant microparticles (MPs) derived from endothelial cells (EMPs) and platelets (PMPs) constitute an enhanced risk for venous thrombosis.
Methods: We studied 22 patients (pts) without history of VTE, 10/12 F/M, aged 24-84 years. There were 13 pts of MM (9 cases of IgG, 2 of IgA, 1 IgM and 1 non secretory MM) and 9 pts with nHL (5 cases of DLBCL, 2 anaplastic T cell lymphoma and 2 FL).All patients underwent routine coagulation tests. Flow-cytometry was used for quantification of endothelial cell (CD133+) microparticles (EMPs) and platelet (CD61+) microparticles (PMPs). In all pts ultrasound examination of venous system of the lower extremities was performed. Both deep and superficial veins of both limbs were evaluated. Conventional and Doppler imagination, as well as elastography and options to detect microcalcifications (micropure) were used.
Results: The ultrasound examination revealed the presence of vein thrombosis in 11 pts (group I, n=11). Five out of 11 pts with bilateral lesions in VSM (*vena saphena magna*, great saphenous vein) and lower leg veins or bilateral in VSM only comprised subgroup Ia. Six pts with unilateral blood clots in VSM formed group Ib. Thrombosis was observed in 7 pts with MM and 4 with nHL, and bilateral thrombotic lesions were demonstrated in 2 MM and 3 nHL pts. The remaining 11 pts showed no thrombosis (group II). The mean percentage of EMPs (CD133+) was 1,04±1,03, and it did not significantly differ between thrombotic (group I) and non-thrombotic patients (group II) 1,22±1,24 *vs* 0,85±0,77, p=0,406), but there was a trend for increased EMPs in the subgroup Ia (1,37±1,17). The percentage of PMPs (CD61+) was 13,14±4,68 and similar in all studied groups: group I 12,82±3,28 *vs* group II 13,45±5,92 (p=0,759),

including subgroup Ia (11,80±2,32). Mean plasma fibrinogen (FBG) concentration was 3,65±1,85 g/L and did not significantly differ in MM and nHL patients with and without thrombosis (4,06±1,90 *vs* 3,24±0,1,80 g/L, p=0,309). But FBG level in subgroup Ia was found to be significantly higher than that in the remaining patients (5,07±2,45 *vs* 3,23±1,48, p=0,047). Mean D-dimer level was 2,59±4,70mg/L, and there was not significantly different in patients with and without thrombosis (4,01±6,40 *vs* 1,16±0,97 mg/L, p=0,159), however D-dimer was elevated in subgroup Ia in comparison with that in group II (2,52±1,29 *vs* 1,16±0,97 mg/L, p=0,034).
Summary/Conclusions: Venous thrombosis was confirmed in 11 of 22 patients with newly diagnosed patients with MM and n-HL. In patients with many thrombotic lesions there was a trend for elevated activity of endothelial cells (EMPs), but not platelets (PMPs).

PB1964

GALECTIN-3 AS A PREDICTOR OF STATIN TREATMENT EFFICACY IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Galectins are a family of lectin molecules that have emerged as key players in inflammation and tumor progression by displaying intracellular and extracellular activities. Increased expression of galectin-3 (Gal-3) has been associated with chronic myeloid leukemia, multiple myeloma, and chronic lymphocytic leukemia. Although modern treatment options for multiple myeloma produce high response rates, the interrelation between elevated Gal-3 and survival rate is not fully understood. Moreover, elevated Gal-3 associates with increased cardiovascular risk. In this context there is possibility to use statins in multiple myeloma patients to prevent unfavorable outcomes under control of Gal-3 level.
Aims: The aim of the study was to investigate an interrelationship between pre-treatment Gal-3 level and one-year survival rate in subjects with multiple myeloma.
Methods: One hundred twelve subjects with multiple myeloma who reached at least partial remission were enrolled in the study. All subjects gave their written informed consent to participation in the study. Patients were divided into 2 groups based on whether or not statins were included in their treatment: a statin group (n=51) and a no statin group (n=61). Among patients in the statin group, 31 patients received 20- mg/day atorvastatin and 20 patients received 40-mg/day atorvastatin. None of the patients had received any lipid-modulating medications, including statins or fibrates, before enrollment. Observation period was up to 1 year. Blood samples for biomarkers measurements were collected. ELISA method for measurements of circulating level of Gal-3, and NT-pro-brain natriuretic peptide (NT-pro-BNP) were used. Concentrations of Gal-3 and NT-pro-BNP for cumulative survival rate were tested.
Results: Within 1 year progressions were reported in 23 patients (8 statin users (15.7%) and 15 never statin users (24.6%) (P<0.05)). 92 cardiovascular events were reported in 36 patients, (12 statin users (23.5%) and in 24 never statin users (39.3%) (P<0.01)). Lipid lowering effect in statin users was associates with declined serum Gal-3 level, whereas in not statin users similar response was not appeared. No any changes in hemodynamics and other biomarkers between both cohorts were found. Univariate logistic regression had exhibited that Gal-3 (odds ratio [OR]=1.13; 95% CI=1.07–1.25; P=0.003), NT-proBNP (OR=1.05; 95% CI=1.03–1.08; P=0.001), and statin therapy (OR=1.06; 95% CI=1.01– 1.10; P=0.001) predicted one-year cumulative cardiovascular events. Gal-3 (odds ratio [OR]=1.09; 95% CI=1.05–1.19; P=0.02), and statin therapy (OR=1.05; 95% CI=1.01– 1.9; P=0.04) predicted one-year progression free survival. After adjustment on statin therapy, Gal-3 remained independent predictor one-year cumulative cardiovascular events (OR=1.07; 95% CI=1.05–1.10; p=0.001). When initial serum Gal-3 level has incorporated into prediction model, statin therapy was found as predictor for improving survival in patients with elevated serum Gal-3 level (>14 ng/ml).
Summary/Conclusions: Elevated pre-treatment galectin-3 level was found a powerful predictor of positive effects of statins in patients with multiple myeloma.

PB1965

CLINICAL SIGNIFICANCE OF OSTEOBLAST PRECURSORS AND OSTEOCLAST PRECURSORS IN EARLIER DIAGNOSIS AND MONITORING OF MYELOMA BONE DISEASE

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Background: Multiple myeloma (MM) is a kind of plasma malignant tumor. Myeloma bone disease (MBD) is a most common complication of MM, with up to 80% of them developing osteolytic lesions.The primary diagnostic procedure for the detection of bone involvement in MM is conventional radiography. Limited sensitivity was an important disadvantage of conventional X-ray in MBD.
Aims: To find more sensitive markers to diagnose bone disease earlier and evaluate the effect of therapy.
Methods: we detected circulating osteoclast precursors (OCPs) and osteoblast precursors (OBPs) by flow cytometry, comparing with special biochemical markers, such as tartrate-resistant acid phosphatase isoform 5b (TRACP-5b), carboxy-terminal cross-linking telopeptide of type I collagen (CTX), osteocalcin (OCN) and procollagen I amino-terminal propeptide (PINP).
Results: The results showed that circulating OBPs in the newly diagnosed MM patients significantly decreased compared with the normal controls (7.14% *vs* 12.82%, *P*=0.045). While circulating OCPs in the newly diagnosed patients and remission patients were significantly increased than the normal controls (2.46% *vs* 0.17%, *P*=0.000; 1.87% *vs* 0.17%, *P*=0.000, respectively). According to X-ray, newly diagnosed patients were divided into stage A and B (without and with osteolytic lesions). Compared with the normal controls, circulating OBPs in stage A and B reduced (12.82% *vs* 7.47%, *P*=0.041; 12.82% *vs* 7.14%, *P*=0.010, respectively), while circulating OCPs elevated (0.17% *vs* 2.71%, *P*=0.001; 0.17% *vs* 2.37%, *P*=0.010, respectively). The levels of TRACP-5b and CTX in the newly diagnosed patients were higher than the normal controls (*P*=0.014, *P*=0.037) and remission patients (*P*=0.025, *P*=0.003), and they were significantly higher in stage B than the normal controls (*P*=0.015, *P*=0.002). However, PINP and OCN levels had no significantly changes in different stages.

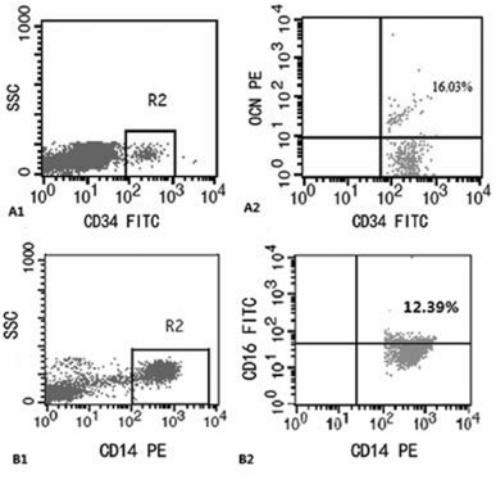

Figure 1.

Summary/Conclusions: In conclusion, abnormal circulating OBPs and OCPs were found earlier before X-ray in MM and still existed in remission patients, indicating that they maybe novel predictive markers for early diagnosing and monitoring bone disease.

PB1966

BORTEZOMIB IN COMBINATION WITH HIGH DOSE MELPHALAN AS CONDITIONING REGIMEN IS SAFE AND IMPROVES THE RESPONSE RATES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA

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Background: High dose therapy followed by autologous stem cell transplantation (ASCT) is the standard of care as first-line treatment in eligible patients with newly diagnosed Multiple Myeloma (MM). High-dose Melphalan (HDM) is the standard conditioning regimen before ASCT. Recent evidence suggests that the depth of response after the induction therapy influences the progression free survival (PFS) and, in most studies, the overall survival (OS). To improve the depth of response after ASCT in transplant–eligible MM patients the introduction of Bortezomib (BOR) to high-dose Melphalan (HDM) in conditioning regimen has shown to be effective without increased hematological toxicity.
Aims: Aim of the study is to evaluate safety and response rates in patients treated with BOR+HDM as conditioning regimen before ASCT.
Methods: In this study we retrospectively analyzed, in a single center, 27 patients treated with BOR-HDM as conditioning regimen as compared to a historical cohort of 21 patients treated with HDM alone in order to evaluate the safety and the response rate of the combination treatment. All patients in both groups were treated with novel agents as part of induction therapy. The conditioning regimen consisted in HDM (100-200 mg/m2, depending on age and comorbidity) administered on day -2, and only for patients in the BOR-HDM group, a single-dose Bortezomib at the dosage of 1.3 mg/m2 on day -1. Stem cells were reinfused on day 0.
Results: Any significant difference was not observed between the two cohorts of MM patients analyzed regarding the median age at diagnosis (61 *vs* 59 years) and the dose of Melphalan. Distribution of ISS stage was similar in the

two groups, as well as that of high-risk cytogenetic or ultra high-risk features (ISS III plus high-risk cytogenetic). Moreover the response rates after the induction therapy was not statistically different in the two groups of patients analyzed. Any significant difference on hematopoietic recovery rates was not observed in BOR+HDM as compared to HDM alone, with a mean time to neutrophil recovery of 12 days (range 9-18) and to platelet recovery of 13 days (range 10-28) in both groups. BOR+HDM conditioning was well tolerated, with no increase of neuropathy occurrence. We then analyzed the response rate after ASCT, showing that the overall response rate was significantly higher in BOR-HDM group as compared to HDM (*P*=0.028) with a higher number of complete response (CR) (54% *vs* 17%). The number of stringent CR was also significantly higher in BOR-HDM as compared to HDM alone (3 *vs* 0).
Summary/Conclusions: In conclusion, this retrospective analysis suggests that BOR-HDM is safe as conditioning regimen with a higher response rate after ABMT in comparison to the standard HDM regimen, giving the rational design for randomized studies needed to assess whether this conditioning regimen is superior to HDM alone.

PB1967

OSTEONECROSIS OF THE JAW IN PATIENTS WITH MULTIPLE MYELOMA IN THE ERA OF MORE POTENT BISPHOSPHANATES
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Background: Long term use of bisphosphonates has been associated with increased incidence of osteonecrosis of the jaw (ONJ). ONJ is commonly precipitated by a tooth extraction or other stomatological procedure in patients treated with long term, potent, high dose intravenous bisphosphonates for the management of myeloma, breast or prostate cancer. ONJ is most commonly reported in patients with MM, but it can occur in all cancer patients treated with bisphosphonates for bone metastatic disease.
Aims: The aim of this study was to evaluate the incidence of ONJ in patients with myeloma treated with bisphosphonates during the last 11 years in our institution.
Methods: We have analyzed 422 patients diagnosed with multiple myeloma in our institution in the period of 2002-2013. Median age at diagnosis was 63 years (range: 36-91), median duration of myeloma was 3.9 years (3 months-14 years). Most common isotype of myeloma was IgG Isotype (62%) and almost 80% of patients had bone lesion at the time of diagnosis. Most common chemotherapy protocol was thalidomide based protocol with more than half of patients treated with thalidomide. Only 351/422 patients (83.2%) were treated with bisphosphonates.
Results: Incidence of ONJ in patients treated with bisphosphonates was 9/351 (2.6%). Median duration of bisphosphonates treatment was 28.5±19.4 months (range: 3-98 months). Most commonly used bisphosponate was i.v. pamidronate 175/351(49.8%), while 218/351 (62.1%) were treated with two or more types of bisphosphonates. Zolendronate was used in 79 patients (22.5%). 83 patients (23.6%) received oral forms of bisphosphonates; 56.2% patients were treated with i.v. forms of pamidronate, ibondronate, clodronate or zolendronate, and 85 patients (24.2%) received combination of oral and i.v. forms of bisphosphonates. Mean duration of bisphosphonates therapy was 33.8±21.7 months. When we compare the incidence of ONJ in the period before and after 2009 (year when we started to use zolendronic acid in the treatment of MM), we could notice a rise in the number of cases with ONJ. In the period 2002-2009 we have registered only 2/190 (1%) cases with ONJ comparing to 7/161(4,3%) patients with ONJ in the period 2009-2013. Despite the evident rise in the number of cases of ONJ in the era of more potent bisphsphonates, these difference was not significant (p=0.058).
Summary/Conclusions: The incidence of ONJ in our institution is similar to already referred incidence in other studies and it has become more common complication of bisphosphonate treatment since we have started to use more potent intravenous forms of bisphosphonates. High cumulative doses of bisphosphonates, type of used bisphosphonate, poor oral health, and dental extractions may be significant risk factors for ONJ development. Preventive measures like stomatological examination before bisphosphonates treatment and regular stomatological check-ups during bisphosphonates treatment must be considered.

PB1968

A PHASE 2 MULTICENTER STUDY OF POMALIDOMIDE IN COMBINATION WITH LOW-DOSE DEXAMETHASONE IN JAPANESE PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA: THE MM-011 TRIAL
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Background: Pomalidomide in combination with low-dose dexamethasone (POM + LoDEX) has shown efficacy in patients with relapsed and refractory multiple myeloma (RRMM). In this patient population, POM + LoDEX prolonged progression-free survival (PFS) compared with POM alone (Richardson et al, *Blood,* 2014) and provided a PFS and overall survival (OS) benefit compared with high-dose DEX alone (San Miguel et al, *Lancet Oncol,* 2013), while demonstrating an acceptable safety profile. To evaluate the safety and efficacy of POM + LoDEX in Asian patients with RRMM, an additional study enrolling only Japanese patients was conducted.
Aims: This multicenter, single-arm, open-label phase 2 trial (MM-011) aimed to evaluate the efficacy and safety of POM + LoDEX in Japanese patients with RRMM.
Methods: Patients with progressive disease on or within 60 days of their last prior therapy and who had received ≥2 prior therapies, including ≥2 cycles of lenalidomide and of bortezomib (either separately or in combination) were included. Patients received oral POM 4 mg per day on days 1-21 and oral LoDEX 40 mg per day (20 mg if aged >75 years) on days 1, 8, 15, and 22, and the course was repeated every 28 days until disease progression, unacceptable toxicity, or withdrawal. The primary endpoint was overall response rate (ORR; ≥partial response) of POM + LoDEX according to the International Myeloma Working Group criteria. All pts provided written informed consent.
Results: Thirty-six patients were enrolled between December 2013 and July 2014 at 13 sites in Japan. The median age of patients was 64.5 years (range, 43-78 years); 11% were aged >75 years. Patients received a median of 6.5 prior anti-myeloma regimens (range, 2-15) and had a high tumor burden (81% had Durie-Salmon stage II or III disease). Thirty-five patients (97%) were refractory to lenalidomide, and 21 patients (58%) were refractory to both lenalidomide and bortezomib. At the data cutoff (February 3, 2015), 16 patients (44%) remained on treatment, and study drugs were discontinued in 20 patients due to disease progression (n=14 [39%]), adverse event (AE; n=2 [6%]), death (n=1 [3%]), and other reasons (n=3 [8%]). The ORR was 42% (n=15 [95% CI, 26% - 58%]), including complete response in 3% (n=1) and partial response in 39% (n=14) of patients. Median PFS was 10.1 months (median follow-up, 7.7 months), and at a data cutoff of September 25, 2015, 1-year OS was 58.5% (median follow-up, 11.3 months). In the 15 responders, median time to response was 1.9 months (range, 0.9-5.5 months), and median duration of response was not reached. The most common grade 3/4 hematologic AEs were neutropenia (n=23 [64%]), anemia (n=15 [42%]), and thrombocytopenia (n=11 [31%]). Pneumonia (n=3 [8%]) and decreased appetite (n=3 [8%]) were the most common grade 3/4 non-hematologic AEs. Peripheral neuropathy (all grades) occurred in 8% (n=3) of patients. No deep vein thrombosis or pulmonary embolism was reported. Overall, AEs were similar to those seen in other POM studies, and no new significant AEs were reported in patients in this study. Nine patients died on study (or within 28 days of the last dose of study drug), 8 due to multiple myeloma and 1 due to AE (pneumonia and aggravated asthma, suspected to be related to study drug).
Summary/Conclusions: POM + LoDEX is an effective treatment in heavily pretreated Japanese patients with RRMM, with an acceptable safety profile that is comparable to those seen on POM studies in RRMM in other regions.

PB1969

IMPACT OF ERYTHROPOIESIS STIMULATING AGENTS ON OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA
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Background: Treatment with erythropoiesis stimulating agents (ESA) in oncologic patients remains controversial. Since 2007, the FDA has included the addition of a black box label warning and the implementation of a risk management program for ESA prescription. Regarding treatment with ESA in patients with multiple myeloma (MM), there are only three studies with discordant results.
Aims: The purpose of our study is to explore the impact of ESA on overall survival in a cohort of MM patients treated in our center.
Methods: Between 2002 and 2015, 212 patients were diagnosed of MM in our center. We excluded 55 patients: 23 patients with diagnosis of smoldering myeloma and 32 patients candidates for palliative care because of age and comorbidities. We collected demographic information, clinical and laboratory data, staging according International Score System (ISS), regimens of treatment, overall survival (OS) as well as information on erythropoietin use for all patients.

Results: One hundred and fifty five patients were included: 63 received recombinant human erythropoietin (rhEPO) at least 1 month. The median total dose was 440000 international units (IU) (range 40000-10920000). Patient's characteristics according EPO therapy are presented in table. Patients treated with rhEPO showed more advanced stages, higher creatinine levels and required hemodialysis more frequently. Nevertheless autologous stem cell transplantation was performed more frequently in non-EPO patients. There was no difference in first line therapy between rhEPO and non-EPO patients. The median OS in all patients was 31 months (CI95% 22.67-39.33) and we did not observe differences between EPO and non-EPO groups (35 *vs.* 31 months, Log Rank p=0.96). However, when looking at the influence of total EPO doses on outcome, we observed differences in OS between patients who received doses of EPO >400000 IU, *vs.* patients with lower doses or untreated (50 *vs.* 23 months, Log Rank p=0.047). Multivariate analysis (Cox Regression) showed, age, albumin, Beta 2 microglobulin and rhEPO doses as independent prognostic factors for OS.

Table 1.

	No rhEPO use	rhEPO use	p value
Patients:	94	63	
Age at diagnosis: median (range)	70 (33-89)	74 (36-87)	0.057*
Male/ Female	49 (51)/ 47 (49)	33 (54.1)/ 28 (45.9)	0.417**
Laboratory findings: median (range)			
Hemoglobin, g/dL	10.05 (4.7-15.7)	10.10 (6.7-14.1)	0.459*
Creatinine, mg/dL	1.11 (0.49-6.63)	2.21 (0.63-13.94)	0.0001*
Albumin, g/dL	3.55 (1.9-5.0)	3.5 (2.1-5.4)	0.647*
Beta-2 microglobulin mg/L	4.794 (1.140-36.247)	7.492 (1.955-48.960)	0.03*
ISS III, No., (%)	40 (41.7)	41 (67.2)	
Treatments: No., (%)			
Hemodialyzed patients	2 (2.1)	20 (32.8)	0.0001**
Autologous Stem Cell Transplant	15 (15.6)	3 (4.9)	0.043**
Melphalan-Prednisone (MP)	21 (21.9)	17 (27.8)	0,162***
Bortezomib Regimens	59 (61.5)	40 (65.6)	
Others Regimens	16 (16.7)	4 (6.6)	
Overall Survival: median (CI95%)	31 (20.58-41.42)	35 (18.61-51.39)	0.963 (Log-Rank test)

**UMW: U-Mann-Whitney, **Fisher test, ***Chi-square test*

Summary/Conclusions: The use of rhEPO does not have a negative effect in MM patients. Our study suggests that treatment with high doses of rhEPO could have a positive impact to improved OS. However, prospective randomized studies to confirm these data are required.

PB1970

FDG-PET/CT IN MULTIPLE MYELOMA A CORRELATION OF SUV AND ISS SCORE

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Background: FDG-PET/CT is a promising methodology for staging, prognostication and response evaluation in multiple myeloma (MM). High intensity of FDG uptake measured as SUV (standard uptake value) at diagnosis is associated to more aggressive disease and reduced overall survival[1,2] However, it is unknown if the informative value is independent of other prognostic markers. 1) Bartel, T. B., *et al.* Blood 114.10 (2009): 2068-76. 2) Zamagni, E., *et al.* Blood 118.23 (2011): 5989-95.
Aims: We aimed to investigate the association of the intensity of FDG uptake in the most intense focal lesions and established prognostic markers in MM, including ISS and revised ISS (R-ISS).
Methods: As a part of a prospective study[3] with evaluation of new imaging technologies in MM we explored the first 35 included patients with treatment demanding MM. Patients were enrolled at diagnosis and studied with standardized baseline 18F-FDG-PET/CT prior to any given anti-myeloma treatment. Patients that had received steroids or bisphosphonates were excluded. Two experienced specialists, a nuclear medicine specialist (ANL) and a radiologist (JTA) evaluated the PET/CT. The lesion with the highest FDG uptake was described by a semi quantitative software ROVER for the values of lesion volume, SUVmax, SUVpeak, SUVmean and SUVmeancorr (SUVmean corrected for partial volume effects). Finally SUVmax was standardized to liver according to the Deauville criteria. Quantitative values were tested for correlation to prognostic indices ISS and revised ISS (R-ISS) using Kruskall Wallis test and Fischers exact test. STATA14 was used for analyses. 3) ClinicalTrials.cov. ID: NCT02187731
Results: One patient had incomplete ISS data. Thus 34 patients were included in the analysis. 19, 11 and 4 patients were stratified into ISS group I, II and III, respectively. 9, 24 and 1 patient were stratified into the revised ISS group R-ISS1, R-ISS2 and R-ISS3, respectively. SUVmax values were between 3.6Mbq/ml and 27.8Mbq/ml and lesion volumes between 0.9ml and 216ml. 6 patients had Deauville score 3, and 12 Deauville score 4, and 6 patients had Deauville score 5. FDG uptake (SUVmax) standardized to liver showed correlation to ISS (p=0.03) but not the R-ISS (p=0.85). Absolute SUVmax, SUVpeak, SUVmean, SUVmeancorr were not correlated to neither ISS nor R-ISS (p values (0.3-0.8).
Summary/Conclusions: 34 newly diagnosed MM patients were tested for association between FDG uptake values and the prognostic indices ISS and R-ISS. Only a marginal significant association between FDG uptake standardized to liver according to the Deauville criteria and ISS was observed. The use of FDG uptake values standardized to liver compensate for the general metabolic activity in the body and may therefore be more informative than absolute SUV values. Our cohort does not show a strong association between FDG SUV values and ISS or R-ISS, which may indicate that FDG PET offers prognostic information independent of R-ISS. At the meeting we will present an extended cohort. The association between FDG uptake values and prognostic factors in MM warrants further studies.

PB1971

INFECTIONS IN MULTIPLE MYELOMA: AN UNDERSTIMATE RISK FACTOR OF COMORBIDITY

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Background: Multiple myeloma (MM) represents the second most common haematological malignancy characterized by the proliferation of monoclonal plasma cells in the bone marrow. The natural history of the disease may be complicated by the occurrence of infections that can be related to the development of neutropenia (mostly therapy related) and/or hypogammaglobulinemia. Immunoglobulin (Ig) classes defect represents a major characteristic of this disease both in the indolent phase (smouldering MM, sMM), where immunoparesis constitute a risk factor for progression to active Myeloma according to the Spanish Group risk model, and in symptomatic Myeloma. Furthermore, this deficit can be worsen by concomitant chemotherapy.
Aims: The aim of this study was to analyse the frequency, the type and the major risks factors of severe infections in our cohort of patients affected by MM.
Methods: A cohort of 291 patients affected by MM (93 smouldering MM, 198 symptomatic MM) followed from 1996 to 2015 was retrospectively studied for the presence of severe infections (infections requiring hospitalization) during the natural history of the disease. Infections were distinguished in neutropenia related infections and not neutropenia related infections according to Absolute Neutrophil Count (ANC <1,000/µl or ANC>1,000/µl respectively). Durie-Salmon (DS) and ISS staging system were used for MM patients staging.
Results: Seventy-three out of 291 patients developed severe infections during the natural history of the disease (98 total events). The majority of these infections occurred in symptomatic MM patients (70 out of 73 patients with 93 out of 98 infections); as a consequence, infections were significantly associated to symptomatic MM ($p<0.001$, $\chi^2=34.76$). Among symptomatic patients, 40 patients (45 infections) developed infectious events at the time of the diagnosis and/or during induction therapy, while the other 30 patients (48 infectious events) received at least one prior therapy before the event. Interestingly, 26 events (28%) occurred in neutropenic patients while remnant 67 (72%) in not neutropenic patients. Furthermore, almost all neutropenia related infections were also therapy related (25/26, 96%), while almost 30% of neutropenia unrelated infections (20 out of 67) occurred in patients out of therapy. In particular, infections represented Myeloma defining event in 11 patients (12% of total infections, 16% of neutropenia unrelated infections). Thirty-nine of remnant 47 infectious events (83%) occurred in patients treated with novel agents with predominance of proteasome inhibitors based therapy with respect to IMIDs based therapy (26 *vs* 11), with 2 events occurring during combination therapy. DS stage III and ISS III were significant associated to severe infections (DS $p<0,05$, $\chi^2=5,5$; ISS $p<0,05$, $\chi^2=6,33$); furthermore, DS stage III and ISS stage III were statistically related to neutropenia unrelated infections ($p<0,05$, $\chi^2=6,3$), while there was no significant relationship to neutropenia related infections.
Summary/Conclusions: Severe infections represent a significant comorbidity in MM, in particular in symptomatic MM, while sMM patients are generally saved. Most of these type of infections involve not neutropenic patients characterized by high risk disease and developed during induction therapy. These results allow to stratify and to identify those patients who may eventually benefit from immunoglobulin supplementation.

PB1972

PREVALENCE OF OCULAR DISORDERS IN MULTIPLE MYELOMA

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Background: Overall survival of multiple myeloma (MM) patients has increased significantly due to the availability of new drugs. However, since MM is an incurable disease, patients are exposed to repeated lines of therapy with different agents. It is therefore increasingly important to monitor the long-term side-effects of treatments. In the present study we focused on ocular disorders.

Aims: Assessing the prevalence of ocular disorders in patients treated or in follow-up for MM.
Methods: 87 symptomatic MM patients were enrolled in a prospective protocol which consisted in a complete ophtalmologic evaluation. Best corrected visual acuity (BCVA) was assessed; lens opacities were classified according to the LOCS III score; intraocular pressure was measured and was considered pathological if >21 mmHg; Schirmer test was pathological if <15 mm. Binary logistic regression analysis (univariate and multivariate) were conducted to relate ocular disorders to age, comorbidities (diabetes, hypertension, smoking, autoimmunity) and MM variables (time from diagnosis to last treatment and to ocular evaluation, cumulative dose of dexamethasone, exposure to lenalidomide, thalidomide, bortezomib and melphalan, previous autologous or allogeneic transplantation and graft-versus-host-disease).
Results: Median age was 63 years. Median number of previous lines of treatement was 2 (range 1-10). Median time from diagnosis to ophtalmologic evaluation was 37 months (range 2-160). Median cumulative dose of dexamethasone was 1600 mg (range 80-8480). Inadequate visual acuity was found in 35/71 pts (49%); severe visual loss (<20/40) was found in 13/71 pts (15%), 10 of which had pre-existing ocular history of lazy eye, myopia, pseudophakia, vitrectomy, retinitis pigmentosa and trauma. No relation was found with cumulative steroid dose, duration or type of treatment. Instead, age and history of hypertension resulted to be independent risk factors for visual impairment, also in multivariate analysis (age OR: 4.6 p=0.004; hypertension OR: 2.8 p=0.05). Retinal disorders (11%) were related to age and hypertension in multivariate analysis. Fifty percent of patients (44/87) had lens opacities of any grade: cataract was observed in 6/87 pts (7%), and in all cases was posterior subcapsular cataract LOCS III >/= 2; moderate lens opacities were observed in 32% pts (28/87), while 23% showed nuclear or concomitant nuclear/cortical opacities (LOCS III=2-3) and 9% posterior subcapsular opacities (LOCS III=1). Lens opacities related with age >63 years (OR: 4.0 p=0.009) and time from diagnosis >37 months (OR: 2.6 p=0.05) in multivariate analysis. No association was found with cumulative steroid dose or other drugs exposure. Only 1 patient showed elevated intraocular pressure. Inadequate tears production was observed in 52% pts (45/87), but no statistically association with treatments or comorbidities was observed.
Summary/Conclusions: Our study shows a high prevalence of visual impairment and early lens opacities in MM patients. We observed a higher prevalence of posterior subcapsular cataract compared to other cataract subtypes, unlike in the healthy population, probably due to the chronic systemic steroids exposure. A high prevalence of inadequate tear production was observed, as well. Interestingly, no significant increase in intraocular pressure was noticed, possibly because of chronic but pulse administration of steroids. Patients with hypertension show an increased risk of retinal disorders. We recommend an adequate ophthalmologist follow-up in treated MM patients.

PB1973

BENDAMUSTINE-BORTEZOMIB-DESAMETASONE (BVD) IN THE MANAGEMENT OF RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A REAL-LIFE EXPERIENCE

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Background: Bendamustine is a bifunctional alkylating agent, with low toxicity, proved to be effective in relapsed, refractory and in new diagnosed Multiple Myeloma (MM).
Aims: It has been evaluated efficacy and tolerance of Bendamustine, in combination with bortezomib-dexamethasone (BVD) in patients with relapsed and refractory MM (rrMM), whose prognosis is particularly severe. A regional retrospective real-life analysis of patients with rrMM who had been treated with BVD as salvage therapy has been performed.
Methods: 47 patients (25 M/22 F), with rrMM, median age at diagnosis 58.4 years (r. 36-82), median age at start of treatment 61.3 years (r.37-83) treated with several lines of treatments (median 6, r. 2-11), every refractory to all the drugs previously received (also Bortezomib), received BVD (Bendamustine 90 mg/sqm days 1,2; Bortezomib 1.3 mg/sqm days 1,4,8,11, Dexamethasone 20 mg days 1,2,4,5,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 9 patients, and in particular one del13q and one t(11;14). All the patients had previously been treated with schedule containing bortezomib and IMIDs, 90% of them with melphalan, 77% with cyclophosphamide, 34% with antracyclines and 30% had also received radiotherapy. 58% of them had undergone at least to a single auSCT. All patients were relapsed and refractory to last therapies received before BVD.
Results: Bendamustine was well tolerated, with grade 3 transfusion-dependent anemia in 29% of patients, and 41% grade 3 neutropenia (no ospedalization was required, no septic shocks were observed). No severe extrahematologic toxicity was observed, only grade 1 gastrointestinal side effect (nausea), treated by common antiemetic drugs. According to IMWG, after a median follow-up of 9 months (r.2-36), ORR was 57% (27/47: 2 CR, 3 VGPR, 14 PR, 8 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rrMM patients. In particular, for 6 patients, BVD was, after having achieved a PR, a bridge to second auSCT, and for one patient a bridge to auSCT. Median time to response was 1.3 months (r.1-3), median OS from diagnosis was 61.4 months (range 6-151), median OS from start of Bendamustine was 9.3 months (range 2-36).
Summary/Conclusions: BVD has shown significant efficacy in a particular severe setting of patients, relapsed and refractory to all avaiable therapeutic resources, and in particular cases it could be considered as a bridge to a second autologous or allogenic BMT.

PB1974

SECOND MALIGNANCIES AND MULTIPLE MYELOMA. A SINGLE-INSTITUTION EXPERIENCE IN SPAIN

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Background: Outcome of patients with Multiple Myeloma (MM) has improved significantly in the past decade. Therefore, the risk of developing second malignancies (SM) has become a relevant aspect to explore. Although the association of several treatments employed in this disease, such as alkylators, lenalidomide or radiotherapy and second malignancies is well known in the literature, subsequent neoplasms may also reflect the effect of other etiologic factors, host characteristics or environmental exposures. In previously reported series, MM patients with SM have consistently showed poorer prognosis.
Aims: Retrospective analysis of a cohort with a consistentof more than 15 years follow-up period.
Methods: We retrospectively analysed the incidence of SM in 296 consecutive patients with MM diagnosis in our institution between 1998 and 2014, focusing on epidemiological aspects as well as on survival analysis. Characteristics of patients: n=296. Gender: male/female: 172/124. Median age at MM diagnosis: 72 years (39-100).
Results: 44 different cancers in 43 patients (15%) were observed. They were mostly male (69.8%) *vs* female (30.2%) The median age at diagnosis was 73 years (46-94). Different neoplasms were separated in those appearing before or synchronously (n=27; 61%) *vs* after the MM diagnosis (n=17; 39%) with a mean developing time to the second malignancy of 67 (0-273) and 42 months (2-161) respectively. The majority presented solid tumors (n=39; 88.6%) in contrast to hematologic neoplasms (n=5; 11.4%). The largest group showed prostate (n=9), gynecologic (n=7), colorectal (n=6) and genitourinary (n=6) as more frequent types of cancer. Myelodysplastic Syndrome (MDS) represented 80% of patients with hematologic processes and typically appeared after the MM diagnosis. No acute leukemia was found in our series. The cumulative incidence of second cancers was 2.6% at 2 years, 10.4% at 5 years and 18.6% at 10 years. No differences were found in overall survival between MM patients with or without an associated second malignancy (p=0.23).
Summary/Conclusions: SM are a growing problem in cancer patients due to their larger survival. From our data, previous and solid neoplasms are most frequent than subsequent and hematologic neoplasms in MM patients. Further studies are necessary to assess the risk and etiology of SM in MM, although they seem not to represent a poorer outcome in terms of survival.

PB1975

A PHASE 2 TRIAL OF SMALL-DOSE BORTEZOMIB, LENALIDOMIDE, AND DEXAMETHASONE (SVRD) AS CONSOLIDATION/MAINTENANCE THERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Consolidation (two to four cycles of combination therapies) and maintenance (continuous therapy, usually with single agents, until the time of disease progression) are commonly used in clinical practice after induction therapy for patients with multiple myeloma (MM). There have been many trials to support the use of consolidation/maintenance to maintain the response achieved after autologous hematopoietic stem cell transplantation or conventional treatments and to improve patient survival with single agent or combination therapy. However, no definitive information is available regarding which drug or which combination of drugs is the most favorable for consolidation/maintenance. The combination therapy with bortezomib, lenalidomide and dexamethasone (VRD) is a powerful regimen for relapsed/refractory as well as newly

diagnosed MM as an induction therapy. However, severe adverse events (AEs) may become a problem when VRD is introduced without dose-reduction as a consolidation/maintenance therapy.
Aims: The aim of this multicenter, open-label, single-arm, phase 2 study was to determine the efficacy and safety of reduced-dose VRD regimen (small VRD: sVRD) in Japanese patients with MM in the consolidation/maintenance setting.
Methods: Eligible patients were age ≥20 and ≤80 years, with measurable symptomatic MM. Patients must have received at least 1 prior regimen and achieved at least a partial response (PR) by the International Myeloma Working Group (IMWG) Uniform Response Criteria. Patients received subcutaneous bortezomib (1.3 mg/m^2 on days 1 and 15), oral lenalidomide (10 mg on days 1-21) and oral dexamethasone (40 mg on days 1, 8, 15 and 22). The course was repeated every 4 weeks for 6 cycles. Patients with at least a PR at the end of cycle 6 could continue sVRD treatment. Patients discontinued therapy if they experienced progressive disease (PD) or unacceptable toxicity, if no more additional benefits could be expected, or if the patient/investigator decided to discontinue therapy for any reason.
Results: Sixteen patients were enrolled. All patients could complete 6 courses of sVRD treatment. The median duration of sVRD treatment was 8.0 courses (range, 6-28 courses), with 56.3% (n=9) and 25.0% (n=4) undergoing >6 and >12 cycles, respectively. The reasons for treatment discontinuation were completion of 6 courses (n=7, 43.8%), disease progression (n=3, 18.8%), secondary primary malignancies (SPM) of acute lymphoblastic leukemia (ALL) (n=1, 6.3%), AE of grade 2 pneumonia (n=1, 6.3%), or other (patient refusal or physician preference) (n=4, 25.0%). The overall response rate and the complete response (CR) rate were 100% and 43.8%, respectively. In particular, one patient with CR and two patients with very good PR (VGPR) at enrollment achieved stringent CR (sCR) during 6 courses of sVRD. In 9 patients with PR at enrollment, 1 achieved VGPR, but 8 remained in PR. Nevertheless, 2 out of 8 patients with PR after 6 courses of sVRD finally achieved VGPR or sCR after a total of 18 or 24 courses of sVRD, respectively. With a median follow-up time of 29.4 months, the median progression-free survival (PFS) and overall survival (OS) were not reached, while the PFS and OS rates at 2.5 years were 66.6% and 77.3%, respectively. Three patients died and their cause of death was disease progression in all cases. It is noteworthy that the three patients who discontinued sVRD treatment due to PD were the same three patients who died in spite of various post-study therapies. Univariate analysis demonstrated that disease progression as a reason for discontinuation of sVRD had a negative impact on OS. One dose modification of dexamethasone from 40 mg/day to 20 mg/day was required because of grade 2 hypertension after the 3rd course of sVRD. One patient had discontinued all study drugs because of grade 2 pneumonia after 6 courses of sVRD. There were no grade 3 or 4 hematologic or nonhematologic AEs. After enrollment, 2 new hematologic malignancies, *i.e.*, ALL in one patient and myelodysplastic syndrome (MDS) in another patient, were diagnosed.

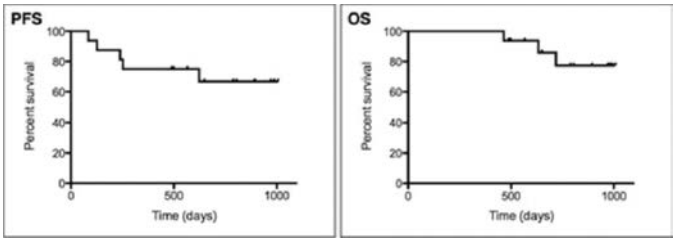

Figure 1.

Summary/Conclusions: Our sVRD regimen as a consolidation/maintenance therapy was well-tolerable and highly effective in patients with MM who achieved at least PR after any induction therapy. We conclude that the dosage of bortezomib and lenalidomide in our sVRD regimen may be able to reduce AEs and have preserved efficacy simultaneously in the consolidation/maintenance setting.

PB1976

BONE MARROW RETICULIN FIBROSIS AS A PROGNOSTIC MARKER IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED IN THE ERA OF NOVEL AGENTS

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Background: Different prognostic strategies are used in order to stratify newly diagnosed multiple myeloma (MM) patients at the time of diagnosis. The survival of MM patients has improved significantly in the last decade, attributed especially to the use of novel therapeutic agents (immunomodulatory drugs and proteosome inhibitors). Bone marrow (BM) biopsies from patients with MM at diagnosis may show different degrees of reticulin fibrosis, but its clinical significance is unclear.
Aims: To evaluate the prognostic impact of the BM reticulin fibrosis in our cohort of patients, treated in the era of novel agents.
Methods: We retrospectively reviewed the medical records of 51 patients treated between 2006 and 2014 at Galilee Medical Center, Nahariya, Israel. BM biopsies were graded for reticulin fibrosis (grades 0-3) based on the the European consensus report. Overall survival (OS) was measured from the time of diagnosis to the date of death or last follow-up. Progression free survial (PFS) was measured from the time of diagnosis to the date of progression or death. Univariate and multivariate survival analysis was performed using Cox Regression Model and Kaplan-Meier method with Log Rank (Mantel-Cox) test.
Results: 51 newly diagnosed MM patients were evaluated. The median age was 69 years (range: 47-88 years); 29 (56.9%) patients were male. The paraprotein type was: 28 (55%) IgG, 12 (23.5%) Light Chain and 9 (17.6%) IgA; 12 (24.5%) patients were ISS I, 12 (24.5%) were ISS II, 25 (51%) were ISS III. 31 (64.6%) patients were treated with chemotherapy, 12 (25%) were treated with chemotherapy followed by ASCT and 5 (10.4%) patients had conservative therapy. 41 patients (80.4%) were treated with novel agents at diagnosis. The median follow-up for the entire cohort after the diagnosis was 30.3 months (range 0.2-112.7 months). At the time of writing, 30 (58.8%) patients were living. For the statistic analysis we separated the patients into two groups: low grade fibrosis (grade 0-1) *versus* high grade fibrosis (grade 2-3). BM fibrosis characteristics did not differ significantly among patients with respect to age, sex, paraprotein type and albumin level. Patients with higher grade of fibrosis were more likely to have lower hemoglobin level (p=0.009), higher creatinine (p=0.007) and calcium level (p=0.008) with ISS III (p=0.002). Univariate analysis of overall survival (OS) showed that only age <65 (p=0.001), and treatment with novel agents (p=0.004) were significantly associated with better survival. Lower degree of fibrosis showed a tendency, not statistically significant for better survival: 72 mo *vs* 40 mo (p=0.14). On univariate analysis of PFS only lower grade of fibrosis was significantly associated with better survival (p=0.024) and a tendency for longer PFS with lower grade of fibrosis was seen in multivariate analysis of PFS (p=0.064). In subgroup analysis for patients treated with novel agents lower degree of fibrosis was still statistically significant for PFS (p=0.02).
Summary/Conclusions: In our cohort of patients, the degree of fibrosis was statistically significant for survival of MM patients, especially for PFS. This survival benefit is seen also in patients treated with novel agents. These results confirm that examination of the grade of fibrosis of the bone marrow at time of diagnosis of MM patients can provide better prognostic significance. These results should be validated in other cohorts.

PB1977

LEPTOMENINGEAL MYELOMATOSIS-A RARE COMPLICATION IN MULTIPLE MYELOMA

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Background: Involvement of the central nervous (CNS) system in patients with Multiple Myeloma (MM) is a rare event, occuring mostly during late stage of disease. However, there are reports about an increasing incidence of extramedullary myeloma manifestations since the introduction of so called novel agents.
Aims: We therefore tried to optimize the diagnostic workup in patients with suspected neoplastic meningitis of Multiple Myeloma - so called leptomenigeal myelomatosis (LMM)-by incorporating different techniques.
Methods: Between 04/2005 and 10/2013 we identified 15 cases with CNS-MM. The involvement was confirmed by magnetic resonance imaging (MRI), cerebrospinal fluid cytology as well as by flow cytometry. Additionally, cIg-FISH and DNA probes mapping to chromosome bands 1q21.2, 9q34, 13q14, 14q32, 17p13, and 22q11 were applied to 4 of the 15 cases. In one case, high-resolution genome-wide screening for genetic alterations using SNP-array analysis (Affymetrix Microarray Human Genome SNP 6.0) was performed on the CNS involvement as well as on the corresponding bone marrow (BM) aspirate.
Results: The median time from initial diagnosis until the occurrence of LMM was 463 days. Only two patients presented with CNS manifestation within 180 days after initial diagnosis. Seven patients were diagnosed at late stage of disease e.g. after high dose melphalan treatment. At diagnosis of LMM, the median age was 59 years. The median cell count in the cerebrospinal fluid was 21/µl (Range 1/µl -1333/µl). All CSF samples showed malignant pleocytosis, confirmed by flow cytometry in 12/15 patients. cIg-FISH presented cytogenetically defined high risk features in all samples tested: 3 of 4 patients showed a translocation t(4;14), one patient had a 17p13 deletion. Using high-resolution genome-wide screening assays revealed different subclones at the two clinical sites (CSF, BM). Treatment for LMM consisted of intrathecal chemotherapy (9 of 15 cases) and radiation therapy (7 of 15 cases). Despite treatment, the outcome of patients with confirmed LMM was dismal with a median overall survival after diagnosis of LMM of 69 days. Only one patient survived longer than 2 years after diagnosis of CNS involvement.
Summary/Conclusions: By combining several technical procedures (MRI, cytology, flow cytometry, cIg-FISH and SNP-array analysis) it is possible to

identify the vast majority of patients with LMM. However, management of affected patients remains challenging and the survival is generally short after diagnosis of LMM.

PB1978

A PHARMACOKINETICS CLINICAL STUDY OF BORTEZOMIB SUBCUTANEOUS INJECTION VERSUS INTRAVENOUS INJECTION COMBINED WITH CHEMOTHERAPY IN CHINESE PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA-INTERIM RESULTS

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Background: Bortezomib was licensed for administration as a bolus i.v. injection. This route of administration has demonstrated efficacy and safety in previously untreated patients with multiple myeloma (MM). However, the i.v. route can be a potential barrier to treatment for patients with poor venous access and limits prescribing flexibility. Subcutaneous (SC) administration of bortezomib showed the advantage in safety and comparable efficacy compared with IV administration.
Aims: However, there is no PK data of bortezomib SC administration for Chinese patients. Our trial was to investigate the pharmacokinetics of the two routes of administration in Chinese population. Demographic and baseline characteristic, pharmacokinetics and safety data till the first VD treatment completion were reported here. (ClinicalTrial.gov Identifier: NCT01812096)
Methods: This was a randomized, single-center, open-label study. The study included the following 4 sections: screening, VD regimen therapy (blood samples collection for PK analysis), extended treatment period (PAD regimen and/or subsequent ASCT) and follow-up period. 20 patients were determined to be randomly assigned in a 1:1 ratio to receive bortezomib subcutaneous or intravenous administration combined with chemotherapy. SC or IV bortezomib was administered at 1.3mg/m2, d1, 4, 8, 11, combined with dexamethasone (10 mg, bid, i.v., d1~2, 4~5, 8~9, 11~12) in the 1st cycle, every 21days, following 2-4th cycles of PAD regimen, bortezomib was SC or IV administered at 1.3mg/m2 on d1, 4, 8, 11; Doxorubicin 40 mg/m2, i.v.d1; Dexamethasone (Dex) 10 mg, bid, i.v., d1~2, 4~5, 8~9, 11~12;.every 28 days. Patients who were eligible for transplant underwent ASCT after the 4th cycle. Patients who were not eligible for transplant continued the 5-6th cycle treatment of PAD regimen. The blood samples for pharmacokinetics analysis were collected at the 15 time points d1, 11-14 of the first VD treatment. Patients had maximum 6 months follow-up after completing or terminating the treatment.
Results: From September 2014 to June 2015, 21 patients (10 patients in SC group and 11 in IV group) were enrolled in our study. 18 patients (10 in SC group and 8 in IV group) enrolled in the pharmacokinetic analysis. Median age in SC group was 56.8 yrs and 57.5 yrs in IV group. 81.82% patients had IgG isotype in the IV group and 40.00% patients had IgA isotype in the SC group. A Higher proportion patient had light chain isotype (20.00%) in the SC group. The median accumulative dose of bortezomib in VD treatment was much the same in each group. Mean maximum plasma concentration (Cmax) was 46.56ng/ml in SC group and 182.45ng/ml in IV group. Median time to Cmax (Tmax) was longer in SC group (0.25 h) than in IV group (0.03h). Mean bortezomib systemic exposure (AUClast) was similar between SC injection and IV administration (234.83vs266.75). Inter-patient variability in Cmax (percent coefficient of variations) was 24.76 in SC group and 30.79 in IV group. Treatment related TEAEs were reported in 7 of 10 patients (70%) in SC group and 9 of 11(81.82%) in IV group. Treatment related Grade≥3 TEAEs were reported in 2 of 10 patients (20%) in SC group and 3 of 11 patients (27.27%) in IV group. TEAE leading to drug discontinuation was only reported in 1 of 11 patients (9.09%) in IV group. No Serious TEAE was reported in either group. The incidence of neutropenia was higher in IV group.
Summary/Conclusions: In conclusion, our interim data suggest that SC administration is a promising alternative to IV administration. The efficacy and safety information will be further presented in the final results.
Acknowledgments: This study was funded by Xi'an Janssen Pharmaceutical Co, Ltd.

PB1979

CHEMOTHERAPY ADHERENCE IS FAVORABLE PROGNOSTIC FACTOR FOR ELDERLY PATIENTS WITH MULTIPLE MYELOMA WHO TREATED WITH FRONTLINE BORTEZOMIB CONTAINING REGIMEN

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Background: Elderly patients with multiple myeloma (MM) are vulnerable to adverse events and the patients' treatment goal may be different from young patients. For elderly patients with MM, the toxicity of bortezomib (BTZ)-based regimen causes frequent dose reduction and interruption of BTZ which could result in poor outcomes.
Aims: This study evaluated the adherence to chemotherapy and treatment outcomes for elderly patients who treated with frontline BTZ containing regimen and non-BTZ containing regimens.
Methods: This study retrospectively analyzed the outcomes of 146 elderly patients (≥65 years) with MM diagnosed from 2007 Mar to 2015 Mar. To evaluate the regimen adherence, the patients who treated with more than 4 cycles were regarded as favorable adherence group and the others as unfavorable adherence group. The effects of toxicity on the adherence to frontline regimen and long term outcomes were also evaluated in the current study.
Results: Among 146 patients, frontline therapy was VMP in 77 patients (52.7%), MP in 50 (34.2%) and CTD in 19 (13.0%). Median age at diagnosis was 70 years (range 65-90 years) and 85 patients (58.2%) were over 70 years old. ECOG performance status showed 2-4 in 49 patients (33.6%). ISS risk groups were stage I in 14 patients (9.6%), II in 64 (43.8%), and III in 68 (46.6%). Median cycles of frontline regimens were 5 cycles (range 1-9 cycles) with VMP and 6 cycles (range 1-77 cycles) with non-BTZ containing regimens (p=0.032). Maximal treatment response was ≥VGPR in 40 patients (51.9%) and 13 (18.8%) in VMP regimen and non-BTZ regimens, respectively (p<0.001). Regimen adherence was favorable in 49 patients (63.6%) and 52 (75.4%) in frontline VMP regimen and non-BTZ regimens, respectively (p=0.126). Major cause of non-adherence was side effects in VMP, while poor response and regimen change were major causes of non-adherence in non-BTZ regimens. Despite poor response to frontline chemotherapy in non-BTZ regimens, 3-year overall survival (OS) rate was not significantly different compared to frontline VMP regimen: 65.3±8.2% and 47.9±7.8% in VMP and non-BTZ regimens (p=0.159) because all the patients who showed poor response to frontline therapy received second-line BTZ containing regimens. However, among the patients with frontline VMP regimen, those with good adherence to chemotherapy resulted in better 3-year OS rate than those with poor adherence: 72.4±9.7% and 52.6±13.2% (p=0.002). In the multivariate analysis, ECOG performance status 2-4 (HR 3.409, 95% CI 1.133-10.261, p=0.029) and non-adherence to chemotherapy (HR 4.896, 95% CI 1.617-14.829, p=0.005) were poor prognostic factors for OS.
Summary/Conclusions: Frontline BTZ-containing regimen showed higher response rate than non-BTZ regimens and good adherence was associated with favorable long-term outcomes. However, regimen adherence was compromized for high incidence of adverse effects with BTZ-contaiing regimen. Therefore, understanding the risk of toxicities and adequate management of side effects needed to have favorable outcomes for the patients with BTZ containing regimen.

PB1980

PROGNOSTIC SIGNIFICANCE OF EXPRESSION OF MULTIDRUG RESISTANCE GENES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH BORTEZOMIB-CONTEINING THERAPHY

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Background: Most drugs used to treat multiple myeloma, are substrates for protein multi-drug resistance. Their effectiveness is reduced in cells with increased expression of genes encoding these proteins. The proteasome inhibitor bortezomib is not a substrate for protein P-gp, encoded by MDR 1 gene.
Aims: The aim of our study was to examine the prognostic significance of the alternative (non-P-gp dependent) mechanisms of multidrug resistance (MDR) in the bone marrow aspirate in patients with newly diagnosed multiple myeloma (MM).
Methods: The study included 15 patients (6 men and 9 women) with established diagnosis of multiple myeloma stage III by Durie□Salmon system. The age of patients ranged from 50 to 78 years. The mRNA expression of MDR genes, 1 MRP, BCRP, LRP was determined by semi-quantitative RT-PCR in mononuclear fraction of the bone marrow aspirate. Numeric value corresponding to the amount of mRNA expression of each studied gene, represented as the ratio of the numerical values of the mRNA expression of each gene to a numeric value of mRNA expression of the gene GAPDH. All patients subsequently received treatment with bortezomib-containing chemotherapy schemes in the amount of 6 courses.
Results: Although the patients had not received cytostatic therapy, detected mRNA expression of all studied genes MDR. The average number of MDR gene transcripts was 1.7±0,24, MRP 1 1±0,14, BCPR 1,04±0,22, LRP 1,47±0,17 points. Because previous studies have shown that the proteasome inhibitor bortezomib is a basic drug for the treatment of MM is not a substrate of P-gp, the analysis of survival in subgroups of patients with the number of LRP gene transcripts above and below the mean. Overall survival of patients with increased expression of the gene was significantly worse than that of whom with reduced expression of LRP (17,1±3,65 *vs* 53, 9±10 months, P<0.005).
Summary/Conclusions: Increased expression of LRP gene exert a poor survival of patients with multiple myeloma for cytostatic treatment, the bortezomib containing chemotherapy programs.

PB1981

THE STRATUS TRIAL (MM-010): ANALYSIS OF THE ITALIAN SUBGROUP OF PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA TREATED WITH POMALIDOMIDE PLUS LOW-DOSE DEXAMETHASONE

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Background: There are few treatment (Tx) options and overall survival (OS) is short for patient (pts) with relapsed/refractory multiple myeloma (RRMM) who have failed or progressed on Tx with agents such as lenalidomide (LEN) and bortezomib (BORT) (Kumar et al, *Leukemia*, 2012). Pomalidomide (POM), a distinct immunomodulatory agent with tumoricidal and immunoregulatory effects, plus low-dose dexamethasone (LoDEX) is approved in the United States and European Union (EU) for the Tx of pts with RRMM who have had ≥2 prior Tx, including LEN and a proteasome inhibitor (BORT in the EU). Tx with POM + LoDEX has shown statistically greater survival benefits than Tx with high-dose DEX (San Miguel et al, *Lancet Oncol,* 2013) or POM alone (Richardson et al, *Blood,* 2014). POM + LoDEX has also demonstrated high overall response rates (ORRs) in pts with RRMM in the STRATUS trial (MM-010), a single-arm, open-label phase 3b study being conducted in 19 countries across Europe, with Italian pts constituting the largest national subset (Dimopoulos et al, EHA 2015).
Aims: To examine the efficacy and safety of POM + LoDEX in the Italian population of the STRATUS trial.
Methods: Pts with RRMM (progressive disease [PD] on or within 60 days of last prior Tx) who had experienced Tx failure with BORT and LEN, had received adequate prior alkylator therapy, and provided informed consent were eligible. Pts received POM 4 mg on days 1-21 of a 28-day cycle in combination with DEX 40 mg (20 mg for pts aged >75 yrs) on days 1, 8, 15, and 22. Thromboprophylaxis was required for all pts. Pts were treated until PD or unacceptable toxicity. Primary endpoint was safety.
Results: In the 219 pts enrolled in Italy, median age was 67 yrs (range, 42-84 yrs), 55% were male, and 37% of pts were International Staging System stage III. The median time since diagnosis was 5.5 yrs. Patients were heavily pretreated, with a median of 4 prior anti-myeloma regimens (range, 2-11), and most pts were refractory to LEN (95%), BORT (82%), or both LEN and BORT (78%). As of May 4, 2015, 2 pts (1%) were not treated, 54 pts (25%) were still on Tx, and 163 pts (74%) had discontinued. The most common reasons for discontinuation were disease progression (50%), death (10%), and adverse events (AEs; 5%). After a median follow-up of 11.3 mos, in the intention-to-treat population, the ORR was 37.9% (range, 31.4% - 44.7%), the median duration of response was 6.8 mos (95% CI, 4.9-10.8 mos), median progression-free survival (PFS) was 5.2 mos (95% CI, 4.4-6.4 mos), and median OS was 12.0 mos (95% CI, 10.6-15.2 mos). The most frequent grade 3/4 treatment-emergent AEs were neutropenia (56.7%), anemia (25.8%), thrombocytopenia (23.5%), and pneumonia (12.4%). Grade 3/4 venous thromboembolism (deep vein thrombosis and pulmonary embolism) and peripheral neuropathy was infrequent (2.3% and 0%, respectively). AEs led to dose reductions or interruptions of POM in 23.0% and 71.0% of pts, respectively, and the median relative dose intensity for POM was 0.90.
Summary/Conclusions: POM + LoDEX was active in this study subgroup, which was representative of the heavily pretreated MM-010 population. Efficacy outcomes, including PFS and OS, were consistent with those seen in previous trials. POM + LoDEX treatment was well tolerated, and no new safety signals were observed. AEs were appropriately managed, and discontinuations due to AEs were infrequent. This study confirms that POM + LoDEX is effective in patients with advanced RRMM, including those who have experienced failure of prior Tx with BORT and/or LEN.

PB1982

RISK OF HYPERTENSION (HTN) AND MALIGNANT HYPERTENSION (MHTN) IN PATIENTS TREATED FOR MULTIPLE MYELOMA (MM)

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Background: HTN is commonly reported in patients (Pts) with MM and may be associated with older age, disease-related complications, or sequelae of MM treatments.
Aims: To evaluate incidence rates (IR) of HTN and mHTN in treated MM Pts in the United States and the risk of mHTN development in MM Pts with pre-existing HTN.
Methods: Newly-treated adult MM Pts were identified from Truven MarketScan claims database from 1/1/05 to 3/31/14 using ICD-9 codes to identify disease state, HTN, mHTN, and comorbidities. Inclusion criteria were new diagnosis of MM with start of MM treatment, ≥12 months continuous enrollment (CE) prior to diagnosis, prescription drug coverage, and ≥30 days of CE following initial diagnosis. Non-MM Pts were matched on age (within+/-5 yrs), gender, and distribution of index dates to MM Pts. Risk of HTN and mHTN based on existing HTN and other cardiovascular (CV) comorbidities were evaluated over time.
Results: Study included 7895 MM Pts (38% with HTN history) and 23685 non-MM patients (24% HTN history). The IR of HTN in MM and non-MM Pts was 260 and 178 per 1000 person-years (PYRs), respectively. The IR of mHTN in Pts with and without HTN history were 10.25 and 3.29 per 1000 PYRs, respectively for MM patients; 4.25 and 1.88 per 1000 PYRs, respectively for non-MM Pts. Risk of HTN (HR: 1.30; 95% CI: 1.22, 1.37) increased 30% in MM *vs* non-MM Pts. MM Pts with (HR: 1.90; 95% CI: 1.26, 2.87) or without (HR: 1.54; 95% CI: 1.04, 2.28) HTN history had a higher risk of mHTN events during the observation period *vs* non-MM Pts. In MM Pts with HTN history, the risk of mHTN was significantly increased with the following comorbid conditions: cardiomyopathy (HR: 2.79; 95% CI: 1.20, 6.48), renal failure (HR: 2.13; 95% CI: 1.36, 3.34), and diabetes mellitus (HR: 1.59; 95% CI: 1.05, 2.39).
Summary/Conclusions: This study confirms that incidence of HTN and mHTN is higher in newly-treated MM Pts *vs* non-MM Pts. Existing HTN is a risk factor for MM Pts developing mHTN. Management of CV risk factors and comorbidities in MM Pts is important based on the increased risk of HTN and mHTN among these Pts.

PB1983

DIARRHEA INCIDENCE IN MULTIPLE MYELOMA PATIENTS TREATED WITH LENALIDOMIDE AND POMALIDOMIDE

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Background: Daily clinical practice in hematology is increasingly relying on novel biological drugs, with new toxicity profiles that hematologists should learn to manage. Immunomodulatory drugs (IMiDs) have a pivotal role in the treatment of multiple myeloma (MM), and are typically administered as a continuous treatment. Thalidomide is a well know constipation-inducing agent. On the contrary, physicians are aware that lenalidomide (Len) may induce gastrointestinal (GI) disorders, in particular diarrhea, but little is known of this side effect. Moreover, no specific data on GI side effects are reported on the third-generation IMiD Pomalidomide (Poma).
Aims: Description of incidence and management of diarrhea in a retrospective cohort of Len- or Poma-treated MM patients.
Methods: One hundred and twenty-six consecutive Len-treated, and 59 Poma-treated MM patients were retrospectively analyzed in terms of diarrhea occurrence. In this analysis we included Len patients consecutively treated from June 2005 to December 2015, and Poma patients consecutively treated from August 2010 to September 2015. A descriptive analysis of diarrhea incidence, onset time, and response to treatments was performed.
Results: The median age of the 126 Len-treated patients was 65 years (range 35-87); 57 (45%) received one or two autologous stem cell transplants, and 12 (10%) patients received an allogeneic stem cell transplant before Len start; 55 (44%) patients received Len as a first line therapy, 32 (25%) patients as a second line therapy, and 39 (31%) as third or subsequent line of therapy. Thirty (24%) patients had diarrhea during Len treatment. Among these patients, 27 (90%) had grade 1 diarrhea, 3 (10%) patients had grade 2 diarrhea, and no patients had grade 3 or more diarrhea. Seventeen (53%) patients had abdominal cramps and 2 (6%) patients had rectal tenesmus. Diarrhea occurred at a median of 10 cycles. Almost all patients (29, 97%) had a correlation of diarrhoeic episodes with assumption of food, and most patients (24, 80%) reported persistent diarrhea during the rest period. Since some patients reported a worsening of diarrhea associated to fat consumption, 14 patients were advised to avoid diary products, and in 9 of them (64%) a benefit was observed. Loperamide was used in all cases, and 19 (63%) patients had a reduction of symptoms. Eleven (37%) patients had a poor control with loperamide and were treated with cholestyramine, which was effective in all cases. Nine (30%) patients had Len dose reduction, and 5 had a reduction of the diarrhea. In 19 cases Len was stopped, resulting in the rapid resolution of diarrhea in all cases. The same analysis conducted in 57 Poma-treated patients did not show any GI symptoms.
Summary/Conclusions: Adverse GI events are common in new targeted

drugs, and a better understanding of the underlying physiopathological mechanism is the basis for a correct management. In particular, IMiDs have a gastrointestinal toxicity profile with peculiar characteristics for the different generations. The first-generation IMiD Thalidomide is well known to be associated with constipation, sometimes very severe. The second-generation IMiD Len has an opposed GI toxicity profile, characterized by diarrhea. In our study diarrhea is mainly mild, but still quite disturbing. However, it seems that a combination of dietary counselling, along with loperamide and cholestyramine treatment may control this symptom. On the other side, we confirm that Poma doesn't have relevant GI side effects.

PB1984

TRABECULAR BONE SCORE (TBS) AND DEVELOPMENT OF FRACTURE IN MULTIPLE MYELOMA

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Background: Osteolytic bone lesions are common complications in patients with multiple myeloma, and have impact on the quality of life because of the risk of fractures. Trabecular bone score (TBS) is a novel texture index derived from dual energy X-ray absorptiometry (DXA) of lumbar spine images, providing information of bone microarchitecture independent of bone mineral density.
Aims: The aim of this study was to test if TBS may be useful to predict bone fractures in patients with multiple myeloma.
Methods: TBS was calculated retrospectively from existing DXA images of the lumbar spine, in 20 patients with newly diagnosed multiple myeloma. We analyzed the development of fractures in these patients.
Results: The median age of the patients was 66 years (range, 49-77), and 15 patients (75%) were female. Fifteen patients had osteopenia (5 patients, 25%) or osteoporosis (10 patients, 50%) by using DXA. Osteolytic bone lesions were observed in 18 patients (90%) at the time of diagnosis. The median duration of follow-up was 31.9 months (95% CI, 20.1-43.7 months), 6 events (long-bone fractures in 5 events, vertebral fracture in 1) of fracture were occurred in 5 patients (25%). The mean TBS of lumbar spine (L1-4) in patients who experienced development of fractures (1.162±0.032 [95% CI, 1.122-1.201]) was lower than patients who did not (1.255±0.154 [95% CI, 1.170-1.3]), however, there was no statistical significance (p=0.061). Among TBS of individual lumbar spines, L2 showed significantly lower score in patient who experienced development of fractures (1.135±0.085 [95% CI, 1.030-1.241] *vs* 1.243±0.169 [95% CI, 1.149-1.336], p=0.032).
Summary/Conclusions: TBS of lumbar spine in patients with multiple myeloma could be helpful to predict development of fractures, however, further investigations are needed.

PB1985

BORTEZOMIB MAINTENANCE TREATMENT IN PATIENTS WITH MULTIPLE MYELOMA-A SINGLE CENTER EXPERIENCE

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Background: The long-term maintenance therapy in multiple myeloma (MM) after definitive therapy may prolong remission and delay relapse. Bortezomib has been used as post-autologous stem-cell transplantation (ASCT) consolidation therapy. Other trials have investigated Bortezomib maintenance for ASCT patients, and for those who are ASCT ineligible.
Aims: To define the role of Bortezomib maintenance therapy (MT) in patients with MM after achieving complete response (CR) or very good partial response (VGPR).
Methods: 37 patients with newly diagnosed symptomatic MM with CR or VGPR after Bortezomib based induction regiments were included in the analysis. 23 patients received MT with Bortezomib 1.3 mg/m^2 once every 2 weeks. 14 patients remained on follow up until progression.
Results: 19 of the patients were men and 18 women with median of age 64,2 years. 11 cases were with IgA myeloma, 20 with IgG, and 6 with light chain myeloma. According to ISS 10 patients were in stage I, 7 in stage II, and 20 in stage III. CR was achieved in 13 (56,5%) of the patients in MT group and in 6 (42,8%) in the group on follow up. Total of 14 patients underwent ASCT - 10 (43,4%) from the patients on MT and 4 (28,5%) on follow up. After a median follow up of 31.8 months (range, 10-65 months) none of the patients on Bortezomibe MT progressed. In contrast, in the patients group on follow up, 12 from 14 patients progressed within median time of 24,17 months (range, 7-60 months). No correlation was found between the progression and myeloma type, ISS stage, type of response and ASCT.
Summary/Conclusions: Our results reveal undoubtedly the impotence of Bortesomib maintenance in patients achieved CR or VGPR.

PB1986

RELAPSED AND REFRACTORY MULTIPLE MYELOMA, EVOLUTION AND COMPLICATIONS SINGLE CENTER EXPERIENCE

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Background: Multiple myeloma (MM) is a malignant plasma cell disorder. It is the second most frequent haematological malignancy and characterized by malignant plasma infiltration or the bone marrow and is associated with an increased level of monoclonal protein in the blood and/or urine. The treatment of MM has undergone significant developments in recent years, and the new agents with potent anti tumor activity has considerably improved the survival of MM patients.
Aims: Retrospective evaluation of the therapeutic results, the evolution and complications of therapy with bortezomib, doxorubicin and dexamethasone (PAD) in the treatment of relapsed/refractory myeloma patients.
Methods: 62 patients were treated for median of four 28-day PAD cycles (1-8). Bortezomib was given at 1.3 mg/m2 (days 1, 4, 8,11), doxorubicin at 9 mg/m2 (days 1-4) and dexamethasone 20 mg p.o. (days 1-4, 8-11).
Results: 62 patients were evaluable for efficacy, 59% had refractory disease and 41% were relapsed. The median age was 65 years (37-80), 59% were male, 41% female. Serum protein electrophoresis revealed a localized band in 74% of patients, and immunoelectrophoresis or immunofixation showed a monoclonal protein in 85%. A monoclonal light-chain was found in the urine in 63%. Non-secretory myeloma was recognized in 3% of patients, whereas light-chain myeloma was present in 16%. Serum albumin less than 3mg/dl was found in 58% of patients. Conventional radiographs showed an abnormality in 81%. Median time from diagnosis was 16 months (2-115) and median number of prior therapy lines was 2 (1-5).Overall response rate of 58% was observed, 28% of patients achieved a complete response (CR), 22% a very good partial response (VGPR), 30% a partial response (PR). Stable disease (SD) was observed in 20%. The median progression free survival (PFS) was 15,2 months. The most common grade 3-4 toxic effects were neutropenia 14%, thrombocytopenia 12%, anemia 12%, infections 16%, peripheral neuropathy 10% and gastrointestinal disturbances 5%. One toxic death (1.1%) due to sepsis was noted.
Summary/Conclusions: The combination of bortezomib, doxorubicin and dexamethasone (PAD) is well tolerated and induced clinically signifiant responses and prolonged remission duration in patients with relapsed and refractory MM.

PB1987

BENDAMUSTINE, LENALIDOMIDE AND DEXAMETHASONE (BLD) COMBINATION THERAPY IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE

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Background: Lenalidomide is an analogue of thalidomide with immunomodulatory properties and is effective and safe in the treatment of Multiple Myeloma. Lenalidomide (Len) plus Dexamethasone (Dex) is approved for the treatment of Relapsed/Refractory MM patients following at least 1 prior therapy. Bendamustine (Ben), a bifuncyional agent, shares properties of alkilanting agents and purine analogs. We report data on efficacy and safety of BLD in patients (pts) with relapsed/refractory MM.
Aims: Our aim was to evaluate effectiveness and feasibility of BLD regimen in pts with relapsed or refractory MM
Methods: From September 2014 through October 2015 9 pts with relapsed or refractory MM with 1-3 prior lines of therapy were treated with BLD regimen. The series included 9 pts, six females and three males; 5 pts with relapsed MM and 4 with refractory MM. Median age was 70 (range 55-73). All patients had symptomatic MM and had previously been treated with bortezomib-based regimen. Five patients received BLD as second line therapy, four as third line.Ben was administered intravenously at a dose of 75 mg/mq on day one and two of each cycle. Pts received lenalidomide 10 mg orally on days 1- 21 and oral dexamethasone 40 mg/day (days 1, 8, 15 and 22). Cycles were repeated every 28 days for a total of 4 courses. Maintenance therapy included lenalidomide 25 mg/day on days 1-21 and dexamethasone 20 mg/die (days 1, 8, 15 and 22) until progression. Pts received concomitant anti-thrombotic (aspirin 100 mg/day) and anti-viral prophylaxis and additional supportive treatment with granulocyte colony stimulating factor (G-CSF) in case of grade 3 or grade 4 neutropenia occurring during cycles.Safety assessment (clinical exhamination, hematological evaluation) was performed weekly during cycle 1 and monthly thereafter. Response assessment was performed after four cycles. Response assessment was based on the International Uniform Response Criteria.
Results: As Grade 3-4 hematological toxicity we observed: neutropenia (55%), thrombocytopenia (44%) and anemia (33%). Grade 3-4 non hematologic adverse events were: infection (22%), hyperglycemia (33%), fatigue (44%) and

diarrhea (11%). No thromboembolic events were reported. We did not observe peripheral neuropathies. One patient discontinued therapy because of prolonged neutropenia and thrombocytopenia. 8 patients were evaluated for response after four courses; 7 pts (87,5%) achieved al least a partial response (PR), including 3 (37,5%) very good partial response (VGPR) and 1 (12,5%) complete response (CR). 3 pts (after achieving VGPR) successfully harvested peripheral blood stem cells (PBSC).
Summary/Conclusions: In conclusion in our study BLD combination was well tolerated with a mild toxicity profile and has shown effectiveness in patients with relapsed or refractory MM. It could represent a reasonable option for relapsed-refractory MM pts, including transplant-eligible patients.

PB1988

OUTCOME OF PATIENTS WITH NONSECRETORY AND SECRETORY MULTIPLE MYELOMA AFTER FIRST LINE TREATMENT CONSOLIDATED WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION THE SINGLE CENTRE EXPERIENCE

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Background: Nonsecretory multiple myeloma (NSM) is an uncommon type of multiple myeloma. It is defined as the presence of monoclonal plasma cells in bone marrow biopsy or in other organs and the absence of monoclonal protein in immunofixation in serum and urine. There are limited data about outcome after first line treatment with consolidation high dose chemotherapy following autologous hematopoietic stem cell transplantation (ASCT).
Aims: We retrospectively evaluated outcomes and predictive factors for overall survival (OS) and progression free survival (PFS) in all myeloma patients, in non-secretory myeloma (NSM) and in secretory myeloma (SM) groups, who received high dose chemotherapy and ASCT between 1998-2015 after first line treatment.
Methods: We identified 114 myeloma patients: 23 (20%) with non-secretory myeloma and 91 (80%) with secretory myeloma. Median time from first treatment to ASCT was 8 months (range 4–28 months). All patients achieved response to first line treatment: complete remission or partial response.
Results: The median OS and median PFS were 41 (range 2- 205) months and 25 (range: 2- 205) months, respectively. Five year OS and 5-year PFS were 70% [95%CI (59%, 79%)] and 42% [95%CI(31%, 53%)], respectively. For NSM and SM groups 5-year OS were 54%[95%CI (31%,77%) and 74%[95%CI(64%,75%)] respectively, (p=0.14) and 5-year PFS were 45%[95%CI(24%,66%)] and 41%[95%CI (29%,53%), respectively. (p=0.79). Age, sex, disease stage, disease status before ASCT, induction chemotherapy, radiotherapy had no influence on OS and PFS for all, NSM and SM groups in univariant analysis. The presence of monoclonal IgG protein in SM patients was associated with better OS compared to OS in NSM group (p=0.04) and the presence of monoclonal protein with kappa light chain in SM patients related to longer PFS compared to PFS in NSM group (p=0.05) in univariant analysis.
Summary/Conclusions: There were no significant differences in OS and PFS for non secretory and secretory myeloma patients after first line treatment consolidated by high dose chemotherapy and ASCT. However, patients with monoclonal IgG protein had significantly better OS than patients with NSM.

PB1989

PLASMABLASTIC LYMPHOMA (PBL) AND EXTRAMEDULLARY PLASMABLASTIC TRANSFORMATION OF PLASMA CELL MYELOMA (PCM): DIAGNOSTIC MORHOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL CHARACTERISTICS

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Background: PBL is an aggressive growth of neoplastic cells that resemble B immunoblasts in morphology and are immunophenotypically indistinguishable from plasma cells and lack B cell markers. Even though it was first described in the setting of immune suppression and in fact underlying HIV infection and/or EBER positivity in the neoplastic cells are frequent, it is now increasingly identified in immunocompetent patients with extreme heterogeneity in terms of nodal and extra nodal involvement. However, extramedullary lesions with plasmablastic morphology are not uncommon in patients with PCM, where they usually develop in the course of a refractory disease. The differential diagnosis may be challenging in initial presentation and the complexity is further magnified as both entities have features that overlap with several other lymphomas with plasmablastic morphology.
Aims: To evaluate and compare morphological, immunohistochemical, molecular and clinical characteristics of patients with PBL and plasmablastic transformation of PCM.
Methods: We present patients with documented PBL, with liver and bone involvement, plasmablastic transformation of PCM with multiple sites of involvement in the oral cavity and the gastrointestinal tract and plasmablastic transformation of orbital soft tissue plasmacytomas.
Results: Histopathological examination showed large confluent plasmablasts with abundant cytoplasm and central oval vesicular nuclei with coarse chromatin pattern and prominent nucleoli. They mostly appear as large centroblasts and/or immunoblasts and cannot be readily classified as a B cell or a plasma cell neoplasm or have a more apparent plasma cell differentiation with a paranuclear hof, eccentric large nuclei and basophilic cytoplasm. Background necrosis, karyorrhexis and increased mitotic figures are prominent. Immunohistochemistry is similar with plasma cell neoplasms, positive for CD79a, MUM-1, CD38 and CD138 and the majority negative for CD45 and negative for CD20, PAX-5 and bcl-6. Perhaps the only difference detected in our cohort of patients was the expression of CD56 and CD10 which were negative in PBL. MYC is expressed in about 50% of cases. All patients have very elevated levels of LDH and poor risk cytogenetics. The clinical course is aggressive and response to treatment is poor, with evolution to plasma cell leukaemia in the non PBL patients and overall survival of no more than 6 months from the diagnosis of PBL or the plasmablastic transformation.
Summary/Conclusions: When an underlying plasma cell dyscrasia is not yet known, the distinction of a high grade aggressive lymphoma with plasmablastic features may be arbitrary, particularly if there are coexistent marrow involvement, presence of paraproteinaemia, bone lesions and a certain degree of plasmacytic differentiation. It is not clear in the literature whether those are entirely different entities or share a common pathogenetic mechanism. Systemic investigation towards the identification of novel markers and the refinement of our current combination of diagnostic tools may enable a more accurate distinction and definition.

PB1990

RETROSPECTIVE COMPARISON OF SAFETY AND EFFICACY OF BUCY AND HDM AS REGIMEN BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Melphalan-based regimen is the standard regimen before autologous stem cell transplantation (ASCT) in the patients with multiple myeloma, but now melphalan is not on the China's market. There were positive data reported in other researches in terms of safety, response rate and long-term survival on busulfan combined with other alkylating agents as regimen before ASCT in MM patients.
Aims: This study retrospectively compare the data in our single center on MM patients with busulfan (BU) and cyclophosphamide(CY) as regimen before ASCT form December to June 2013 to December 2014 with the high-dose melphalan (HDM) regimen. long-term survival on busulfan combined with other alkylating agents as regimen before ASCT in MM patients.
Methods: 20 cases with BUCY and 17 cases with HDM regimen before ASCT in MM patients were compared in terms of incidence of adverse reaction, the rate of hematopoietic reconstitution, therapeutic effect evaluation and survival.
Results: There is no significant differences between age, gender, duration from diagnosis to transplantation, the type of disease and ISS stage, the number of cycles of induction chemotherapy and disease status before transplantation in the two groups. A 10-day of median myeloid hematopoietic recovery time was observed in both groups, and the median megakaryocyte hematopoiesis reconstruction time was 10-day and 11-day (P >0.05), respectively, in BUCY and HDM group. The incidence of liver function injury above grade 2 in BUCY group was higher than that in HDM group (30% *vs* 0, *P* <0.05), but there were no hepatic veno occlusive disease (VOD) occurs in both groups. And there was no significant difference in other all regimen toxicity, especially above grade 2 toxicity, and treatment related mortality (TRM) within 100-day of two groups were 0. The partial response(PR) rate after transplantation in BUCY group was significantly decreased (6/20 *vs* 0/20, *p*<0.05), while the patients in the HDM group were not observed this phenomenon. The median PFS of HDM group was 17 months, and the median PFS of BUCY group and median OS of both groups had not been reached yet.

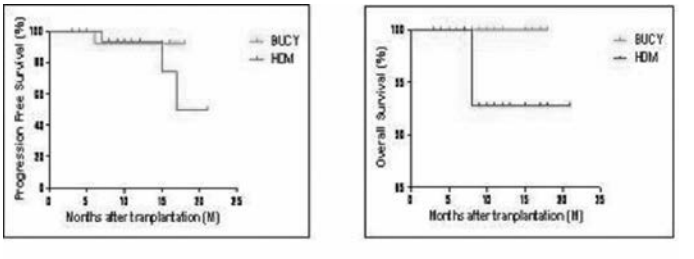

Figure 1.

Summary/Conclusions: BUCY regimen did not show weakness in safety and survival compared with HDM regimen, which prompt the possibility of BUCY regimen replacing the HDM regimen as the standard regimen in China.

PB1991

CYCLOPHOSPHAMIDE, THALIDOMIDE AND DEXAMETHASONE (CTD) AS INITIAL THERAPY FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: Major progress has occurred in multiple myeloma (MM) treatment in recent years. However, due to limited healthcare resources, newer agents are not readily available. In this setting traditional therapy such as oral cyclophosphamide, thalidomide and dexamethasone (CTD) represents an alternative for treatment in newly diagnosed MM.
Aims: To assess the clinical efficacy and toxicity of cyclophosphamide, thalidomide and dexamethasone.
Methods: Retrospective study with the regimen CTD (cyclophosphamide 400mg/m2 for 5 days, thalidomide 100mg/d increasing to 200 mg/day if tolerated, and dexamethasone 40mg weekly; in 28-day cycles), in patients with newly diagnosed MM treated at Instituto Nacional de Enfermedades Neoplasicas in Lima, Perú, between January 2008 and July 2013. Survival outcomes were estimated by Kaplan-Meier method.
Results: Fifty-nine patients were found to meet the selection criteria. Mean age was 56 years (27-68). Fifty-nine percent (n=35) were male. Salmon Durie stage III disease was present in 88.1%. The median number of treatment cycles delivered was 11 (range 4-12). After a median of 31 months follow-up (range 5-81), the overall response rate was 69.5%, with a 39% stringent complete response (SCR), complete response (CR) and very good partial response (VGPR), one patient (1.7%), three patients (5.1%), and nineteen patients (32.2%) respectevely. Median progression free survival (PFS) was 35 months. Five-year overall survival (OS) was 58.5%. The most common adverse events include neutropenia of all grades (44.1%), febrile neutropenia (grade III/IV 18.6/11.9%), severe infection (8.4%), deep venous thrombosis (6.8%). Out of 37 patients eligible for HSCT, 8 (21.6%) proceeded with it after this treatment regimen and 9 patients (24.3%) out of 37 are in maintenance. Treatment-related deaths occurred in 4 patients (6.7%) whose ages were 62, 64, 74, and 76 years-old.
Summary/Conclusions: CTD achieves durable responses with tolerable toxicity. This regimen represents a feasible and effective approach for MM patients in low income healthcare settings with tolerable side effects profile.

PB1992

PROGNOSTIC FACTORS IN PATIENTS WITH BENCE JONES MULTIPLE MYELOMA

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Background: Bence Jones Multiple Myeloma or Light Chain Multiple Myeloma (LCMM) is characterized by the production of only lights chains. It can be found approximately in 15% of MM patients and it is considered to have a worse prognosis than Intact Immunoglobulin Multiple Myeloma.
Aims: The aim of this study is to evaluate the prognostic value of serum free light chains (sFLC) at presentation in newly diagnosed LCMM patients.
Methods: 43 patients with LCMM were included in this study during a period of five years. sFLC levels were measured by nephelometry (Freelite, The Binding Site, Birmingham, UK) and sFLC ratio was calculated as K/L. Clinical and laboratory variables including sFLC, albumin, beta-2-microglobulin (B2M), creatinine, hemoglobin, calcium, LDH, M-protein size, plasma cell infiltration, presence of plasmacytoma and presence of lytic bone lesions) were recorded and evaluated for their impact on the patient's outcome. Statistical analysis was performed using SPSS 23. Overall survival (OS) was analysed by Kaplan-Meier method and curves compared by Log-Rank test.
Results: The median age of the patients included in the study was 69 years (range 59-75) and 51% were female. The median follow-up was 32 months (range 17-45 months) and during the period of study there were fourteen disease-related deaths. According to ISS; 28% patients had stage 1, 41% had stage 2 and 30% had stage 3. At diagnosis, the proportion of patients with renal impairment (creatinine>2 mg/dL), anemia (Hb<10 g/dL), hypercalcemia (Ca>11 mg/dL) and presence of bone lesions were 28%, 35%, 12% and 70%, respectively. The median percentage of BM plasma cells was 18% and 19% of patients had plasmacytoma at diagnosis. Altered sFLC ratio at diagnosis defined by K/L ratio <0.26 or >1.65 was observed in all the patients. Median sFLC levels in patients with kappa light chain restriction (n=20) was 413 mg/L (range 128-1612 mg/L) and in those with lambda light chain restriction (n=23) was 985 mg/L (range 270-2858 mg/L). The median sFLC ratio for kappa secretors and lambda secretors were 43 and 0.01, respectively. The cohort of patients was separated in two groups based on sFLC ratio cut-off of <0.01 or >43. The 5-years OS was significantly inferior in patients with high sFLC ratios (<0.01 or >43, 12% 5y-OS), compared with those with low sFLC ratios (between 0.01 and 43, 74% 5y-OS) with median survivals of 45 months and NR, respectively (p=0.017, HR=3.70, CI95% 1.15-11.87). Involved sFLC (iFLC) levels above the median values (kappa≥413 mg/L and lambda≥985 mg/L for kappa and lambda secretors, respectively) predicted a worse prognosis. The 5-years OS was 70% in patients with iFLC levels below the median and 0% in those with iFLC levels above the median, with median survivals of NR and 45 months, respectively (p=0.009, HR=4.33, CI95% 1.31-14.28). Other variables significantly associated with adverse outcome were B2M>3.5 mg/L (p=0.006) and albumin<3.5 g/dL (p=0.023). There was no significant correlation with the others variables.
Summary/Conclusions: sFLC ratio and involved FLC levels above median values at diagnosis are important risks factors of worse prognosis in patients with Bence Jones Multiple Myeloma.

PB1993

COMORBIDITY AS A FACTOR OF PROGNOSIS IN MULTIPLE MYELOMA

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Background: Despite the modern possibilities of therapy, multiple myeloma (MM) is an incurable disease with a wide variety of outcomes, depending on the presence of risk factors. MM Classification based on the criteria of Durie/Salmon and the ISS, include, primarily, the risk factors associated with the disease itself. However, factors associated with the patient, such as comorbidities and organ dysfunction which further affect the outcome of the tumor, is still not fully integrated into the predictive model.
Aims: To analyze the effect of prognostic factors: age, sex, medical condition and comorbidities, as well as indices of comorbidity and Sharlson Freiburger, overall survival (OS) in patients with MM.
Methods: 206 patients with MM (91 men and 115 women) analyzed. Patients ranged in age from 36 to 81 years (median 68 years). The statistical analysis was used software package SPSS 23. Effect of studied factors on the long-term prognosis was estimated by odds ratios (confidence interval 0.95). In developing the model predicting the likelihood of an unfavorable outcome were used methods of correlation, factor and regression analysis.
Results: during the factor analysis, all investigated prognostic factors, such as somatic status, GFR <30 mL/min/1.73 m2, the presence of any tumor or metastasis of solid tumors, and had a significant effect on the agents. The prevalence of comorbidity in patients with MM at diagnosis was - 80%. The most common diseases have been associated disease of the gastrointestinal tract (88.4%), cardiovascular (78.6%), urinary (44.5%) and endocrine systems (26.8%). In assessing the prognostic impact on overall survival and an index of comorbidity Sharlson Freiburger index shows that both indices significantly affect the survival of patients with MM, depending on the increase in their score on the scales (P <0.001). Median survival in the evaluation of Freiburger index was 55.0, 29.5 and 19.5 months for 0, 1 and 2-3 scores, respectively. Sharlson comorbidity index also indicated a significant difference in the survival rate of patients corresponding to 0, 1-2, and ≥3 points.
Summary/Conclusions: so comorbidity patients affects the survival and effectiveness of treatment in patients with MM, reducing these figures.

PB1994

PLASMA CELL LEUKEMIA: A 10-YEAR SINGLE CENTER EXPERIENCE

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Background: Plasma cell leukemia (PCL) is a rare disorder representing less than 5% of malignant plasma cell diseases and defined by the presence of clonal plasma cells in the peripheral blood with an absolute count of >$2x10^9$/L and/or >20% of the white blood cells. It presents either as primary disease (pPCL) or as a secondary leukemic transformation (sPCL) of multiple myeloma, and usually has an aggressive course and poor outcome.
Aims: To evaluate clinical features, prognostic factors and treatment outcome in patients with PCL.
Methods: A single center retrospective case-series study was performed. Data from patients diagnosed with PCL between January 2006 and December 2015 were collected and analysed.
Results: We identified 15 patients with PCL (5 male/10 female, median age

68 years, range 44-78) of whom 11 had pPCL and 4 sPCL. In sPCL the median time to leukemic progression was 22.3 months, with a median of 3 previous therapeutic lines. The subtype of monoclonal component found at diagnosis was IgG (n=7), IgA (n=2), IgE (n=1), light chain only (n=3) and non-secretory (n=2). Median plasma cell count in peripheral blood was $4.05x10^9$/L (range 0.3-60.5) with a median proportion of plasma cells of 32% (range 11-85).
At baseline, most of the patients had poor performance status with ECOG³2 (n=9), advanced stage disease classified at stage III according to International Staging System (n=11) and Durie-Salmon Staging System (n=10), and at least one end-organ damage (n=14). Extramedullary disease was present in 5 cases. Elevated lactate dehydrogenase was observed in 11 patients (median 338U/L, range 118-2610, N<248U/L) and β2-microglobulin in 12 (median 17.7mg/L, range 1.0-62.1). In 8 patients cytogenetic studies were carried out by conventional cytogenetic analysis and by FISH. Seven of them presented high risk cytogenetic alterations. Four patients received anthracycline-based regimens as first-line treatment, 2 single alkylating agents and 6 bortezomib or lenalidomide as additional or unique treatments. In 3 patients, with very aggressive disease and poor performance status, early death occurred, allowing only palliative treatment with steroids. Two patients underwent autologous hematopoietic stem cell transplantation after first line treatment. Eight patients achieved complete or partial response. The median overall survival (OS) was 4.5 months and OS at 2 years was 12.5%. Significantly longer OS was observed in patients responding to first-line treatment *versus* those who did not respond (median 9.6 *vs* 0.9 months, p=0.002); in patients who received bortezomib during the course of the disease (median 23.8 *vs* 0.9 months, p=0.022), and in patients with good performance status (ECOG <2) at the time of diagnosis (9.6 *vs* 4.1 months, p=0.024).
Summary/Conclusions: PCL is a rare disease with a poor prognosis, aggressive clinicobiological features and a low response rate to conventional treatment. Although our conclusions are limited by the small sample size, our study shows that good performance status and response to first-line treatment present a positive impact on survival. The use of bortezomib also appears to improve outcomes by lengthening OS.

PB1995

A SINGLE CENTRE EXPERIENCE OF DPACE-BASED THERAPY WITH LENALIDOMIDE OR THALIDOMIDE +/- BORTEZOMIB PRIOR TO AUTOLOGOUS TRANSPLANT IN MYELOMA

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Background: DPACE (infusional Cisplatin, Doxorubicin, Cyclophosphamide, Etoposide and oral Dexamethasone) with Thalidomide and Bortezomib is used within the Arkansas Total Therapy Regimen for myeloma. We have used DPACE-based therapy for high-risk and poorly responsive myeloma prior to autologous transplant (ASCT).
Aims: To establish the toxicity and efficacy of DPACE based chemotherapy with a combination of thalidomide or lenalidomide and/or bortezomib as a stand alone therapy or prior to consolidation with autologous stem cell transplant in high risk or poorly responsive myeloma.
Methods: We conducted a retrospective review of 22 patients treated at a single centre with DPACE-based therapy between 2010-2015 incuding two who received DPACE-based therapy as two separate treatment lines. Overall survival and time to next treatment from commencement of DPACE-based therapy were estimated using a Kaplan-Meier method and the impact of categorical variables assessed using a log rank analysis. Median follow-up was 2.1 years.
Results: PS was 0 (72.7%) or 1 (27.3%). ISS at commencement of DPACE was I (27.3%), II (22.7%) and III (36.4%). 15 of 22 patients had FISH performed prior to DPACE of which 40% had 2 or more adverse abnormalities (gain 1q, loss 1p, loss 17p, t(4;14), t(14;16), t(14;20)). Patients received DPACE with an Immunomodulatory agent (Lenalidomide 62.5% or Thalidomide 37.5%) and 66% received Bortezomib. DPACE-based therapy was used as primary therapy in 4/24 (16.6%) for and as salvage for suboptimal response in 20/24 (83.3%). Two cycles were given in 22/24 (92%). Patients received a median of 2 units of red cells per cycle (range 0-8 in cycle 1, 0-12 in cycle 2). Median platelets transfusions equalled 1.5 (0-6) in cycle 1 and 3.5 (0-9) in cycle 2. All patients experienced grade 4 neutropenia with median duration of 6 days in cycle 1 and 5 days in cycle 2. Grade 3-4 sepsis occurred in 9/24 (37.5%) in cycle 1 and 7/22 (31.8%) in cycle 2. All planned patients harvested successfully. From start of DPACE-based therapy, median OS was 23.3 months. Median TTNT was 12.6 months. Treatment-related mortality was 1/24 (4.1%) due to sepsis. Following DPACE-based therapy, 12/24 (50.0%) achieved CR or VGPR. 15/24 (62.5%) were consolidated with transplant (one allogeneic). Consolidation with ASCT demonstrated a clearly superior OS (77.1% *vs* 0% at 2 years, p<0.001) and improved TTNT (median 33.1 *vs* 4.3 months, p=0.007). Lenalidomide did not impact on toxicity or response rates. There were no reported second primary malignancies in any patients.
Summary/Conclusions: DPACE-based therapy is an effective induction or salvage therapy prior to ASCT in high risk or poorly responsive myeloma. Toxicity is manageable and stem cell harvest is achievable. Time to next treatment and overall survival is significantly longer in those consolidated with stem cell transplant indicating that there is limited value in using PACE-based chemotherapy without stem cell transplant. Initial data does not indicate a difference between Lenalidomide or Thalidomide in toxicity, response rates or survival although longer follow-up is warranted to investigate this further.

PB1996

FLOW CYTOMETRY BASED MRD ANALYSIS VERSUS IMMUNOFIXATION ELECTROPHORESIS FOR RESPONSE ASSESSMENT OF MYELOMA PATIENTS ON TREATMENT

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Background: Minimal residual disease (MRD) analysis by multiparametric flow cytometry (MFC) is a sensitive method to evaluate treatment efficacy in patients of plasma cell myeloma (PCM), and also as a tool for predicting patient outcomes and guiding therapeutic decisions. In the present study, we compared MRD levels in patients of plasma cell myeloma after chemotherapy or autologous stem cell transplant (ASCT), assessed by MFC, with immunofixation electrophoresis (IFE) and also the serum M-band status.
Aims: To evaluate the utility of MFC (six colour - three tubes) assay for detection of residual disease in patients of PCM and compare it with results of serum protein electrophoresis (SPEP) for M-band and immunofixation electrophoresis (IFE) done at same time.
Methods: Twenty six PCM patients (16 males & 10 females) were included in the study with mean age of 55.5 (range 37-80) years. Nine patients were post-ASCT (day +100), and 17 patients were on chemotherapy (majority treated with combination of cyclophosphamide, bortezomib and dexamethasone) and had completed induction at time of MRD analysis. MRD was analyzed using a dual laser BD FACS Canto II. Pre-titrated cocktail of CD38, CD138, CD19, CD45, cytoplasmic Kappa and lambda light chain, CD56, CD81, CD27, CD28 and CD200 were used in 6-color combination of three tubes for MRD analysis. IFE and M-band detection were also performed and compared.
Results: Ten out of 26 patients had detectable residual disease by MFC and/or IFE. MRD by MFC was detectable in 8 patients with mean of 1.79% (0.004-6.44%), all eight belonging to post-induction chemotherapy group. Only 4 of these 8 patients (50%) revealed both IFE positivity and detectable M-band. Two out of these 10 patients were MRD negative, however revealed IFE positivity as well as detectable M-band in one, and the other patient was only IFE positive (Table 1). Remaining 16 patients did not show evidence of residual disease by any of the three methods. Statistical analysis between MRD-positive (8/26) and MRD-negative (18/26) groups revealed no significant difference between age and sex distribution, mean values of hemoglobin, leukocyte count, platelet count, serum creatinine, serum calcium and serum albumin and the mean percentage of plasma cells on bone marrow aspirate smears *(Mann-Whitney U test)*.

Table 1. Comparison of results of the 10 patients showing persistent residual disease by SPEP/ IFE/ MFC, along with plasma cell percentages on bone marrow aspirate smears.

S. No.	SPEP	IFE	MRD (%)	Plasma cells on morphology (%)
1.	Negative	Negative	0.07	2
2.	Negative	Negative	0.11	1
3.	Negative	Negative	0.41	1
4.	Negative	Negative	0.7	4
5.	Positive	Positive	0.004	1
6.	Positive	Positive	3.2	15
7.	Positive	Positive	3.4	0
8.	Positive	Positive	6.44	22
9.	Positive	Positive	0	2
10.	Negative	Positive	0	1

Summary/Conclusions: MRD detection by multiparametric flow cytometry is a sensitive technique and should be routinely performed in addition to immunofixation electrophoresis and serum protein electrophoresis for more accurate assessment of treatment-response, better prognostication and further management of plasma cell myeloma patients.

PB1997

DIAGNOSIS, PROGNOSIS AND RISK ASSESSMENT OF MULTIPLE MYELOMA: IDENTIFYING GAPS AND ADDRESSING EDUCATION NEEDS FOR HEMATOLOGISTS AND ONCOLOGISTS

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Background: The diagnostic and prognostic criteria for multiple myeloma (MM) have rapidly evolved as the therapeutic landscaped has advanced. Furthermore, in 2014, the MM diagnostic criteria were revised to incorporate biochemical parameters in addition to traditional symptomatic criteria. These emerging

changes require that the clinician interpret and integrate this new information into clinical practice.
Aims: A 2-part study was conducted with the first part focused on identifying clinical gaps in prognosis and diagnosis of patients with MM and the second part focused on determining the effect of educational intervention designed to address the identified clinical gaps.
Methods: Education interventions for hematologists/oncologists were developed and posted online (http://www.medscape.org/sites/advances/multiple-myeloma). Two of the interventions used a 25-question self-assessment focused on identifying clinical practice gaps in MM while the third comprised a 20-minute video of a two MM expert discussion designed to narrow the knowledge and competence gaps of hematologists and oncologists. Learning in the video-based intervention was assessed by comparing each learner's responses to pre-education questions with responses to the same questions posed after education. The McNemar's chi-square test was used to assess whether the mean pre-assessment score was different from the mean post-assessment score. Activities were posted online July 2015 and data were collected through September 2015.
Results: Responses of 434 hematologist/oncologists who completed all assessment questions in at least one intervention during the study period were analyzed. At baseline, identified clinical gaps included: the inability to recognize clinical features of high-risk disease (82% incorrect), inability to identify tools for assessing prognosis (73% incorrect), inability to distinguish unfavorable (44- 56% incorrect) or favorable (47% incorrect) cytogenetics, inability to assign the International staging system (ISS) score (26% incorrect), inability to detect clinical relapse (49% incorrect), and inability to interpret the IMWG diagnosis criteria (40-79% incorrect). For the second phase of the study, the education intervention focused on IMWG guidelines in newly diagnosed MM and utilized a study group of matched hematologist/oncologist learners (N=58). After participation in educational intervention, 84-95% of hematologist/oncologists selected the correct answer regarding diagnostic criteria (Table 1).

Table 1. Post-education Outcomes.

IMWG Diagnosis Criteria Topic	Pre-education % Correct	Post-education % Correct	% Improved Learners	P-value
Abnormal Bone Marrow Plasma Cells	41%	95%	53%	P<.05
Serum Free Light Chain Ratio	60%	95%	38%	P<.05
Number of Lesions as Detected by MRI	31%	84%	55%	P<.05

Summary/Conclusions: Despite the publication and dissemination of recent guideline updates, clinician show gaps in knowledge of clinical diagnostic and prognostic criteria for MM. This study identified specific education needs of physicians treating patients with MM regarding diagnostic, prognostic and risk stratification and demonstrated increased knowledge after participation in a targeted online intervention. Learners showed a mean improvement of 49% (range: 38-55%) in knowledge of IMWG diagnostic criteria in post assessments. Future education is warranted on practical application of diagnosis criteria, laboratory thresholds, prognostic, and monitoring tools, and proper risk assessment in order to enhance prompt diagnosis and effective disease management.

PB1998

IDENTIFICATION OF MULTIPLE MYELOMA IN PATIENTS ATTENDING EMERGENCY SERVICES WITH SEVERE BONE PAIN

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Background: Bone metastases are due to a variety of primary tumors that include Multiple Myeloma (MM) and solid tumors (lung, breast, prostate). Its effects result in pain refractory to conventional analgesic treatments and osteolysis leading to pathological bone lesions. Sometimes, patients with age>50 years, intense and repetitive bone pain are treated with analgesics without assessing the possibility of a MM at Emergency Services (ES). Typically, after several visits to the ES because of progressive increase of pain and evidence of bone damage the patient is admitted to study a possible MM. Early study of the pathological bone lesions is crucial for a correct diagnosis and to increase the survival time of patients. The protocol "SPE+FLC" that uses serum free light chains determination (FLC) and serum protein electrophoresis (SPE) enables sensitive quantification of a possible monoclonal component in the study of MM.
Aims: The aim of our work is to study the diagnostic utility of the protocol "SPE+FLC".
Methods: During a period of 12 months, we studied 44 patients with age>50 years old, intense bone pain and recurrent visits to Emergency Service where imaging methods (X-Rays, CT scan and MRI) showed osteolytic lesions, vertebrae collapse and pathological fractures that may be associated a MM or metastasis from a primary tumor of unknown origin (TU). The protocol (SPE+FLC) was applied to every patient to study a possible MM and the determination of tumor markers to discard a TU with bone metastasis.
Results: The diagnosis was: MM in 16 patients (36%), TU with bone metastasis in 14 patients (32%) and 14 patients without tumoral pathology (32%). In MM patients, the median age was 68 years (range 58-75) and the median time from symptoms to diagnosis was 5 months (range 2-7) with a median number of visits to Emergency Service of 3. The diagnosis was intact immunoglobulin MM in 13 patients and Bence-Jones MM in 3 patients. According to ISS system for MM; there were 2 patients in stage 1 (12%), 4 patients in stage 2 (25%) and 10 patients in stage 3 (63%). During the study there were 3 MM related deaths. The protocol "SPE+FLC" had a sensitivity of 100%, specificity of 97%, PPV of 94% and PNV of 100%.
Summary/Conclusions: In patients with age>50 years, intense bone pain with pathological bone lesions, the application of the protocol "SPE+FLC" allow us to detect a possible MM in order to apply an early treatment and increase the survival time of the patient.

PB1999

LIGHT-CHAIN ESCAPE IN MULTIPLE MYELOMA: A NEW PATTERN OF DISEASE RELAPSE CHANGING BIOLOGY. A SINGLE-CENTER EXPERIENCE OF THREE CASES

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Background: Multiple Myeloma (MM) is characterized by the production of a monoclonal protein which can be an intact immunoglobulin, free light chain (FLC), both or, in rare cases, neither. The introduction of novel drugs has changed the natural history of the disease, leading to new manifestations of relapse. Light chain escape (LCE) defines a kind of relapse in which the FLC increase is not accompanied by a concomitant raise of the original monoclonal component (MC).
Aims: Disease biology and progression in MM is now thought to be the result of Darwininan-like evolution process: multiple pathological clones are present at MM diagnosis, possibly producing different monoclonal proteins. The cases presented here indicate that disease progression and relapse may be associated with selective outgrowth of a specific FLC producing clone.
Methods: Here we report three cases diagnosed with aggressive LCE between 2001 and 2015 at a single institution. We even include an interesting case of IgG lambda monoclonal MM who relapsed after double autologous stem cell transplantation (ASCT)changing face presenting like FLC MM lambda with a new rare IgD lambda monoclonal pattern.

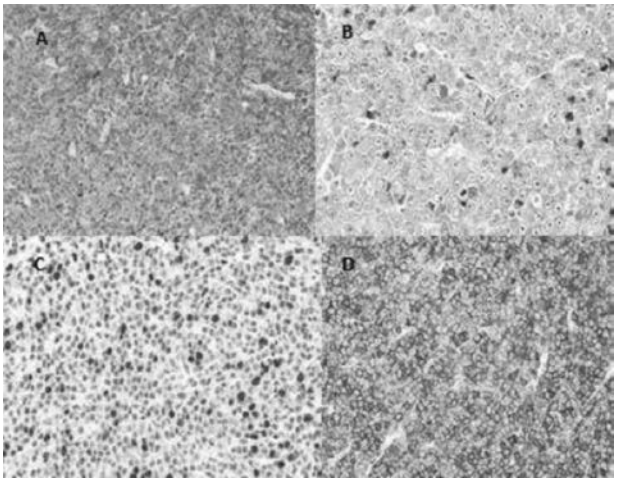

Figure 1. A) Histopathological examination of inguinal lymph node revealed plasmocytoma with immunostaining positive for chain lambda (40x). B-C-D) Bone marrow biopsy demonstrated plasmocytoma (Giemsa 40x), with Ki67 expression in bone marrow plasma cells >20% and immunostaining positive for lambda chain (20x). Case 1.

Results: Patient 1. A 63-year old woman was diagnosed with IgG-lambda (stage IA, ISS2) with trisomy of chromosome 5 and 9. Before the "new drug era"the patient received 3 cycles of chemotherapy according to VAD regimen, followed by tandem ASCT; she achieved a VGPR. After 9 years the patient developed an enlargement of right inguinal lymph node and then painless subcutaneous mass above left shoulder. Biopsy of the 2 nodular lesions confirmed extramedullary plasmocytomas (Fig1A). The patient did not show classic monoclonal protein but a new little MC IgDlambda protein with an abnormal secretion of serum FLC lambda. Bone marrow plasma cells were dysmorphic (Fig 1B), with high proliferation index measured by immunohistochemical staining Ki67>20% (Fig1 C) and positive for lambda chain assessement (Fig 1D). Serum

LDH levels at recurrence resulted high. Patient 2. A 64-year old man suffering from IgG-kappaMM stage IIIA, ISS2 underwent double ASCT and then was enrolled in PANORAMA study because of relapsing disease. He then started salvage therapy with lenalidomide and dexamethasone, but after 23 cycles he showed progressive disease with the features of LCE kappa. He presented a complex karyotype with trisomy of chromosome 9, 15 and gene rearrangement of 14q32, increase of LDH and plasmoblastic medullary cytomorphology. Patient 3. A 51-year old woman presented IgG-lambda MM stage IIIA ISS3 with extramedullary involvement (L5-S1) and deletion in 17p chromosome. She was treated according to VTD therapy followed by double ASCT and dexamethasone maintenance. She remained in remission of disease for 60 months until an increased amount of serum FLC lambda appeared and rapidly increased, with multiple vertebral bodies collapses and bone marrow infiltration of 40% atypical plasma cells showing various prognostically unfavorable genetic signs (1q amplification, 17p deletion, 13 deletion, 14q32 rearrangement).
Summary/Conclusions: These cases represent a documented series of a rare but clinically important mode of relapse. They suggest the potential selective pressure exerted by novel agents and highlight the importance of FLC monitoring in MM patients undergoing ASCT and/or treatment with biological and new drugs. LCE underlines the intraclonal heterogeneity of MM; it was recently associated with shortened overall survival. Hematologists should keep in mind this possibility of evolving biology of disease in the "high dose and novel agent era".

PB2000

DOMESTIC SUBCUTANEOUS SELF-INJECTION OF BORTEZOMIB IN MULTIPLE MYELOMA

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Background: Bortezomib-based therapies are suggested as standards of care in management of patients with newly diagnosed and relapsed multiple myeloma. The recommended dose and schedule of Bortezomib is 1.3 mg/mq administred on days 1, 4, 8, and 11 of 21-day cycles, a regimen active and well tolerated. Subcutaneous administration of bortezomib could be a good option for patients, particularly those with poor venous access. It's known that home intravenous administration of bortezomib is feasible to adequately informed patients, because we have recently demonstrated that a solution of bortezomib powder in normal saline stored at 4°C remains stable for nearly one month.
Aims: Since 2009, in our unit all patients requiring bortezomib for the treatment of multiple myeloma perform intravenous injection of the drug at home, after having been supplied with the exact dose in saline solution, in ready-to-use plastic syringes, appropriately prepared under hood in sterile conditions. This procedure reduces the time spent by patients in hospital, improving convenience for patients and physicians. However, in some patients venous access may be difficult or sometimes unfeasible. As the drug is not histotoxic, subcutaneous administration is feasible, and this possibility is particularly attractive in domestic settings.
Methods: Safety and efficacy of subcutaneous injections of bortezomib at the same dose as i.v. administrations (1mg/sm, days 1, 4, 8, 11), but dissolved in smaller saline volume (max. 1ml), in association with oral dexamethasone 20mg/dd. 1-2,4-5, 8-9, 11-12, was verified in 108 patients affected by multiple myeloma, with poor venous access. In particular, the efficacy, evaluated as reduction of the monoclonal component during the i.v. period *vs* the s.c. period was performed in a subgroup of 12 patients.
Results: Results indicated an equivalence between the two administration modalities, according to other larger controlled studies. Based on these reports, 108 patients, requiring Bortezomib as part of anti Myeloma regimens, have been systematically treated by subcutaneous injections, performed at home. No significant side effects have been reported so far and quality of life is particularly improved. In particular, in 37 of them we have evaluated an equivalence between the subcutaneous and intravenous administration, according to other controlled studies.
Summary/Conclusions: The possibility to perform an antineoplastic regimen at home is particularly well accepted by all patients affected by multiple myeloma, with an achievement of a very good quality of life.

PB2001

AL AMYLOIDOSIS: REAL WORLD EVIDENCE FROM ARGENTINA

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Background: AL amyloidosis is a challenge in clinical practice due to the difficulties in diagnosis and treatment. We present our experience from the RIA Amyloidosis Registry managing this patients.
Aims: To evaluate the characteristics and outcomes of patients with Amyloidosis in Argentina.
Methods: Ambispective cohort observational study of patients with AL Amyloidosis included in the Amyloidosis RIA Registry from January 2006 till July 2015. Review of electronic medical records by the amyloidosis team. Data were analysed with SPSS program using conventional descriptive statistics and Kaplan Meier survival curves.
Results: The RIA Registry includes 140 patients: 6% (9) AA amyloidosis, 7% (10) FAP amyloidosis; 8%(11) senil amyloidosis; 24% (36) localized amyloidosis; 18% (27) unidentified and 34% (47) AL amyloidosis. Multiple myeloma patients with amyloidosis were excluded from the Registry. We included in this study the 47 patients with confirmed AL amyloidosis. 64% (31) were males with a median age of 58 years (35-81). The main reasons for consultation were heart failure (81%); proteinuria (62%); nephrotic syndrome (30%); renal failure (26); peripheral neuropathy; autonomic disfunction and gastrointestinal complaints (10%). In the laboratory workup only 56% of patients had a measurable M protein spike. Nevertheless, all patients had detectable clonal free light chains by inmunofixation or serum free light chain assays. The most frecuente light chain involved was lambda. Data regarding proBNP or BNP were available in 34 patients and only 4 patients had normal results. Due to delay in the diagnosis 9 patients died before starting proper treatment. Other 6 patients were treated in other hospitals (no data available). From the 32 patients treated in our hospital: 5 patients received upfront cardiac transplantation followed by CYBORD; 20 patients received low dose CYBORD; 4 patients received IMIDs and 3 patients conventional chemotherapy (prior to 2008). Autologous bone marrow transplantation was performed in 10 patients. The response rate was: complete response in 18 patients; partial reponse in 7 patients; progressive disease 1 patient; not evaluable 6 patients. There were 7 deaths; 3 in complete reponse and 2 in partial response. The main causes of death were cardiac failure (5) and sepsis (2).The median overall survival of patients treated in our hospital was 86 months (IC95 27-144).
Summary/Conclusions: AL Amyloidosis is underdiagnosed in Argentina. The consequent delay in diagnosis and start of treatment has a huge impact in the quality of life and survival of this patients. Low dose CYBORD is a good treatment option with and adecuate response rate and safety profile considering the difficulties associated with amyloid organ damage. M spike and free light chains response do not always correlates with organ response which usually occurs much later. Therefore it is important to consider heart transplantation in young patients with severe amyloid myocardiopathy as it remains the main cause of death in this patients.

PB2002

RETROSPECTIVE STUDY OF THE EFFICACY, ECONOMY AND SAFETY OF TREATMENT WITH SUBCUTANEOUS INJECTION OF BORTEZOMIB IN DE NOVO PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple myeloma(MM) is a clonal plasma cell malignancy. NCCN guidelines recommended bortezomib-based therapies as the first front-line treatment in patients with newly diagnosed and relapsed multiple myeloma. Oakervee H E, et al proved that PAD combination (bortezomib/doxorubicin/dexamethasone) can improve survival for previously untreated MM patients.Peripheral neuropathy(PN) is a well-known and unavoidable side effect of bortezomib, which is limited to the usage for some patients. The multi-centers clinical research suggested that subcutaneous administration of bortezomib was noninferior to the standard intravenous route of delivery but could reduce sideeffects of bortezomib, particularly in peripheral neuropathy. But China is lack of data about the usage of bortezomib.
Aims: To explore the efficacy and safety of subcutaneous injection of bortezomib in the treatment of de novo MM patients.
Methods: A total of 57 MM patients treated with bortezomib, adriamycin and dexamethasone form June 2008 to January 2015 were analyzed. Among them 28 received conventional PADiv with the intravenous bolus of bortezomib and another 29 received PADih with the subcutaneous injection of bortezomib. The efficacy and safety of two groups were evaluated.
Results: The overall response rate was 94.6% of all patients after four courses of PAD induction.67.9%, 32.1% in PADiv group and 60.7%,30.4% in PADih group achieved response>=VGPR and sCR/CR. The median follow-up of PADiv group was 40(1.5-73) months and PADih was 20(1.5-30) months. There was no difference of TTP and DFS between Two groups. But PADih group showed a more favorable OS (p=0.004)even though with a shorter period of follow-up. The statistical results showed that the most common hematologic toxicities of grade 3/4 in the PAD group were granulopenia(67.9%), leukopenia(67.9%), thrombocytopenia (67.9%) and anemia (35.7%), and non-hematologic toxicities of grade 3/4 mainly included infection (39.3%), constipation (25%), diarrhea (21.4%), peripheral neuropathy(21.4%), fever (21.4%), VZV infection (17.9%) and anorexia (17.6%). Accordingly, the hematologic toxicities of grade 3/4 in the PADih group were granulopenia(55.2%), thrombocytopenia (51.7%), leukopenia(41.4%), anemia(41.4%) and non-hematological toxicities were mainly constipation(34.5%), nausea(27.6%), anorexia(17.2%), diarrhea (17.2%) and vomiting(10.3%). A total of 27 patients(47.4%) treated with Velcade suffered from peripheral neuropathy. Incidence of PN of two groups even grade 3/4

showed no statistic difference. 39.3% PADiv patients were infected during the treatment and 3 died from severe pneumonia. The incidence of infection in PADiv and PADih group shows great difference(P=0.000). Among other grade 3/4 adverse reactions, only data of leukopenia(P=0.045) and VZV infection (P=0.023) show differences. Remarkably, six patients (21.4%) receiving PADiv due to severe PN reduced dose of Velcade or Suspended treatment. Patients treated with PADih did not appear serious infection. Average length of hospital stay per circle of PADih and PADiv group is 24, 15 days, respectively (P=0.000). Comparison of supportive therapies including G-CSF, platelets transfusion, resuscitation, prevention of infection, nutrition and bortezomib reduction or suspension through chi-square analysis showed that proportion of platelet transfusion and prevention of infection was statistically significant (P=0.094; P=0.091) when alpha=0.1. Ratio of resuscitation, anti-infection drugs, suspension or reduction of bortezomib in PADiv group is higher(P=0.009; P=0.002; P=0.011).

Summary/Conclusions: The PADih regimen by changing bortezomib from intravenous bolus to subcutaneous injection significantly reduced adverse events, improved the safety of clinical application of bortezomib without affecting curative effect, and had greatly improved the overall survival due to lower incidence of infection in the PADih patients. In addition, subcutaneous bortezomib could obviously reduce the length of stay in hospital per circle and incidence of infection and decrease the cost of treatment.

PB2003

THROMBOTIC MICROANGIOPATHY ASSOCIATED WITH BORTEZOMIB TREATMENT IN MULTIPLE MYELOMA

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Background: Thrombotic microangiopathy (TMA) is a very diverse medical condition defined by the presence of microangiopathic hemolytic anemia (MAHA), thrombocytopenia and microvascular thrombosis, being characteristic of the MAHA schistocytes in peripheral blood. From an etiopatogenics point of view, it can be classified as primary, when there is clear evidence of a disorder caused by TMA, as the TTP and HUS, and secondary, when the TMA is a symptom of underlying conditions such as tumor processes, pregnancy, HIV infection, bone marrow transplant and exposure to certain drugs. Today, drug induced TMA is clearly recognized and it has been reported that at least 22 drugs involved in this process, including various chemotherapeutic agents (cyclosporine, mitomycin C, gemcitabine, bevacizumab, ect) and other substances such as vaccines, herbal products and homeopathic drugs.
Aims: We show the sixth case reported to date of bortezomib-induced thrombotic microangiopathy in MM.
Methods: 52 year-old woman diagnosed with multiple myeloma stage IIIA IgG Lambda ISS II with anemia and without thrombocytopenia, creatinine 0.84 mg/dL and glomerular filtration rate of 81 mL/min, discreet elevation of LDH and Beta2 microglobulin, monoclonal component of 2.2 g/dL IgG lambda and urine 6390 mg/dl, bone lesions in dorsolumbar vertebrae and medullogram with infiltration for plasmatic cells monoclonales of 25-35%. She starts first-line treatment with bortezomib, lenalidomide and dexamethasone. The eleventh day of treatment presents sudden increase of creatinine and a decrease in the filtration rate, oliguria, and the progressive decrease of platelets. In addition, hypertransaminasemia with hyperbilirubinemia and increased PCR, gradual rise in LDH and decreased haptoglobin documented. In the peripheral blood smear the platelet count was verified and frequent presence of schistocytes was reported. Within the analytical study, not immune anemia is found with a negative Coombs test, normal coagulation tests, serology, Shiga toxin in feces and negative pregnancy test, ADAMTS13 activity of 33% and negative ADAMS13 antibodies, and complement levels were normal. The treatment was started with plasmapheresis until all 12 sessions were completed with progressive improvement. After 5 additional cycles of chemotherapy with dexamethasone and lenaidomida without bortezomib, and an autologous stem cell transplant, she reached very good partial response.
Results: So far, 5 cases of post TMA bortezomib have been described in the literature (Table 1). The acute immunological reaction and the drug toxicity are the two recognized hypothesis that explain the development of thrombotic microangiopathy secondary to drugs. The variavility in the time of the beginning of the TMA in five cases, ADAMS13's different values and the absence of antibodies in one of them, suggests that not only the immune mechanism is involved in his etiopatogenia. Nevertheless, other drugs such as ticlopidine, produce MAT by a clearly immune mechanism unleashing a severe decrease of the activity of ADAMS13 by the production of antibodies anti ADAMS13. Though, the immunomodulatory capacity of the bortezomib inside the bone marrow microenvironment was demonstrated, still it is not known if such a condition could have any relation with the development of MAT; of fact, a case of microangiopathy successfully treated with bortezomib has been reported, arguing the blockage of plasma cells and B-lymphocytes autoreativos. On the other hand, other medicines like the monoclonal antibodies anti VEGF (ej. bevacizumab) that alter directly the angiogenesis have been associated with TMA. Bortezomib in addition from blocking the action of the proteasome, also suppresses the production and secretion of VEGF, which could be related to an altered angiogenesis and endothelial damage characteristic of TMA. For the treatment of MAT diverse empirical therapies were used, without restarting the bortezomib in any of five cases and the clinical being solved in all of them.

Table 1. Case reports of TMA associated with BTZ.

Study	Drug	Age/Sex	Time to onset of TMA	ADAMS 13	ANTI ADAMS13	TTO
Morita et al. 2008	TASPE BTZ	54/M	Day 8	36,5%	Negative	FFP (240ml/d) + Haptogobin suspension BTZ
Moore et al. 2011	BTZ	57/F	Day 2	12%	---	
Salmenniemi et al. 2012	TASPE BTZ	52/F	Day 11 after 5 cycles	---	---	PE (12)
Mehta et al. 2012	BTZ	70/F	Day 2 after 9 dose	31%	---	PE (1)
Kah-Lok et al. 2015	BTZ	61/F	Day 10 after 5 cycles	25%	---	Suspension BTZ
HCSC. 2015	BTZ	52/F	Day 11	33%	Negative	PE (12)

BTZ: Bortezomib, FFP: fresh frozen plasma , PE: plasma exchange

Summary/Conclusions: TMA must be recognized as a potentially serious secondary side effect to its administration of bortezmib in patients who develop MAHA, thrombocytopenia and acute renal failure.

PB2004

A SINGLE CENTRE REAL LIFE EXPERIENCE ON MULTIPLE MYELOMA (MM) PATIENTS: SUBCUTANEOUS (SUBQ) VERSUS INTRAVENOUS (IV) BORTEZOMIB (BOR)

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Background: The outcome of multiple myeloma patients (MM) has significantly improved over recent years, mainly due to the discovery of novel antimyeloma agents together with a better knowledge of the biology of the disease [Kumar *et al.* 2008]. One of these novel agents is bortezomib, the first in the class proteasome inhibitors introduced in the clinical practice approximately one decade ago. However, toxicity, especially peripheral neuropathy, as well as the intravenous route required for its administration are the two most significant bortezomib-related issues. To try to reduce the peripheral neuropathy, new guidelines for its management and the introduction of weekly schedules of administration have contributed to significantly decrease its incidence and the subcutaneous (subQ) administration has been recently introduce to avoid the intravenous (IV) route. Results obtained in phase I/II and III studies have confirmed that subcutaneous administration is feasible and represents an additional step towards the optimization of bortezomib use, resulting in a probably more convenient method than the IV route that is at least as effective.
Aims: To evaluate efficacy and tolerability of Bortezomib (IV *vs* sub Q) in standard combination myeloma regimens. Moreover, we compared the incidence of peripheral neuropathy.
Methods: We reviewed data of 50 consecutive MM patients treated at our Hematologic division between October 2008 to November 2015. Standard criteria were applied to evaluate response rate and neurotoxicity (NCI CTCAE national Cancer institute common terminology criteria for adverse events version 4.0).

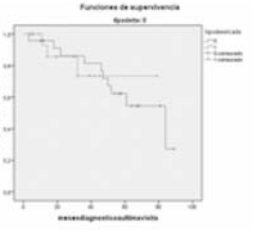

Figure 1.

Results: Our case serie is composed by 50 patients diagnosed with MM and treated in 1º line with combination regimen including Bortezomib (subQ or IV). Median age 75 years (range 39-89). 17/50 (34%) were administered VD regimen (7 subQ *vs* 10 IV), 27/50 (54%) patients were administered VMP (15 subQ *vs* 12 IV), 2/50 patients (4%) received poliQT VMCP/VBAD (IV Bortezomib) while 4 patients (8%) received VDT regimen as induction to transplant consolidation with subQ Bortezomib. Overall survival (OS) of the serie was 84 months (47-120 range) (Figure 1) with a free progression survival (FPS) of 23 months (19-30 range): no difference was found between subQ or IV Bortezomib regimen in terms of survival. There were 12 cases (24%) of neurotoxicity in our serie: 2/12 (16%) patients with VTD (subQ Bor) where the role of the inmunomoduladory drug in the toxicitiy must be considered; 6/12 (50%) cases

with IV Bor and 4/12 (33%) cases with subQ Bor: all 5/6 cases of neurotoxicity in IV Bortezomib were grade III with need of suspension of the drug. In subQ population there were 1 case with grade I toxicity and 3 cases of grade II neurotoxicity with reduction of the dose.
Summary/Conclusions: In our real life experience OS and FPS in our serie is notable, with 84 months and 23 months described. In terms of toxicity there were slightly more cases of neutorotoxicity in IV Bor population with higher grade of toxicity than the subQ population. In conclusion, the subQ formulation of bortezomib represents an additional step towards the optimization of bortezomib use, resulting in a more convenient route that is at least as effective as the IV route.

PB2005

REAL WORLD EXPERIENCE OF BORTEZOMIB RE-TREATMENT FOR PATIENTS WITH MULTIPLE MYELOMA AT FIRST RELAPSE

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Background: In the United Kingdom (UK), bortezomib (Velcade, Janssen) based triplet regimens are commonly used as first line therapy for both transplant eligible and ineligible patients. The efficacy of re-treatment with bortezomib monotherapy or bortezomib plus dexamethasone in subsequent lines of therapy has been previously reported. However there are limited data on re-treatment at first relapse, particularly with bortezomib-based triplet regimens which are more commonly used in current practice.
Aims: This study aimed to evaluate the efficacy of bortezomib triplet regimens at first relapse in patients previously treated with first line bortezomib. The safety of bortezomib as re-treatment was also assessed.
Methods: This was a retrospective analysis of MM patients at first relapse treated with bortezomib, who had also received bortezomib as first line therapy. Twenty three sequential patients from a single UK center who had achieved at least a partial response (≥PR) and a treatment free interval (TFI) of a minimum of 60 days from first line bortezomib treatment (*i.e.* not refractory) were identified. Response to treatment and disease progression were defined by the International Myeloma Working Group criteria.
Results: A triplet bortezomib combination was used as first line therapy in 78% of patients (n=18/23) with a median of five cycles given (range 4-8). No patient received maintenance therapy. The median time to best response (TTBR) was 3.5 months (range 0.7-9.0) and the median duration of response (DOR) was 14.9 months (range 4.7-44.5). The overall response rate (ORR) to bortezomib at first relapse was 87% (PR 39%, VGPR 35%, CR 13%). The median TTBR was 4.1 months (range 0.7 -15.0) and median DOR was 11.5 months (range 1.0-18.5). After first line bortezomib, the median time to progression (TTP) was 18.9 months (range 7.3-49.6) whilst it was 14.4 months (range 1.4-16.6) at first relapse. The median TFI was 20.7 months (range 2.0-62.8) after first line bortezomib and 10.4 months (range 1.0-14.0) after bortezomib re-treatment. Ten patients received ASCT at first line compared to 11 at first relapse. Treatment-related toxicity was also evaluated. At first line, eleven patients (48%) experienced peripheral neuropathy (PN) (all grade 1). There were three (13%) grade 3-4 non-hematological adverse events (AE), which were all infections. All but one patient restarted bortezomib at full dose at relapse. Only two patients (11%) required a subsequent dose reduction (for PN). Eleven patients (61%) experienced PN with first relapse treatment (all grade 1-2). There were two grade 3 AEs (diarrhoea and febrile neutropenia). All patients who relapsed for a second time were able to proceed to a third line regimen which was most commonly lenalidomide and dexamethasone.
Summary/Conclusions: This single center study in real life patients demonstrates that bortezomib combinations used at first line and sequentially at first relapse represent a valid treatment strategy for selected patients.

PB2006

FIRST EXPERIENCES WITH GENERIC BORTEZOMIB ADMINISTRATION ONE CENTRE EXPERIENCE

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Background: Multiple myeloma (MM) is malignant haemato-oncology disease characterised by clonal proliferation of plasmocytomas. Disease treatment is started with induction therapy in the symptomatic phase of the disease. Regimens with combination of vincristine, doxorubicin and corticoid were used in the last century. From 2002 are used in the Czech Republic "new drugs" as thalidomide and bortezomib (several years later also lenalidomide). As standard induction regimen is VTD regimen recommended (bortezomib, thalidomide and dexamethasone). Original drugs with bortezomib (Velcade) are still used, but generics drugs with equivalent active substance are also on the market now. These generics should be equivalent with originals. According our first experiences none serious adverse events were presented, other complications were of similar character as with original bortezomib.
Aims: First experiences in administration of generic bortezomib, adverse events, patient's toleration.
Methods: During 11-12/2015 generic bortezomib was administered to 28 patients (altogether 80 applications) at our department
Results: Adverse events after generic bortezomib applications occurred in 28 patients. Anaemia (c.18%) and thrombocytopenia (c. 14%) were between most often complications. From complication of lower grade occurred tiredness (17%), infection (3%), neuropathy (8%) and diarrhoea (3%).
Summary/Conclusions: We administered altogether 80 generic bortezomib applications to 28 patients at Haematology and oncology department in 11-12/2015. During this time none serious complication occur and most of adverse effects were of I. and II. grade. These complications were similar to original bortezomib complications. Other comparison with original product in longer time is necessary.

Myeloproliferative neoplasms - Biology

PB2007

DEVELOPMENT OF RAS-INDUCED ZEBRAFISH LEUKEMIA MODELS

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Background: The zebrafish has emerged as a versatile novel experimental model for studies on developmental hematopoiesis and leukemogenesis. Several oncogenes involved in human leukemia have been successfully overexpressed in zebrafish embryos. However, despite first encouraging results, these models often fail to fully recapitulate human myeloid malignancy, perhaps due to early lethality caused by transgene expression or lack of secondary events necessary for full malignant transformation.
Aims: Here we present a RAS overexpression zebrafish model that we plan to use for investigations on molecular mechanisms involved in leukemia initiation and progression and for drug screening that can identify novel anti-leukemic compounds.
Methods: We take advantage of the Gal4/UAS binary system and of existing transgenic lines and overexpress human oncogenic *HRAS* in zebrafish hematopoietic cells under the control of specific promoters (*fli.1, pu.1, runx.1, mpeg1*). The generated *HRAS*-transgenic fish lines are followed microscopically until the time-point of death or sexual maturity and hematopoietic cell development is studied correspondingly at embryonic (*fli.1*, *pu.1*), larval (*pu.1*, *runx1*) and adult stages (*runx1*) also by *in situ* hybridization/real-time PCR analysis of hematopoietic gene expression, flow cytometry, immunohistochemistry and/or blood smear morphological assessment.
Results: Different phenotypes were observed depending on the promoter driving the oncogene expression. *HRAS* induction via the early hematopoietic promoter *fli.1* affects primitive hematopoiesis inducing myelo-erythroid proliferation and delayed erythrocyte maturation resulting in an expansion of hematopoietic tissues (Alghisi *et al.* 2013). Unfortunately, studies at later stages are not possible in these fish due to their early lethality resulting from vascular defects and cardiac edema. Alternatively, *HRAS* expression driven by *runx1*, *pu.1* and *mpeg1* allows survival at these early stages permitting studies on larval and adult hematopoiesis. Interestingly, at 1 month, *runx1-HRAS* fish displayed a cellular expansion of hematopoietic stem/progenitor cells (HSPC) in the kidney marrow (KM), the zebrafish definitive hematopoietic compartment. Cytospin preparation and flow cytometric analyses revealed high numbers of undifferentiated stem/progenitor cells in both KM and peripheral blood of HRAS transgenic fish, suggesting that *HRAS*-overexpression in HSPCs expands this compartment by inducing its proliferation and perhaps additionally by impairing differentiation capacity. Furthermore, *mpeg1*-HRAS fish showed increased numbers of blood progenitors in the KM and abnormal gene expression of progenitor markers as demonstrated by qRT-PCR. Analysis of *pu.1*-HRAS juvenile fish is under way.
Summary/Conclusions: We are currently further investigating the effects of *runx1*-driven *HRAS* on the hematopoietic compartment and generate tools to explore potential cooperation of *HRAS* with other oncogenes during leukemogenesis. A screen analyzing compounds successfully suppressing the effects of HRAS on zebrafish hematopoiesis is underway and the results will be presented at the meeting.

PB2008

MOLECULAR AND CYTOGENETIC PROFILE OF PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: Primary Myelofibrosis (PMF) is hematopoietic stem cell malignancy characterized by clonal proliferation of myeloid-lineage cells. The molecular basis of this event includes mutations in *JAK2, MPL* and *CALR* genes, called clonal markers (CM), cytogenetic abnormalities and epigenetic disorders. The variability of the clinical course among patients with PMF due to different CM and epigenetic status requires close analysis for possible stratification by risk groups.
Aims: The aim of our study was to estimate overall survival (OS) in PMF patients depending on the type of CM, cytogenetic and epigenetic features.
Methods: We have examined 89 patients with PMF. Median age was 59 years (range 19-82). For all patients the detection of V617F mutation of *JAK2* was done. *JAK2*-negative samples were tested for *MPL* 515 codon mutations (PCR-RFLP) and 9th exon mutations of *CALR* gene by direct sequencing. Seventy-three patients underwent the analysis of mutations in *EZH2* and *ASXL1* genes with high resolution melting method followed by direct sequencing of probably mutated samples. Karyotype research was done for 39 patients with available bone marrow samples.
Results: CM were detected in 64 patients: *JAK2*+ 46.1% (41/89), *CALR*+ 21.3% (19/89), *MPL*+ 4.5% (4/89) cases. No clonal markers were found in 28.1% (25/89) patients considered triple-negative (TN). Median survival of TN patients was the shortest and amounted to 4 years (p=0.041), *JAK2*+ 11.9 years. In *CALR*+ and *MPL*+ patients during follow-up of 10 and 4 years, respectively, median survival has not been reached. According to the results of cytogenetic analysis patients were divided into 2 groups: the first group included 20 patients with normal karyotype (NK) and 5-with del(13)(q22), del(20)(q12), add(6)(p25), del(6)(q15) single features; the second group consisted of 13 patients with complex abnormalities and unfavorable aberrations (+8, -7/7q-, i(17q), inv(3), -5/5q-, 12p-). Median survival in the first group was 7.4 years, in the second group-4.5 years. It should be noted separately that unfavorable karyotype frequency was distinct in groups with different CM: *CALR*+ 0% (0/8), *JAK2*+ 35% (6/17), TN 54% (7/13) cases (p=0.053). We have detected 18 mutations of *ASXL1* gene in 16 patients (21.9%): fourteen patients had single mutation and two patients harbored 2 mutations at once. Mutation frequency was significantly higher in *CALR*+ patients compared to *JAK2*+ (37.5% (6/16) and 6.9% (2/29), p=0.037, resp.) and in TN patients compared to *JAK2*+ (33.3% (8/24) and 6.9% (2/29), p=0.044, resp.). Two mutations in *EZH2* gene (2.2%) were observed in TN, *ASXL1*+ patients. Both cases characterized of high-risk progression of PMF. The first patient (men, 61-year-old, complex karyotype) progressed to blast phase rapidly from the diagnosis and died after 8 months. The second patient (men, 75-year-old, NK) had severe thrombocytopenia and died from hemorrhagic stroke after 5 months since the diagnosis.

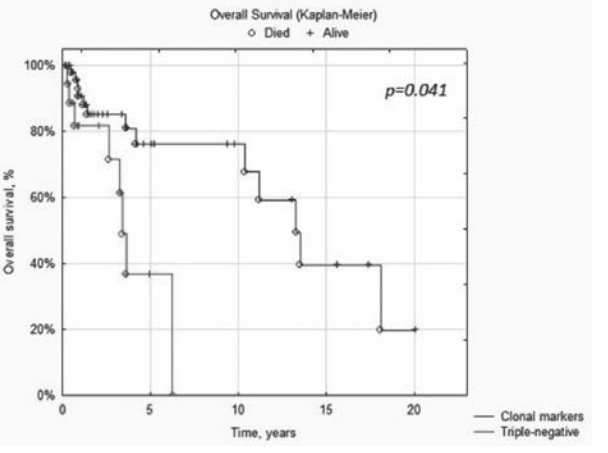

Figure 1.

Summary/Conclusions: Type of CM, cytogenetic aberrations and epigenetic changes can be correlated with different prognosis of PMF. The absence of CM and unfavorable karyotype are associated with reduced overall survival. The impact of epigenetic status on the prognosis in PMF patients requires further study.

PB2009

ASSESSMENT OF THE INTERLABORATORY VARIABILITY AND ROBUSTNESS OF JAK2V617F MUTATION ASSAYS: A STANDARDIZATION STUDY INVOLVING A CONSORTIUM OF 19 ITALIAN LABORATORIES

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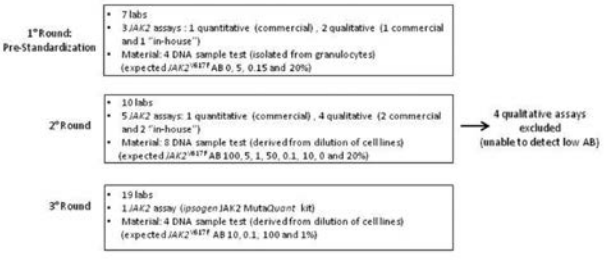
Background: In chronic myeloproliferative neoplasms (MPNs), the quantification of the *JAK2*^{V617F} allele burden (AB) is crucial for diagnosis and prognosis assessment, and also for disease monitoring after allogeneic stem-cell transplantation. To-date, a plethora of techniques for *JAK2*^{V617F} determination is used over different molecular laboratories, with substantial differences in specificity and sensitivity. Given the need to provide reliable and comparable molecular results, the standardization of molecular techniques is of utmost importance.
Aims: The aims of this multicenter study were: 1) to evaluate the inter- and intra-laboratory variability in *JAK2*^{V617F} quantification in 19 Italian molecular laboratories; 2) to identify the most robust assay for the standardization of the molecular test; 3) to allow consistent interpretation of individual patient analysis results.
Methods: A network of 19 Italian molecular laboratories was established. The study was coordinated by the Institute of Hematology "L. and A. Seràgnoli", Bologna, and was developed in 3 different rounds (Fig. 1). In routine practice, 2 laboratories did not assess *JAK2*^{V617F} mutation whereas 7 used a qualitative approach and 10 performed a quantitative evaluation. Both reagents and DNA samples were provided by Werfen-IL SpA and QIAGEN. Raw data and runs validity were checked according to handbook recommendations. Statistical analysis was carried out by QIAGEN/Bologna University.
Results: In the 1st round, we aimed to investigate the inter-laboratory variability on different mutation loads. All laboratories using a quantitative approach were able to determine the expected *JAK2*^{V617F} AB. Conversely, laboratories using a qualitative approach did not detect the positivity of samples with a low AB (0.15%). To further investigate the inter-laboratory variability on low-positive samples, we developed a 2nd round, in which 3 additional laboratories were included. In this 2nd round, each laboratory performed 2 runs with *ipsogen* JAK2 Muta*Quant* kit and 2 runs with their routinely used method. None of the laboratories using qualitative "in-house" methods were able to detect low-positive samples, while quantitative results by *ipsogen* JAK2 Muta*Quant* kit showed only a small variability among different laboratories at low AB (0.1 and 1%; CV =0.42 and 0.24, respectively). The 3rd round was intended to confirm the robustness of the *ipsogen* JAK2 Muta*Quant* kit in a larger cohort of laboratories. The study was therefore extended to 9 additional laboratories. "Home-made" methods were excluded and all laboratories performed 2 runs with the *ipsogen* JAK2 Muta*Quant* kit. Quantitative results were well reproducible across all mutation loads. Only one laboratory failed to quantify 0.1% sample in one run. Importantly, all laboratories clearly distinguished between the 0.1 and 1% mutated samples (0.1 and 1%; CV =0.46 and 0.77, respectively).

Table 1. Design of the study.



Summary/Conclusions: The first result of the study is that a qualitative approach is not sensitive enough to detect the *JAK2*^{V617F} mutation at a low (≤1%) burden. Conversely, the *ipsogen* JAK2 Muta*Quant* kit resulted highly efficient and sensitive in the quantitative detection of all mutation loads. This study sets the basis for the creation of an Italian network of molecular laboratories focused on the diagnosis of MPNs, including not only *JAK2*^{V617F}, but also Calreticulin and *MPL* mutations. The network will aim to identify/standardize the most efficient and cost-effective techniques for the evaluation of these mutations, so to produce reliable and reproducible molecular data.

PB2010

PHARMACOLOGICAL ACTIVITY PROFILING OF PACRITINIB IN THE BIOMAP® HUMAN PRIMARY CELL PLATFORM

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Background: Pacritinib is an orally available kinase inhibitor with specificity for JAK2, FLT3, IRAK1 and CSF1R. Comprehensive mapping of the pharmacological profile of pacritinib should enable a better understanding of the mechanistic basis of pharmacological action and support the utility of pacritinib in physiologically-relevant therapeutic areas.
Aims: The main goal of this study was to explore the effects of pacritinib on biomarkers relevant to human disease using BioMAP® Human Primary Cell Systems. A secondary goal was to compare and contrast the profile of pacritinib using this platform with that of other clinically available kinase inhibitors, to gauge the potential for differentiating effects of pacritinib in the clinical setting.
Methods: Pacritinib was evaluated in the BioMAP® Diversity PLUS panel by DiscoverX Corporation. Twelve human primary cell based systems were stimulated with one or more well-described biological factors to activate multiple disease-relevant signaling pathways. Each primed system was then tested with four concentrations of pacritinib ranging from 26-711 nM, encompassing physiologically and clinically relevant concentrations of the free drug (~200 nM in plasma). Anti-proliferative activity of pacritinib was determined for several human primary cell types and 148 biomarkers were measured by high-throughput immune-based assay. Biomarkers impacted by pacritinib treatment were evaluated for dose-dependency, and compared to a reference database of compounds including ruxolitinib (Jakafi), a JAK2 inhibitor approved for use in myelofibrosis.
Results: Exposure to pacritinib resulted in anti-proliferative activity in human endothelial cells, T cells, B cells, and coronary artery smooth muscle cells at 711 nM. This effect was most pronounced in B cells, the only cell type in which proliferation was also impacted at the lower, clinically relevant range. Cytotoxicity was not observed in any of the cell types. In addition, pacritinib had prominent immunomodulatory activities in the BT system modeling T-cell dependent B-cell activation. In the BT system, pacritinib resulted in statistically significant, concentration-dependent decreases in soluble TNFα, IgG, IL-17A, IL-17F, IL-2 and IL-6. Statistically significant, but less pronounced effects were also observed on inflammatory, tissue remodeling, and hemostasis-related activities. A comparison of pacritinib's profile at 711 nM with that of 1 μM ruxolitinib in the BT system demonstrated that pacritinib had more inhibitory effects on immune biomarkers, with fewer effects on biomarkers in other systems modeling different tissue biology.
Summary/Conclusions: BioMAP® analysis indicated that pacritinib impacts several disease biomarkers in a manner that may translate to differentiated therapeutic benefit. For instance, pacritinib's marked inhibition of inflammatory cytokines IL-17A and IL-17F and TNFα is potentially beneficial in rheumatoid arthritis, atherosclerosis, and/or psoriasis. The information obtained in the BioMAP® platform provided valuable insights that enabled selection of follow-up studies to perform in relevant animal disease models. Moreover, pacritinib's profile was distinct from that of ruxolitinib, which may underlie some of the comparative differences observed in the clinical setting. In order to further characterize pacritinib's effects on human disease models in comparison to other compounds, an expanded follow-on BioMAP® study is planned.

PB2011

A NOVEL REAL-TIME PCR ASSAY SET FOR DETECTION OF DIVERSE CALR MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Mutations in the *CALR* gene have been found in a large proportion of patients suffering from myeloproliferative neoplasms (MPN). While 52bp deletions (Type 1) and 5bp insertions (Type 2) constitute >80% of *CALR* mutations, a variety of other insertion-deletion mutations have been reported, all within a 66bp mutational hotspot in exon 9 of *CALR*. Reliable and efficient detection of those somatic driver mutations is crucial for MPN diagnosis and patient management.
Aims: We previously characterized our MPN patient cohort for insertions and deletions in the *CALR* gene using a PCR-based fragment sizing assay in combination with Sanger sequencing. Here we compare the characteristics of this fragment analysis with a novel real-time PCR (qPCR) based assay set (*ipsogen* CALR RGQ PCR Kit, provided pre-launch by QIAGEN, Hilden, Germany).
Methods: Fragment analysis of *CALR* exon 9 was performed using high-res-

olution sizing of fluorescent dye labeled PCR products on a 3130*xl* Genetic Analyzer (ABI, Foster City, CA). The *ipsogen* CALR RGQ PCR Kit was applied according to the manufacturer's instructions in 7 separate hydrolysis-probe-based qPCR reactions per sample within the same run. The kit identifies Type 1 and Type 2 mutations using ARMS PCR, in which a primer mismatch prevents elongation on wild-type (WT) DNA. Minor variants of *CALR* mutations are detected through a PCR clamping approach using 3'-phosphate blocked oligonucleotides complementary to WT sequence. For both fragment analysis and the *ipsogen* assay we determined dynamic range and detection limits through serial dilution of mutant genomic DNA (gDNA) in WT gDNA, generating *CALR* mutational burden standards down to 0.01%.
Results: Using the *ipsogen* CALR RGQ PCR Kit, we re-tested gDNA from 48 MPN patients (10 Type 1, 10 Type 2, 18 minor variants, 10 *CALR* mutation negative) previously evaluated by fragment analysis. Concordance between both assays was 100%. While the *ipsogen* assay formally requires a per reaction input of 50ng gDNA freshly isolated from peripheral blood, our samples were from a mixed cohort of various storage time and isolation protocols. Moreover, we applied the *ipsogen* assay on a large proportion of our samples using DNA inputs of 25ng and 10ng, respectively. *CALR* mutational status could be confirmed for all those cases. We next determined the limit of detection (LoD) of Type 1 and Type 2 mutations for both the *ipsogen* assay and fragment analysis. While limit-of-blank-based calculation of LoD revealed 0.39% and 0.08% for Type 1 and Type 2 *ipsogen* assays, respectively, fragment analysis could reliably detect 2.5% but not 1% *CALR* mutational burdens. Determination of the dynamic range revealed linearity for the fragment analysis down to the 2.5% detection limit. For the *ipsogen* assays, linearity on the log-scale could be observed down to 1% and 0.5% *CALR* mutant burden for Type 1 and Type 2 assays, respectively. Within these dynamic ranges, standard curves could be produced with a correlation coefficient of >0.98.
Summary/Conclusions: While fragment analysis represents a rapid, cost-effective assay for screening larger cohorts in a multi-well format, the specific identification of Type 1 and Type 2 mutations requires Sanger sequencing in addition. The *ipsogen* CALR RGQ PCR Kit offers, at once, reliable detection of *CALR* mutations and specific identification of Type 1 and Type 2 mutations, requiring minimal processing and equipment (Rotor-Gene Q MDx, QIAGEN). Moreover, the *ipsogen* assay showed superior sensitivity over fragment analysis and might therefore be most suitable for tracking the dynamics of the malignant MPN clone.

PB2012

THE S100 PROTEINS AS MEDIATORS OF INFLAMMATION ARE INCREASED IN MYELOPROLIFERATIVE NEOPLASM AND DOWNREGULATED BY JAK2 INHIBITION

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Background: Chronic inflammation is associated with sustained myeloproliferation, while S100 proteins have been shown to regulate cell proliferation, differentiation and inflammation in pathological conditions. S100A8, A9 and A12 produced by cells of myeloid origin were mediators of inflammation, while S100A4 regulated cell proliferation in neoplasia. S100A4 and A12 are produced via activation of the JAK-STAT pathway, constitutively activated in myeloproliferative neoplasm (MPN).
Aims: This study analyzed the inflammation linked S100 proteins in MPNs: polycythemia vera (PV, n=16), essential thrombocythemia (ET, n=16), primary myelofibrosis (PMF, n=20), according to JAK2V617F and CALR mutation status, and healthy controls (n=8), and their regulation in *JAK2V617F* mutated human erythroleukemia cell line (HEL) during MPN simulated therapy.
Methods: *S100A4*, *A9* and *A12* mRNA levels in granulocytes and HEL cells are measured by real time PCR, while protein expression of S100A4, A8, A9 and A12 are examined in granulocytes and plasma of MPN using immunoblotting and immunoassay, respectively. In addition, S100A9 is determined by immunohistochemistry in bone marrow. Also, levels of S100A4, A9 and A12 are measured by real time PCR and immunoblotting in HEL cells incubated 48 hours with hydroxyurea and specific JAK2 inhibitor 1,2,3,4,5,6-hexabromocyclohexane (HBC). Mutations of *JAK2V617F* and *CALR* exon 9 are analyzed by DNA sequencing and allelic PCR.
Results: *S100A12* mRNA level is significantly increased in *JAK2V617F* heterozygous ET patients, but downregulated in *JAK2V617F* heterozygous PV patients and PMF without mutation (p<0.05). However at protein level, S100A12 is significantly increased only in granulocytes of ET patients without mutation (p<0.05) and plasma of PV and PMF without *JAK2V617F* mutation (70 ng/ml, p<0.01). Besides non significant modification at mRNA levels, S100A4 and S100A9 protein expression demonstrated a common significant increase in granulocytes (p<0.01), followed by increased quantity of S100A9-positive cells in bone marrow and S100A8/A9 proteins in plasma of MPN patients (51 and 28 ng/ml, p<0.01). Presence of *CALR* mutation augmented S100A8/A9 levels in plasma and granulocytes of ET and PMF patients. After 48 hour, hydroxyurea and specific JAK2 inhibitor HBC significantly decreased *S100A4* mRNA levels in HEL cells (p<0.01). Further on, protein expression of S100A4 and S100A9 are also significantly downregulated by hydroxyurea and JAK2 inhibitor HBC in HEL cells (p<0.05).
Summary/Conclusions: S100A4, A9 and A12 mRNA and protein levels are not generally influenced by *JAK2V617F* mutant allele burden. S100A4 and anti-inflammatory S100A8/A9 protein levels demonstrated stable elevation in contrast to sporadic pro-inflammatory S100A12 appearance in MPNs. Moreover, JAK2 inhibition reduced S100A4 and S100A9 levels in HEL cells. S100A8/A9 could serve as a clinical biomarker and therapeutic target in MPNs, with existing S100A8/A9 blockers permitted for clinical testing.

PB2013

HIGH SENSITIVITY MLPA ASSAY FOR THE DETECTION OF THE RECURRENT CALR, JAK2, KIT AND MPL MUTATIONS WITH ≥1% ALLELE BURDEN IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPNs) are chronic hematopoietic stem cell malignancies, characterized by clonal proliferation of blood cells. Recurrent mutations in CALR, JAK2, KIT and MPL genes are important molecular markers for classification and prognostication of MPNs. Multiplex Ligation-dependent Probe Amplification (MLPA) is a widely used technique for gene copy number detection, also allowing simultaneous identification of known point mutations. However, the sensitivity of standard MLPA is limited to a mutant allele burden of ≥10%. To overcome this limitation, we have introduced a high sensitivity MLPA assay enabling detection of an allele burden as low as 1% for the most frequent mutations in MPN.
Aims: To demonstrate the feasibility of a modified MLPA assay to detect ≥1% mutant allele burden using artificial positive DNA and cell line samples. Validation of this assay is done with MPN patient samples.
Methods: Salsa MLPA P520 MPN probemix was designed and optimized to detect ≥1% mutant allele burden of the eight most frequent mutations in MPNs: **CALR**, 52-bp deletion and 5-bp insertion in exon 9 (L367fs*46 and K385fs*47), **JAK2**, deletions in exon 12 (N542_E543del and E543-D544del), **JAK2**, substitution in exon 14 (V617F), **KIT**, substitution in exon 17 (D816V) and **MPL**, substitutions in exon 10 (W515L and W515K). The performance of the newly developed MLPA assay was optimized and tested on artificial positive DNA samples with 1% mutation burden. The performance of the assay was further validated using commercial reference DNA samples with 1% allelic burden of JAK2 V617F and KIT D816V mutations, as well as by using dilution series of a JAK2 V617F positive, UKE-1 cell line, where the allelic burden was confirmed by the Ipsogen MutaQuant assay. Validation on diagnostic MPN patient samples (n=167) was performed in a single-blind setting.
Results: Results obtained by high sensitivity MLPA assay were concordant with an allele-specific PCR results in MPN patient samples. Moreover, 2 novel cases with 1.4-5% JAK2 V617F burden, which were not detected by allele-specific PCR, were identified with our novel assay and confirmed by the Ipsogen MutaQuant assay. Furthermore, no false positive calls for mutations were obtained when testing on healthy human DNA samples (n=143).
Summary/Conclusions: Our results demonstrate that the P520 MPN MLPA assay is a reliable method for simultaneous detection of eight frequent mutations in MPNs, even when the patient DNA sample has a low (1-5%) mutant allele burden. These results merit further consideration of MLPA as a possible alternative for mutation testing for newly diagnosed MPN patients.

PB2014

FUNCTIONAL STUDY OF TWO NEW POINT MUTATIONS OF EPOR GENE IN PRIMARY FAMILIAL CONGENITAL POLYCYTHEMIA

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Background: Primary Familial Congenital Polycythemia (PFCP) is extremely rare. It is caused by mutations in the EPO receptor (*EPOR*) gene (locus 19p13.3-p13.2) resulting in hypersensitivity to erythropoietin EPO stimulus. In PFCP, in spite of reduced levels of serum EPO, mutated *EPOR* is ''switched on'' to stimulate red blood cell production by erythroid progenitor cells but without ''switch off'' mechanism.
Aims: Evaluate the functional effect of two *EPOR* gene novel mutations.
Methods: In our huge cohort of patients with erythrocytosis, we found 2 unrelated patients carrying novel mutations in *EPOR* gene. We performed a functional assay to evaluate the activation of EPOR signaling in these mutations:

first we created mutated plasmids to transfect K562 cell as model. 48h after electroporation, we performed a stimulating assay with EPO following a time course at 0', 5' 15' 30' and 45'. We finally studied the signaling cascade activity with Western Blot analysis.
Results: Both observed mutations were found in exon 8 of *EPOR* gene: c.1013G>A, p.Cys338Tyr and c.1022C>T, p.Thr341Met. The EPOR signaling cascade resulted more active in mutated cells than in WT cells when stimulated with EPO. 5' and 15' after stimulation, transfected cells with C338Y mutation showed higher phosphorylation of STAT5 compared to T341M mutation. We observed also a similar phosphorylation pattern of ERK occurring earlier than STAT5 (at 0' and 5'), while no signal of activity was detected in JAK2 and AKT cascades.
Summary/Conclusions: In this study, we report two new missense mutations of the *EPOR* gene, located in exon 8 as all the 22 previous described functional mutations. Exon 8 encodes the C-terminal negative regulatory domain of the protein. Both mutations here described impair the C-terminal negative regulatory domain of *EPOR* resulting in a gain-of-function in the EPOR signalling cascades, more relevant for C338Y than T341M mutation. These novel mutations, causing hyperactivity, increase proliferation and differentiation, and decrease of apoptosis of erythroid progenitor, sustain the erythrocytotic phenotype of our patients.

PB2015

Abstract withdrawn.

PB2016

Abstract withdrawn.

Myeloproliferative neoplasms - Clinical

PB2017

CLINICAL-BIOLOGICAL PROFILE AND RESPONSE TO IMATINIB IN MYELOID AND LYMPHOID NEOPLASM ASSSOCIATED WITH EOSINOPHILIA AND IDIOPATHIC HYPEREOSINOPHILIC SYMDROME
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Background: Primary eosinophilic diseases are a broad and heterogeneous subgroup of hematological disorders with a very low incidence (SEER database age-adjusted incidence rate 0.036 per 100.000). They have been generally defined as peripheral blood eosinophilia (≥1.5x109/L), tissue infiltration and end-organ damage. According to the OMS-2008 classification, primary eosinophilic diseases are subdivide in a mayor category of myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA/B or FGFR1(MNP-Eo); chronic eosinophilic leukemia not otherwise specified (CEL-NOS), idiopathic hypereosinophilic syndrome (HES) and lymphocytic T variant hypereosinophilia (HES-L).
Aims: The MPN-Eo associated with abnormalities of PDGFRA/B frequently respond to low doses of Imatinib, while is controversial the role of Imatinib in idiopathic HES. In this study we show our series of HES, with the aim of describing the clinical-biological profile and the rate of responses to Imatinib in both subtypes of HES, with and without PDGFRA/B abnormalities.
Methods: We performed a retrospective and descriptive analysis in GAMFIN cooperative group (Andaluz philadelphia-negative chronic myeloproliferative neoplasm group) of diagnosed patients with hypereosinophilic disease (OMS-2008 and HDWG Workshop 2010 classification) and differential diagnostic algorithm of Tefferi *et al.* (Mayo Clin. Proc. 2010; 85:158-164). From 2005 to 2015 clinical and biological profile is analyzed with statistical software IBM SPSS.19
Results: We have analyzed a total of 20 cases of MPN-Eo, with an age-adjusted incidence rate of 0.029/100.000 habitants/year (2014 Andalucía Population Statistic), subdivided according to the OMS-2008 classification of eosinophilic diseases in:7 cases ofMPNs-Eo associated with abnormalities of PDGFRA(6)/PDGFRB(1), 1 case of CEL-NOS(t(8;13)(p12;q12)+21 and FISH FGFR1 positive), 1 HES-L case and 11 cases of idiopathic HES. Our series shows a similar distribution by sex (11M/9F) and a lower age at diagnosis in MPN with PDGFRA/B(41,14 *vs* 54,6; p=0.044). The most frequent clinical presentation was: constitutional syndrome (60%),lung disease (40%), heart disease (35%:71,4% vs18,2%, 0.049; in PDGFRA/B and HES), skin involvement (35%), bowel damage (25%), rhinitis/asthma (20%), adenopathy (10%) and myalgias(5%); 35% of patients with splenomegaly(71,4% *vs* 9,1%, p=0.013; in NMP-PDGFRA/B and Idiopathic HES) (p=0,013). Bone marrow karyotype was normal in all but 2 patients: 1 PDGFR-B (5q33) case and another CEL-NOS(8p). 25% cases were initially treated with Imatinib, and another 16 patients (55%) received it in second line. With a median follow-up of 94,33 months in the 6 cases of PDGFRA, 100% reach early hematological complete response (HCR: 6,20+/-4,60 weeks) and molecular response in 4 evaluable cases by FISH(MCR5+/-4,12 moths), 1 case PDGFRB, after 1 moth of follow-up, reach partial response (PR) and 1 case CEL-NOS(FGFR1+), is treated with low doses of PEG-IFN, but 6 months later progressed to lymphoblastic lymphoma. In the 11 cases of idiopathic HES, with a median follow-up of 28+/-29,16 months, 9 cases received Imatinib and six of them reached CHR (66%), while three patients discontinued the therapy (1: no response, and 2 intolerance). Finally, only three cases of Idiopathic HES treated with hydroxycarbamide reached CHR.
Summary/Conclusions: The incidence rate of HES, gender and age distribution, and clinical-biological profile, in our community of Andalucía, are similar to that previously described in other series. In the majority of patients with MPN-Eo we must tested a therapy with Imatinib, regardless PDGFRA or PDGFRB abnormalities, because a significant subgroups of patients respond, while responses in patients with PDGFRA or PDGFRB abnormalities are more frequents, deepest and fastest. The prognosis and survival depend on the tissue damage, especially cardiac injury, presents at diagnosis in 71,4% of patients with PDGFRA/B abnormalities, frequently irreversible. This suggests a clear recommendation to treat these types of MPN-Eo early, mainly with Imatinib at diagnosis, in order to avoid cardiac damage

PB2018

BASELINE CHARACTERISTICS AND RISK FACTORS FOR LEUKEMIA-FREE AND OVERALL SURVIVAL IN BULGARIAN PATIENTS WITH PRIMARY MYELOFIBROSIS-A NATIONAL COHORT STUDY
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Background: Primary myelofibrosis (MF) is a rare form of BCR-ABL1(-) myeloproliferative neoplasm (MPN). Comprehensive research on the characteristic of this life-threatening condition has not been done in Bulgaria yet. Therefore, the Working Group of MPN of the Bulgarian Society of Hematology initiated a nationwide study.
Aims: To determine the prevalence of MF in the country, and to provide data on the baseline clinical and laboratory parameters and factors for leukemic transformation and overall survival (OS) of a national cohort of patients (pts) with the disease.
Methods: Data on 284 MF pts diagnosed and/or treated in 2014-2015 in 10 clinical centers covering the territory of the country with a population of 5242000>25-yrs of age were analysed. Major demographic, clinical and laboratory parameters, risk profiles according to the international prognostic system (IPSS) and the version for dynamic evaluation of risk (DIPSS and DIPSS-PLUS), and therapeutic approaches were evaluated at baseline as well as in regard to leukemia-free (LFS) and OS in 250 pts.
Results: A male/female ratio of 1.6:1 was determined. The mean age at diagnosis was 64.24 yrs (range 25-86 yrs). In 250 (88%) pts primary MF was diagnosed and in the other 34 (12%) secondary MF developed after another MPN. The most common symptoms were enlarged spleen (78%), anemia (58%), ≥1 constitutional symptoms (41%), thrombocytopenia (21%) and leukocytosis (20%). In 252 (91.2%) pts the diagnosis was confirmed with bone marrow histology and the severity of fibrosis was determined with silver stain in 77% of the cases. Molecular analysis was conducted in 179 (63%) pts and 113 (63.1%) were positive for JAK2 V617F mutation, including as expected 84% of secondary *vs* 60% of primary MF (p=.014). In primary MF, analysis revealed a significant association only between JAK2 mutation and platelet counts <100x10^9/L (p=.018) and transfusion dependence (p=.043). In 20 (7.4%) pts blast transformation was registered regardless of the primary or secondary nature of MF. Univariate analysis of parameters at the time of diagnosis revealed a significant association between inferior LFS and peripheral blood blasts >1% (p<.0001), leukocytosis >20x10^9/L (p<.0001), platelet counts <100x10^9/L (p=.001), hypercatabolic symptoms (p=.01), splenomegaly (p=.01), and treatment history with hydroxyurea (p=.03). Median OS was 6.2 yrs and as expected, it was significantly shorter in pts with leukaemic transformation (3.6 yrs *vs* 7.3 yrs, log rank test p=.002). According to the IPSS, DIPSS and DIPSS-PLUS pts were categorized predominantly in intermediate-high (IHR) and high risk (HR) groups: 68%>79%>87%, respectively. A significant association was confirmed between the OS and risk profiles (p<.0001). In total, 71% of pts required treatment (e.g. hydroxyurea, erythropoetin, androgen, interferon, corticosteroids, ruxolitinib). Splenectomy was performed in 4% of the pts, while RBC transfusions were indicated in 59%. Interestingly, therapy of any kind was significantly associated with better survival in all DIPSS-PLUS risk categories except for HR group who might be the candidates for novel therapies (p=.009 in low risk *vs* p=.002 in IHR groups *vs* p=.127 in HR).
Summary/Conclusions: The study provides, for the first time, nationwide data on the 5.4/100000 prevalence and basic features of Bulgarian pts with MF in regard to clinical and laboratory presentation as well as to prognostic parameters and OS.
Contributions: LG, MG, GM, GB designed the study and contributed equally to this work. MG, YT conducted data analysis. DT, DT, GD, GT, LS, MZ, NP, SD, TD, VG, VS, YP performed research and contributed equally to this work.

PB2019

DISEASE CHARACTERIZATION AND TREATMENT PATTERNS OF PATIENTS WITH MYELOFIBROSIS: ANALYSIS OF US CLAIMS DATABASES

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Background: Myelofibrosis (MF), a rare myeloproliferative neoplasm, is characterized by clonal myeloproliferation, ineffective erythropoiesis, bone marrow stromal changes, hepatosplenic extramedullary hematopoiesis and aberrant cytokine expression. Patients (pts) present with splenomegaly, constitutional symptoms, moderate to severe anemia, thrombocytopenia and leukocytosis. MF has a profound negative impact on survival and quality of life. Presentation may be primary (PMF) or MF transformation from essential thrombocythemia (ET) or polycythemia vera (PV), or may be secondary from diseases such as myelodysplastic syndrome, leukemia, and lymphoma (Other MF). Management options include allogeneic stem cell transplantation, hydroxyurea, interferon alpha, alkylating agents, splenectomy, splenic radiotherapy, and the JAK1/2 inhibitor ruxolitinib.
Aims: Characterize disease and treatment patterns in pts with MF using 2 US health insurance claims databases.
Methods: The Truven Marketscan (Commercial Claims and Encounters and Truven Medicare) database was retrospectively analyzed to identify pts with MF diagnosed between 2006 and 2015 using ICD-9 codes 238.76 and 289.83. Pts aged ≥18 years with ≥1 month of history prior to diagnosis were included. Pts were categorized as PMF, post-PV/ET MF, or Other MF based on earliest MF diagnosis code. Demographic characteristics, constitutional symptoms, platelet counts, and treatment patterns were summarized. Treatment regimens were analyzed according to the following hierarchy (highest to lowest; "± O" referring to combination with any other treatment listed lower in the hierarchy): ruxolitinib±O, hydroxyurea±O, chemotherapy±O, stem cell transplant±O, splenectomy±O, radiation±O, best supportive care (BSC)±O, and steroids only. Sub-analysis of ruxolitinib uptake post launch (in 2011) will be presented.
Results: We identified 6795 pts with MF diagnosis between 2006 and 2015. Based on the exclusion criteria, 6123 pts were included; 3211 (52.4%) were male, and median age at diagnosis was 66 years. A total of 1425 (23.3%), 840 (13.7%), and 3858 (63.0%) pts had PMF, post-PV/ET MF, and Other MF, respectively. Prevalence of splenomegaly at baseline was 11.1% in PMF, 15.5% in post-PV/ET MF, and 7.8% in Other MF. Platelet counts at baseline (± 90 days of index MF diagnosis) were available for 149 (2.4%) pts; mean and median platelet levels were 270,323/μL and 218,000/μL, respectively; 16 (10.7%) pts had platelet counts of <50,000/μL and 116 (77.9%) had platelet counts of >100,000/μL (Table). Overall 3502/6123 pts (57.2%) received ≥1 regimens for MF. Mainstays of initial treatment included hydroxyurea±O (964 treated pts; 27.5%), and BSC±O (753; 21.5%). Only 425/3502 treated pts (12.1%) received ruxolitinib±O as first-line treatment; by category, pts receiving first-line ruxolitinib±O included 194/1390 treated pts (14.0%) with PMF or post-PV/ET MF and 231/2112 treated pts (10.9%) with Other MF. Hydroxyurea±O was the most common first-line regimen for pts with PMF or post-PV/ET MF (463/1390; 33.3%); BSC±O (517/2112; 24.5%) and steroids (615/2112; 29.1%) were the most common first-line regimens for pts with Other MF.

Table 1.

Table. Demographics, baseline characteristics, and treatment of pts with different types of MF at presentation

	Pts		
	PMF n = 1425	**Post-PV/ET MF n = 840**	**Other MF n = 3858**
Male, n %	829 (58.2)	401 (47.7)	1981 (51.4)
Age at diagnosis, median years (range)	67 (20-102)	65 (24-97)	66 (18-103)
Pts with splenomegaly within 1 year before and 90 days after MF diagnosis, n (%)	159 (11.1)	130 (15.5)	299 (7.8)
	PMF or Post-PV/ET MF n = 2265		**Other MF n = 3858**
Pts receiving treatment for MF, n (%)	1390 (61.4)		2121 (55.0)
Pts receiving ruxolitinib ± O as first-line treatment for MF, n/n pts receiving treatment (%)	194/1390 (14.0)		239/2121 (11.3)
Pts with baseline platelet counts, n (%)	**PMF n = 26**	**Post-PV/ET MF n = 15**	**Other MF n = 108**
Platelet count /μL within 90 days of diagnosis			
< 50,000	3 (11.5)	2 (13.3)	11 (10.2)
50,000-75,000	1 (3.8)	1 (6.7)	7 (6.5)
75,000-100,000	0	1 (6.7)	7 (6.5)
> 100,000	22 (84.6)	11 (73.3)	83 (76.9)

Summary/Conclusions: MF was typically diagnosed in pts aged ≥65 years, and was associated with splenomegaly or thrombocytopenia at baseline in a minority of pts. In the present database analysis, ~55-60% pts received treatment. Although ruxolitinib was approved in the US in 2011 for treatment of high- or intermediate-risk PMF or post-PV/ET MF, there is limited information in the database on its clinical use as a first-line treatment.

PB2020

RISK FACTORS AND OUTCOMES OF ASIAN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS (MPNS) IN LEUKAEMIC TRANSFORMATION

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Background: Progression to acute leukaemia is a rare event in the natural his-

tory of the MPNs, namely essential thrombocythaemia (ET), polycythaemia vera (PV) and myelofibrosis. Prognosis in MPN patients with leukaemia transformation (LT) is almost universally fatal and no effective treatment is available.
Aims: As data on Asian patients with MPN in LT is lacking, we evaluated the risk factors and outcomes of Asian MPN patients with LT in this retrospective single centre analysis.
Methods: We reviewed the case records of 824 Asian patients with MPNs from our institution's IRB approved MPN registry. Patients with LT were compared with other patients in the registry who did not have LT on follow up.
Results: Among 824 MPN patients, 30 were identified to have LT from 2002 to 2015. The median follow up was 6.2years. Median age of LT was 72years and occurred at a median of 33.2years after diagnosis. 3.9% (n=22) of ET, 0.5% (n=1) of PV and 11.5% (n=7) of myelofibrosis patients progressed to acute leukaemia (p <0.05). Time to LT was longest for ET (11years), followed by myelofibrosis (3.9years) and PV (1.5years) (p=0.018). There was no impact of JAK2, Calreticulin and MPL mutations on LT (p=0.55). Patients with LT had lower Hb at diagnosis (12.7g/dL *vs* 14.8g/dL, p=0.001). A trend to higher presenting leukocyte count (15.5x 10^9/L *vs* 12.6x10^9/L, p=0.053) and older age of diagnosis of MPN (59.5years *vs* 56years, p=0.061) was seen in patients with LT (p=0.053). On log-rank test, MPN subtype (p<0.05), presenting Hb <10g/dL (p<0.05), presenting WBC ≥13x10^9/L (p=0.009), presenting platelet count ≥1000 x10^9/L (p=0.045) and age at diagnosis of MPN ≥60years (p=0.001) were factors for shorter time to LT. Use of hydroxyurea (p=0.17) and exposure to more than one cytoreductive agent (p=0.45) had no impact on LT. Using all covariates with a p value ≤0.10, we developed a model using Cox proportional hazard model to determine the relative effect of these variables on LT. The model included the above variables and presence of splenomegaly at diagnosis (p=0.071). A presenting leukocyte count ≥13x10^9/L (HR 5.73, 95% Confidence Interval [CI] 1.84-17.88, p=0.03) and presenting Hb <10g/dL (HR 8.05, 95%CI 1.66-38.95, p=0.01) were independent poor prognostic factors for LT. At LT, 43.3% (n=13) had complex/ adverse cytogenetics. 30% (n=9) had clonal progression compared to karyotype analysis at diagnosis (seven did not have prior cytogenetics results). Median survival after LT was 2.5months. A diagnosis of LT after 2009 (when azacytidine became available at our institution) was not shown to affect survival after LT (p=0.73). Outcomes were not different whether LT occurred below or above the age of 60years (p=0.73). One patient (3.3%) was lost to follow up. 63.3% (n=19) received supportive treatment including palliative chemotherapy. 16.7% (n=5) received azacytidine-based chemotherapy, while 13.3% (n=4) received an AML-type regimen/ allogeneic stem cell transplant and one (3.3%) received experimental treatment. Survival for the different treatment modalities was not statistically significant (p=0.19): palliative chemotherapy/ supportive care 1.5months, azacytidine-based 3.1months, intensive chemotherapy/ allogeneic stem cell transplant 8.1months.
Summary/Conclusions: As in published literature, outcomes for Asian MPN patients in blast phase are dismal. In this study, a presenting Hb <10g/dL and presenting WBC ≥13x10^9/L were predictors of LT in MPN. Existing treatment modalities are ineffective in attaining long term remission and have not improved survival over time.

PB2021

SAFETY AND EFFICACY OF RUXOLITINIB IN CLINICAL PRACTICE FOR PRIMARY AND SECONDARY MYELOFIBROSIS

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Background: Ruxolitinib, a JAK1 and JAK2 inhibitor, has been tested and approved for the treatment of primary and secondary myelofibrosis (MF). Reduction of spleen volume, improvement of constitutional symptoms and improved quality of life have been reported as major findings in sponsored randomized clinical trials.
Aims: Aim of our study is to report safety and efficacy of ruxolitinib in 98 patients affected by MF treated outside clinical trials.
Methods: Patients were collected and treated consecutively by the Lazio Cooperative Group for Ph negative myeloproliferative diseases.
Results: There were 45 males and 53 females, median age was 61.8 years (range 35.3-88). Forty-five patients were diagnosed as primary MF and 53 as evolution from a previous MPN: 31 after Polycythemia Vera, 20 after Essential Thrombocythemia and 2 were not specified. Median splenomegaly at baseline was 6 cm. At baseline, IPSS stratification revealed 4 patients with low risk, 27 with intermediate-1 risk, 42 as intermediate-2 and 23 as high risk. Seventy-seven patients (78.5%) experienced constitutional symptoms at baseline and out of 80 patients tested, 58 (72%) were $JAK2^{V617F}$ mutated. Overall, 40 patients received hydroxyurea as firstline treatment, 30 patients received other chemotherapeutic approaches, whereas 28 were treated with ruxolitinib frontline. Median time from diagnosis to start of ruxolitinib in the whole cohort was 34.6 months. All patients at the time of treatment were intermediate-2/high risk according to IPSS. Hematological parameters pre-treatment were: median haemoglobin level 10.4 gr/dl, median WBC count 11x10^9/l, median platelet count 239x10^9/l. Median splenomegaly pre-treatment was 10 cm. As regards initial daily dose, according to platelet count, 5 patients started with 5 mg BID, 7 patients with 10 mg BID, 26 patients with 15 mg BID and 60 patients with 20 mg BID, with a median initial daily dose of 20 mg BID. Fifty-eight patients (59%) required a dose modification during the first 3 months, which consisted in a dose reduction due to haematological toxicity in the majority of cases. After 24 weeks of treatment, 48% of patients experienced a clinical benefit with some degree of reduction in spleen volume: in particular, according to revised IWG-MRT criteria, 5 patients (5%) achieved a complete response (CR), 8 patients (8%) a partial response, 6 (6%) a clinical improvement (CI), 28 (28.5%) a spleen response, whereas 25 patients did not achieve any response and 24 were not evaluable. At 48 weeks, 52% of patients obtained a clinical benefit: of them 7 patients (7%) had a CR, 10 (10%) a PR, 6 patients (6%) a CI and 28 patients (28.5%) a spleen response. Overall, 66% of patients had disappearance of baseline symptoms burden. After 1 year, of 72 evaluable patients, 52% achieved and maintained a clinical benefit. Adverse events of special interest at any grade included anemia (39.7%), thrombocytopenia (25.5%), infections (16.3%, of which 10 were bronchopneumonia), fluid retention (3%), diarrhea (2%) and abdominal pain (2%). After a median follow-up of 16 months from start of ruxolitinib, median daily dose decreased to 10 mg BID and 21 patients (21%) discontinued the drug (8 for toxicity, 4 for progression, 4 lack of efficacy, 1 patient underwent BMT, 1 second neoplasia, 3 deaths).
Summary/Conclusions: The results of this retrospective multicentric analysis confirmed the efficacy of ruxolitinib outside clinical trials with more than half of treated patients achieving and maintaining a clinical benefit and most of them reporting relief from symptoms.

PB2022

RESULTS IN ELDERLY CHRONIC MYELOMONOCYTIC LEUKEMIA PATIENTS TREATED WITH 5-AZACYTIDINE

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Background: Two hypomethylating (HMT) agents,5-azacytidine (AZA)and decitabine have been approved by the FDA for the treatment of CMML. Initial data on HMT efficacy came from studies in patients with high-risk myelodysplastic syndrome (MDS), including, however, only a few cases of CMML. Hypomethylating agents are associated with overall response rates of approximately 30% to 40%, with complete remission rates of about 15%.
Aims: Evaluate efficacy and security of AZA in CMML patients.
Methods: we retrospectively collected clinical data of 8 patients (7 males/1 females) with a median age of 80 years (range72-86) diagnosed with CMML and treated with AZA, in our institution between 2010-2015.
Results: 5 (63%) patients were diagnosed as CMML-1 and 3 (37%) patients as having CMML-2 subtype. AZA was administered at 75mg/m2 daily for 7 days, every four weeks. Response was assessed after median number of 6 cycles. Our patient cohort received a median number of 17 cycles (3-30) of therapy. Overall survival was 42 months (25-74 months). Progression free survival was 23 months (4-28 months). The median time to first response was 4 months. Causes of death were: 3 patients (38%) did evolution to AML (median time to progression 3 months). 3 patients had a sepsis. 1 patient had a lung cancer.
Summary/Conclusions: AZA in elderly CMML patients is efficacy and safety. The response were quickly and cytopenias were controlled.

PB2023

ONLINE STREAMING YOGA IS A FEASIBLE NON-PHARMACOLOGIC MANAGEMENT STRATEGY IN MYELOPROLIFERATIVE NEOPLASM PATIENTS

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Background: Up to 84% of Myeloproliferative Neoplasm (MPN) patients report a reduced overall quality of life (QoL). While MPN patients often experience an array of disease and treatment-related symptoms, fatigue is the most frequently reported symptom (93%). Yoga may be an effective approach to improving symptom burden (i.e., fatigue) and QoL in MPN patients based on evidence suggesting yoga may improve fatigue and QoL in other cancer types. (e.g., breast, endometrial).
Aims: To examine the feasibility (i.e., acceptability, demand, practicality) of a home-based, online-streamed yoga intervention in MPN patients. A secondary aim was to examine the effects of yoga on self-reported symptom burden and QoL outcomes.
Methods: MPN patients were recruited nationally using social media. Participants were asked to complete 60 minutes of online-streamed yoga per week

and to complete online self-report surveys administered via Qualtrics (demographics, symptom burden (MPN-SAF TSS), and QoL (i.e., pain, anxiety, depression, sleep, sexual function via PROMIS). Surveys were administered at baseline (wk 1), mid-point (wk 7), and post-intervention (wk 12). Weekly yoga minutes were self- reported and collected weekly with adherence defined as achieving ≥60 min/wk.
Results: *Patients:* Two hundred and forty-four MPN patients completed the eligibility survey, 134 were eligible, 55 completed the informed consent and 38 participated in the 12-wk study. The most common reasons for ineligibility were a diagnosed mental illness (n=55) and already regularly performing mindful activity (n=46). Thirty patients had completed the intervention at the time of this analysis. Among those (N=30; mean age=55.8±9.2 years; mean BMI=25.0±4.4 kg/m^2;·86.7% female), polycythemia vera was the most prevalent diagnosis (43.4%), followed by essential thrombocythemia (36.6%) and myelofibrosis (20.0%). The majority of participants were diagnosed >3 years ago (63.3%) and were JAK*V617F* positive (70%). Baseline measures were as follows: MPN-SAF TSS mean=34.6±14.0; Fatigue mean=6.6±2.5; Anxiety mean=51.9±7.0; Depression mean=47.5±7.6; Sleep mean=49.7±6.8. *Yoga Participation & Safety:* Just over 43% (13/30) of study participants averaged ≥60 min/wk of yoga. Overall yoga participation averaged 59.3±32.0 min/wk. Overall, 68% of participants were either satisfied (32%) or very satisfied (36%) with online yoga and 75% of participants agreed (46%) or strongly agreed (29%) that they felt safe from injury while practicing. Only one adverse event was reported (irritated enlarged spleen). *Impact of Yoga Intervention:* From baseline to post-intervention, there were significant improvements in self-reported symptom burden (MPN-SAF TSS mean reduction of 4.77±8.29; p=0.004), fatigue (MPN-SAF TSS mean reduction of 0.83±2.15; p=0.003), anxiety (PROMIS mean reduction of 5.02±7.97; p=0.002), depression (PROMIS mean reduction of 2.89±7.73; p=0.049), and sleep (PROMIS mean reduction of 3.76±3.60; p<0.001). When examining the differences between those that averaged <60 min/wk compared to those that averaged ≥60 min/wk, there were no significant differences in outcomes.
Summary/Conclusions: A 12-wk, home-based, online-streamed yoga intervention is feasible for MPN patients. Although the sample size of this study was small, this study suggests that online yoga may be effective for improving MPN related symptoms and self-reported QoL outcomes (i.e., anxiety, depression, sleep). Interestingly, outcomes were not significantly different between those that averaged <60 min/wk of yoga compared to those that averaged ≥60 min/wk of yoga. Future research should explore the minimal dose needed for improved symptom burden and QoL in MPN patients. Additionally, a randomized controlled trial with an active control group will help to determine the effectiveness of yoga for self-reported patient outcomes.

PB2024

ASCITES A MANIFESTATION OF AGGRESSIVE SYSTEMIC MASTOCYTOSIS CAN SUCCESSFULLY BE TREATED WITH MIDOSTAURIN (PKC412)
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Background: Systemic mastocytosis (SM) is a myeloproliferative neoplasia caused by uncontrolled proliferation and accumulation of pathological mast cells in one or more extracutaneous organs. The 2008 WHO classification divides SM into several subtypes including aggressive SM (ASM) with at least one "C finding." Among the four different "C findings" is palpable hepatomegaly with impairment of liver function, ascites and/or portal hypertension. Midostaurin (PKC412) is a new and so far not approved drug shown to have a strong inhibitory activity on neoplastic human mast cells carrying the D816V *KIT* mutation and with an acceptable side-effect profile.
Aims: The primary objective was to assess the clinical effect of PCK412 on ascites in patients with ASM. The secondary objective was to study the effects of PCK412 on other symptoms and findings in ASM patients.
Methods: All our patients with ASM and ascites were receiving midostaurin on a "compassionate use program" provided by Novartis. The 4 patients were ≥18 years and had a verified ASM according to the 2008 WHO classification. Liver biopsies, demonstrating mast cell aggregates, were performed in two of the patients. Portal hypertension was verified by abdominal ultrasound. All the patients had ascites and carried the D816V *KIT* mutation. The effect of PKC412 on ascites was evaluated by means of clinical examination, abdominal computed tomography and/or abdominal ultrasound.
Results: In all of the patients, PKC412 had a significant clinical effect on ascites. From having a need of pleuracentesis weekly or every second week, all of they became independent of pleuracentesis. Their quality of life enhanced dramatically. The patient with the most frequent need of pleuracentesis nearly died by peritonitis probably associated to all his interventions. The initial dosage of midostaurin was 100 mg twice daily. Two of the patients, further confirmed the efficacy of midostaurin by revealing relapse of ascites after having stopped with midostaurin because of sickness. In both cases the production of ascites ceased, and the need of pleuracentesis ended by reintroducing PKC412.
Summary/Conclusions: In all these patients with ASM, midostaurin had an excellent effect on ascites. Although this material is very small, all 4 out of 4 patients with ASM and ascites responded and became independent of paracentesis. The correlation between relapse of ascites when stopping midostaurin and disappearing when the medication was reintroduced, strongly supports a positive effect on ascites, which is a clinical finding that troubles many patients with ASM. It became nearly fatal for one patient in our material. In order to validate a significant effect of PCK412 on ascites in patients with ASM, studies on a larger population is needed.

PB2025

QUALITY OF LIFE AND SYMPTOM BURDEN IN MYELOFIBROSIS PATIENTS: A REAL WORLD STUDY
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Background: Myelofibrosis (MF) is associated with poor prognosis, significant symptom burden, and worsened quality of life (QoL). Assessment of patient-reported outcomes is an effective way to identify patients' risks/benefits of MF treatment.
Aims: We aimed to study QoL and symptom burden in MF pts in a real world setting as well as to make comparisons in the groups depending on treatment modality.
Methods: A total 93 pts with MF were enrolled in the multicenter real world QoL study-64 (69%) with primary MF, 14 (15%) post-essential thrombocytopenia MF, 15 (16%) post-polycythemia Vera MF. Median age-59 years (range 28–90); male/female-37/56. All pts received the best available treatment (BAT, n=62) or ruxolitinib (n=31) for at least 6 months (median 12 months, range 6–238). High proportion of pts (79%) had IPSS intermediate or high risk. All pts completed the SF-36 QoL questionnaire, symptom assessment questionnaire CSP-MF and Patient Global Impression of Change (PGIC) tool; physicians collected information about disease and treatment from medical records. Integral QoL Index was calculated on the basis of SF-36 QoL questionnaire. For statistical analysis ANOVA, multiple regression and $\chi 2$ test were applied.
Results: 23 pts (24.7%) from 93 pts were full spleen reduction responders, 31 pts (33.3%)-moderate responders, 39 pts (42%)-non-responders. Number of full responders was higher in pts receiving ruxolitinib as compared to BAT (48.4% *vs* 12.9%, p<0.0001). QoL in all SF-36 scales was significantly worse in pts with MF comparing to healthy controls; the most pronounced impairment was observed in role physical functioning (39.8 *vs* 76.9), physical functioning (58.1 *vs* 85.3) and role emotional functioning (52 *vs* 77.1); Integral QoL Index in pts was lower than in healthy controls-0.3 *vs* 0.55 (p<0.0001). More than one third of pts had significant or severe QoL impairment: Integral QoL Index was 7 times less as compared to healthy controls. Pts receiving BAT showed more pronounced QoL impairment as compared to pts receiving ruxolitinib: they had worse physical functioning (51.3 *vs* 74.7), role physical and role emotional functioning (32.2 *vs* 61.3, 43 *vs* 75.3), general health (40.9 *vs* 53.6), vitality (43.9 *vs* 60.2), social functioning (58.4 *vs* 81), and mental health (52.1 *vs* 67), p<0.05; Integral QoL Index was significantly lower-0.24 *vs* 0.47 (p<0.0001). Positive changes in health condition measured by PGIC were reported in 67% pts receiving ruxolitinib *vs* 40% patients receiving BAT (p=0.01). The majority of pts (74%) exhibited at least one moderate-to-severe symptom; the most prevalent among symptoms were fatigue, inactivity, pain in bones/muscles, insomnia, dizziness and feeling of worry. These symptoms were more pronounced in pts on BAT comparing to pts on ruxolitinib (p<0.05). Inactivity appeared to have the most significant negative impact on QoL; multiple linear regression model indicated significant relationship between symptom severity and Integral QoL Index (R-squared=0.65, p<0.001) with the highest regression coefficient for inactivity (Beta-coefficient=0.6).
Summary/Conclusions: Patient-reported outcomes such as QoL and symptom burden in MF pts during treatment in a real world setting may be a solid supplement to clinical outcomes and may contribute to better disease control and improved quality of care of this difficult patient population. The data of this real world study demonstrate benefits of ruxolitinib therapy from patient perspective and support the results of clinical studies.

PB2026

BFIBRINOGEN G-455-A GENE POLYMORPHISM: IS IT A FACTOR FOR ASPIRIN PLATELET INSENSIVITY IN PATIENTS WITH POLYCYTHEMIA VERA?
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Background: Polycythemia vera (PV) is a myeloid neoplasm characterized by platelet activation and thrombotic risk. According to PVSG, the antithrombotic therapy includes low dose ASA. However, there are insensivity ASA PV patients. It is debated if inherited thrombophilia increases the polycythemic platelet activation and, hence, the ASA platelet insensivity.
Aims: Therefore, we evaluated βFibrinogen G-455-A gene polymorphism, as thrombophilic molecular mutation associated with increased platelet aggregation, platelet count, hematocrit (HCT), β-thromboglobulin (β-TG) and platelet factor 4 (PF4) as markers of platelet activation, fibrinogen (Fg), Platelet functional activity (PFA) as indicator of ASA platelet sensitivity and clot formation time (CFT), as indicator of aspirinated platelets contribution to clot firmness. We studied 40 patients (28 men, 12 women; mean age 64 years, range 35_85 years) with PV according to WHO criteria. Fifty subjects served as controls.
Methods: The mean duration of disease was 9 years. All patients were on phlebotomy and ASA (100 mg once day). The βFibrinogen G-455-A genotype was determined using a commercialized polymerase chain reaction kit with sequence-specific primers. Platelets and HCT were measured by automated analyzer. βTG and PF4 were determined by ELISA. PFA and CFT were measured by Platelet Function Analyzer (PFA-100) and by ROTEM delta, respectively.
Results: All patients had heterozygous βFibrinogen G-455-A. The mean platelet and mean HCT value were 428±180 x109/L and 47±3%. All patients had normal Fg (244±47mg/dl), high β-TG and PF4 (133±47 IU/ml *vs* 20±11 IU(ml and 45±21 IU/ml *vs* 6±2 IU7ml, respectively) (p<.0001 and p<.001, respectively), prolonged C/EPI closure time (CT, units: s, n.v. 84-160 s) (252±48 s) and normal CFT (CFT, units: s, n.v. 30-110 s) (50±7 s).
Summary/Conclusions: These findings suggest that βFibrinogen G-455-A gene polymorphism is not associated with ASA platelet insensitivity in patients with polycythemia vera.

PB2027

ESSENTIAL THROMBOCYTHEMIA WITH JAK2 V617F OR CALR MUTATION: TWO DIFFERENT PHENOTYPES

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Background: The JAK2 V617F mutation is found in 50-60% of cases of essential thrombocythemia while 30% of cases carry calreticulin gene (CALR) and 5% MPL substitution. Recent studies have analyzed the clinical and hematologic features in essential thrombocythemia and have demonstrated the phenotypically differences between JAK2 V617F and CALR mutations.
Aims: We analyzed a Spanish cohort of 101 cases of essential thrombocythemia with JAK2 V617F or CALR mutations. We studied the hematologic and clinical features, including the thrombotic and hemorrhagic events and we compared both groups in order to study different phenotypes in essential thrombocythemia.
Methods: We retrospectively analyzed a cohort of 101 cases of essential thrombocythemia diagnosed between 1990 and 2015. We examined the frequency of JAK2 V617F and CALR mutations, the clinical (Age, gender, thrombotic and hemorrhagic events) and hematologic features (platelets, leukocytes and hemoglobin counts) and the overall survival in these patients. The mutation load of JAK2 V617F was determinated with real-time quantitative PCR. For the detection of CALR exon 9 mutations, fragment analysis have been used. Statistical analyses were performed with SPSS software.

Table 1.

	JAK2 mutated	CALR mutated	p
Age at diagnosis, years	63 (range 24-90)	52 (range 28-83)	**0,007**
Gender (Male)	40%	60%	
Hemoglobin, (g/dL, mean ± SD)	14,3 ± 1,6	13,8 ± 1,4	0,227
Leukocytes (10^9/L, mean ± SD)	9,7 ± 3,5	9,4 ± 2,7	0,721
Platelets (10^9/L, mean ± SD)	707,794 ± 256,3	1.006,950 ± 482,2	**0,001**
Hematocrit (Percentage, mean ± SD)	42,4 ± 4,4	42,6 ± 3,1	0,914
Cardiovascular risk factors			
- Hypertension	45%	55%	0.044
- Mellitus Diabetes	85%	15%	1
- Dyslipidemia	69%	31%	0.084
- Obesity	92%	8%	0.712
- Smoking	67%	33%	0.832
Thrombosis	68,3%	31,7%	**0.020**
Bleeding	10%	90%	**0.012**
Antiaggregation	54,5%	45,5%	0.343

Results: In our study we identified 80% with JAK2 V617F mutation and 20% with CALR mutation (type 1, 70%; type 2, 25%; and others, 5%). The mean age of patients with CALR mutation was 52 years (range 28-83) and JAK2 patients was 63 years (range 24-90). The CALR group had more males (60%) compared to JAK2 group (40%) and were younger at diagnosis (P=0,007). The CALR group had higher platelet counts (1.006,950±482,2 x10^9/L *versus* 707,794±256,3L; p=0,001), lower hemoglobin concentration and lower leukocyte counts, not being statistically significant. Venous and arterial thrombosis were more frequent in JAK2 patients (30%; p=0,030) who had also more cardiovascular risk factors. However, thrombosis in these patients was not related to the platelet counts. Hemorrhages were more frequent in CALR patients (90%; p=0.012) and strongly associated with higher platelet counts (p=0.001) and less with low hemoglobin levels (p=0.227). We also observed that the two groups have similar overall survival.
Summary/Conclusions: In our study, we have observed that patients with CALR mutation were younger and they had higher platelet counts compared with JAK2 mutation group. CALR mutation patients showed a higher risk of developing hemorrhagic events while JAK2 mutation-positive showed a higher risk of venous thrombosis. Thrombosis events were also related to higher leukocyte counts and the presence of cardiovascular risk factors, which were more frequent in the JAK2V617F group. However, we did not find differences in overall survival between both groups. In conclusion, this study confirms that essential thrombocythemia with CALR or JAK2 mutations have biological and phenotypical differences. Therefore, it might be useful for a better understanding of the prognosis and treatment in this disease.

PB2028

CLINICAL IMPORTANCE OF B2 GLYCOPROTEIN I ANTIBODIES IN BCR-ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Arterial and venous thrombotic events are observed to be higher in BCR-ABL negative Myeloproliferative Neoplasms (MPN) than the normal population. These complications are most important causes of morbidity and mortality. β_2 glycoprotein I antibody (β_2-GPI Ab) (anti-apolipoprotein H Antibodies) is an important marker of thrombosis in autoimmune diseases.
Aims: In our study, we aimed to evaluate the association of thrombosis and levels of β_2-GPI Ab in BCR-ABL negative MPN patients without no genetic predisposition to thrombosis.
Methods: Seventy one patients with BCR-ABL negative MPN and 62 controls were included. Exclusion criteria were present of cardiovascular risk factors, malignancy, active infection, renal failure, and being smaller than 18 years old. Genetic and laboratory testing (such as Factor V Leiden, prothrombin G20210A, activated protein C resistance and homocysteine) for thrombophilia were done in all patients and controls in order to evaluate accurately association between thrombosis and β_2-GPI Ab.
Results: Average level of β_2-GP Ab was 187 U/ml in all 133 persons (patient and control groups). Average level of β_2-GP Ab was 217 U/mL in 71 patients with BCR-ABL negative MPN and 160 U/mL in 62 controls. This difference was statistically significant (p=0.006). Subgroup analysis in patients are seen in Table 1. There was no difference between patients with or without history of thrombosis according to the β_2-GPI Ab levels (p=0.144).

Table 1. Relationship of β2-GPI Ab Level and History of Thrombosis in Patient Group.

		β2 Glycoprotein I Ab (U/mL) **		
History of Thrombosis	Number (%)	Median	Min-Max	p *
Present	27 (38 %)	187	15-696	0.144
Absent	44 (62 %)	242.5	34-763	
Total	71 (%100)	217.0	15-763	

Summary/Conclusions: Despite the known increased risk of thrombosis and hemorrhage in MPNs, mechanisms are still not fully clear. To our knowledge, our study is the first study evaluating the relationship between β_2-GPI Ab and thrombosis in BCR-ABL negative MPNs after genetic risk factors excluded. While β_2-GPI Ab level was significantly higher in patients with MPN than in normal subjects; no significant difference was determined between patients with or without thrombosis. Alongside β_2-GPI Ab, assessment of antibodies against domain IV-V of β_2-GP may be useful.

PB2029

NEW PROPOSED WHO CRITERIA FOR THE PV DIAGNOSIS: A CLINICAL OVERLOAD PROBLEM?

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Background: There are controversies regarding on which cell parameter may be more conveniently used as a surrogate criteria of an increased red cell mass (RCM), that is, hemoglobin (HB) level and hematocrit (HCT) value. According to the 2007/2008 WHO classification, HB level should be >18.5 g/dl in men and >16.5 g/dl in women and/or increased HCT. It has been argued that application of these criteria may result in an underdiagnosis of PV by excluding patients with actual RCM mass that is 25% above mean predicted value, but whose HB and HCT levels are below the WHO guidelines. Therefore, recently the WHO revised the criteria and in 2015 made a new proposal lowering the HB/HCT threshold to 16.5 g/dl/49% in men and 16 g/dl/48% in women.
Aims: We aimed to apply the proposed WHO 2015 criteria in order to determine whether this contribution in the identification of masked PV would compensate the work overload in our laboratory and clinical practise.
Methods: We selected samples of patients from routine analytical test that meet the new proposed WHO criteria from 22/12/2015 to 23/01/2016 and studied the presence of JAK2V617F mutation as mayor diagnosis criteria for PV. JAK2V617F mutation was determined qualitatively by amplification refractory mutation system polymerase chain reaction assay. We collected white blood count, erytrocytes and platelets levels as well. For statistical analysis has been used ShapiroWilk test to check the normality of the data of quantitative variables. The statistical program used was R Core Team (2014).
Results: In our study, there were 48 patients: 47 men and 1 woman. The median age of the patients was 48,5 years (range: 17-73). The median of HB was 17.7 g/dL (16.5-18.3) and the median HCT was 51.35% (48.1-55.4%). The number of positive JAK2V617F mutation was cero. The rest of analytical characters were: Erythrocytes (average 5.44 10e6/uL, 1.36-6.52), leykocytes (average 9.09 10e3/uL, 3.87-19.8) and Platelets (average 259.02 10e3/uL, 134-416).
Summary/Conclusions: Amont the 48 patients, none of them vas positive for JAKV617F mutation. Admission of new criteria would means a high increase in the numbre of patients to be evaluated, implying an enhane human and laboratory resource consumption, with a significant economic impact. Based on our results, we wonder whether these new proposed WHO criteria should be approved.

PB2030

PREVALENCE OF THE JAK2 V617F MUTATION IN SOME COHORTS OF THE CENTRAL SIBERIA (KRASNOYARSK REGION) POPULATION

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Background: Somatic mutation of the JAK2 V617F is associated with the pathogenesis of myeloproliferative neoplasms (MPNs) and it is an important diagnostic marker. However, V617F JAK2 was detected also at 0.2-1% of the adult population, without the MPN when using highly sensitive allelic load test (Xu X, et.al., 2007; Nielsen C., et.al., 2014). The V617F JAK2 mutation significantly increases the risk of both arterial and venous thrombosis, including cerebral vessels, visceral intestinal veins and especially Budd-Chiari syndrome (Smalberg JH, et.al., 2012). These causes of hospitalization may be the first manifestations of MPN.
Aims: Evaluate the frequency of the JAK2 V617F mutation in different cohorts of hospital patients and blood donors.
Methods: Allele-specific RT PCR was performed to detect of the JAK2 V617F allele load in whole blood samples among the following groups: healthy blood donors, patients who were included in the program of routine inspections, patients who were are hospitalized in general hospitals, as well as those who were directed by hematologist with suspected to MPN.

Table 1. JAK2 V617F mutation frequency among cohorts.

Cohort	Total surveyed	JAK2-V617F positive patients, n (%)	JAK2-V617F allele load, (%) (Me (Min-Max))	Age (Me (Min-Max))
Blood donors	1149	8 (0.7)	0.47 (0.07-2.58)	39 (18-67)
In baseline medical examination	1515	17 (1.12)	0.26 (0.05-0.18)	53 (45-80)
From not hematological hospital departments	1290	24 (1.86)	0.84 (0.04-48.8)	57 (16-90)
Directed by hematologist	903	301 (33.3)	32.0 (0.06-97.0)	54.0 (16-100)

Results: The frequency of the JAK2 V617F mutation was maximal when the patients were directed by a hematologist with MPN suspected (Table 1). Minimum prevalence was observed in healthy blood donors. Among patients from non-hematological hospital departments 12% cases (3 of 24 patients) with JAK2-V617F had ischemic stroke. Participation in voluntarily medical examination reveals patients with mutation but who have no any hematological abnormalities in 95% cases were directed by hematologist with suspected to MPN.
Summary/Conclusions: High risk of thrombosis and of the MPN development, as well as the potential risk of transmission of the transformed cell clone to recipients of the bone marrow and blood, raises the issue of screening for JAK2 V617F among some cohorts of patients. An analysis of "benefit - harm", taking into account the effectiveness of preventive measures will be the subject of additional studies.

PB2031

CLINICAL FEATURES OF LATENT/MASKED POLYCYTHEMIA VERA (SINGLE CENTER EXPERIENCE)

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Background: Polycythemia vera is a heterogeneous group of diseases. In patients who don't meet the World Health Organization (WHO) criteria for overt polycythaemia vera (PV), a diagnosis of latent/masked PV (mPV) can be determined. MPV is characterized by JAK2V617F positive, morphological features of bone marrow for PV according to WHO, but hemoglobin <18,5 g/dL in men and <16,5 g/dL in women.
Aims: The aim of this study was to identify clinical features of mPV as a separate group of PV.
Methods: The study included 81 patients observed in the outpatient department of National Research Center for Hematology from 2014 to 2015, 50 patients with PV and 31 patients with mPV.
Results: Distribution of patients by gender was statistically comparable. Patients with PV was older compared to mPV: median age was 56 and 44. Between the groups of patients with mPV and PV obvious difference in red blood cells ($5.37x10^{12}$/L ($4.1-6.5x10^{12}$/L) *vs* $6.94x10^{12}$/L ($5.4-8.8x10^{12}$/L)); hemoglobin (14,8 g/dL (10,0-16,7 g/dL) *vs* 17,8 g/dL (13,6-24,7 g/dL)); hematocrit (45% (30-52%) *vs* 53% (42-70%)). Median platelet counts higher in the group of patients with mPV compared with PV: the median was $644x10^9$/L ($179-1978x10^9$/L) *vs* $636x10^9$/L ($137-2437x10^9$/L). Differences of white blood cells was not reveale in the two groups. All the patients were V617F JAK2 positive. Determination of allele burden JAK2V617F performed 29 patients with mPV and 37 patients with PV. JAK2 allele burden was significantly higher in patients with PV compared to mPV: median 14% (3-57%) and 55.5% (24-86%) respectively. Thrombosis revealed in 38% (12 patients) with mPV and in 16% (10 cases) in the PV. It was mainly venous in the case of mPV with a high frequency of splanchnic vein thrombosis. Arterial thrombosis detected only in 4 cases.
Summary/Conclusions: Masked PV is a separate nosological variant of PV.

PB2032

CLINICAL FEATURES AND MOLECULAR MARKERS IN ESSENTIAL THROMBOCYTHEMIA

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Background: Particular molecular marker detection (*JAK2 V617F, MPL, CALR*) or its absence (triple-negative (TN)) in essential thrombocythemia (ET) can be served as a basis of different biological neoplasm behavior.
Aims: The aim of this study was to investigate interactions (differences) between the presence of each molecular marker, clinical features and course of ET.
Methods: One hundred and fifty ET patients, who had been diagnosed at our institution according to WHO 2008 criteria. The following parameters were assessed: age, gender, complete blood count, clinical symptoms (weakness, headache, dizziness, arthralgia, constitutional symptoms, erythromelalgia, splenomegaly), thrombotic complications and bleeding. Overall survival (OS) in ET patients with different molecular markers were analyzed by Kaplan-Meyer method and compared between groups. Log-rank, ANOVA Kruskal-Wallis test and the Chi-square test with Yate's contingency correction were used for statistical analysis.
Results: Median age was 56 years (range 19-82), 110 patients (73%) was female. Median follow-up was 28 months (range 1-168). The following mutations were detected: *JAK2V617F (JAK2+)* n=115 (76,7%), *MPL*+ n=1 (0,7%), *CALR*+ n=14 (9,3%) and triple-negative (TN) molecular status was registered in n=20 (13,3%) patients. Complete blood count mean values (standard deviations) at initial ET diagnosis were: *JAK2*+: Hb 14.2 (17.6) g/dL, WBC 9.7 (3.7) $x10^9$/L, PLT 831 (273) $x10^9$/L. *MPL*+: Hb 12.3 g/dL, WBC 7.1$x10^9$/L, PLT 2079 $x10^9$/L. *CALR*+: Hb 13.8 (16.7) g/dL, WBC 9.3 (3.9) $x10^9$/L, PLT 1086 (453) $x10^9$/L. TN: Hb 13.6 (15.6) g/dL, WBC 11.4 (4.7) $x10^9$/L, PLT 777 (230) $x10^9$/L. The presence of *CALR* mutation was associated with significant higher platelets level (p=0.03). The symptoms frequencies according molecular markers groups were as followed: *JAK2*+: weakness-34.8%, headache/dizziness-20.9%, arthralgia-19.1%, splenomegaly -16.5%, erythromelalgia-8.7%, pruritus-5.2%, constitutional symptoms-4.4%. *MPL*+: weakness only (100%). *CALR*+: weakness-21.4%, headache/dizziness-7.1%, splenomegaly-21.4%, erythromelal-

gia-14.3%. TN: weakness-25.0%, headache/dizziness-25.0%, arthralgia-25.0%, splenomegaly-10.0%, erythromelalgia-10.0%, pruritus-5.0%. Thrombosis rates according molecular markers groups were: *JAK2*+ - 48.6%; *CALR*+ - 14.2% - and TN-30.0%. Bleeding frequency in *CALR*+ patients was 21.4% and for *JAK2*+ patients 6.9%. Overall survival (OS) was significant higher in *CALR*+ group compared to others (*JAK2*+ and TN, p=0.019).

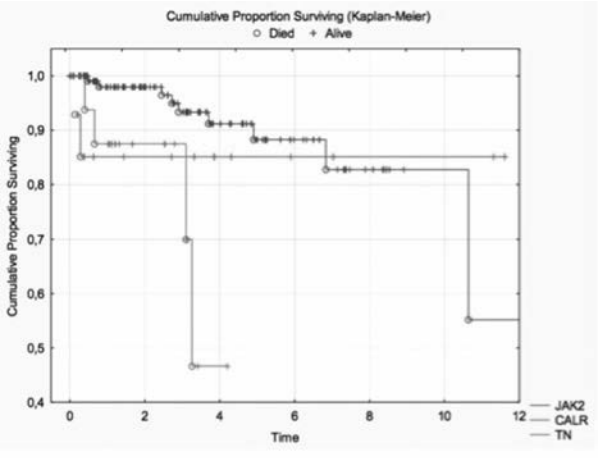

Figure 1.

Summary/Conclusions: The presence of *JAK2V617F* mutation in ET patients is associated with higher thrombosis risk, despite the fact of *CALR*+ patients had higher platelets level. OS in *CALR*+ ET patients was significant higher compared to *JAK2*+ and TN.

PB2033

IMPLEMENTATION OF THE JAK2 V617F MUTATION ANALYSIS IN THE DIAGNOSIS OF SUSPECTED MYELOPROLIFERATIVE NEOPLASMS IN CAPE TOWN

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Background: The Philadelphia-negative myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell malignancies characterized by overproduction of one or more myeloid lineages. The discovery of the JAK2 V617F (JAK2) mutation in 2005 was a crucial step towards unraveling the pathogenesis of the MPN and finally provided a clonal marker for the diagnosis of Polycythaemia Vera (PV), Essential Thrombocytosis (ET) and Primary Myelofibrosis (PMF). Previously diagnostic guidelines of MPN were fraught with controversy due to the absence of exactly such a defining feature. The JAK2 mutation makes it possible to definitely diagnose >95% of PV, and 50-60% of both ET and PMF cases.
Aims: We retrospectively analysed our tertiary care institution patient population in whom this test was requested since its inception in 2007. JAK2 was requested in two groups: patients presenting with thrombosis and with probable MPN. We looked at the role of the JAK2 mutation analysis in the diagnostic algorithm of MPN and in patients presenting with thrombosis.
Methods: We created a database with detailed folder review of 267 patients who had undergone the JAK2 test between 2007 and 2013 in Groote Schuur Hospital. The initial presentation of each patient was analysed for two variables, namely raised cell counts (cytoses) or thrombotic complications, or both. The diagnostic algorithm for MPN implementing the JAK2 analysis was applied to each patient. In addition to JAK2, patients with sustained erythrocytosis were investigated with serum erythropoietin (s-EPO), red cell mass (RCM) and bone marrow examination (BME).
Results: Mean age at testing were 51.2 years. Male gender predominated (n=153; 57.3%). In our population 30% (n=80) of all patients and 64.5% (n=78) of MPN patients were JAK2 positive; the other 2 JAK2 positive patients had mixed myelodysplastic-myeloproliferative syndrome. One-hundred-twenty-one patients were diagnosed with an MPN: ET patients (n=38) tested JAK2 positive in 34%; PMF (n=27) in 63% and PV (n=56) 86% positive. Of the 103 patients investigated for a sustained erythrocytosis, only 56 (54%) could be confirmed as PV. RCM and s-EPO indicated a relative erythrocytosis in 24 patients, the other 23 patients had erythrocytosis of undetermined origin. Sixty-seven patients presented with thrombotic complications: 26 with MPN diagnosis (39%) and 51 (61%) where MPN was ruled out. The diagnosis of MPN was associated only with those patients who exhibited sustained cytoses while presenting with thrombosis. MPN patients presenting with thrombosis were 0.7 times as likely to be JAK2 positive as negative, the difference was not significant.
Summary/Conclusions: The JAK2 mutation analysis was positive in the majority of our MPN population in accordance with published results but in a significant minority other methods of diagnosis remain important. The percentage JAK2 positivity in the PV population was lower than previously reported. Furthermore, almost half of patients investigated for sustained erythrocytosis did not fulfil criteria for PV. These patients with relative or undetermined erythrocytosis form a significant minority where current diagnostic and pathogenetic methods are lacking. Our analysis indicates that employing the JAK2 test to uncover an underlying MPN in patients presenting with thrombosis is unlikely to yield a positive result in the absence of sustained cytoses. Proven MPN patients presenting with thrombosis were just as likely to be JAK2 negative as positive.

PB2034

RUXOLITINIB IN MYELOFIBROSIS: A TWO-CENTRE EXPERIENCE

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Background: Ruxolitinib, an oral Janus Kinase (JAK)1 and JAK2 inhibitor, was approved in the European Union in August 2012 for treating disease-related splenomegaly and constitutional symptoms in adults with primary myelofibrosis (PMF), post-polycythaemia vera myelofibrosis (PPV-MF), and post-essential thrombocythaemia myelofibrosis (PET-MF), following the results of two phase III trials (COMFORT-I and COMFORT-II). The James Paget University Hospital and Ipswich Hospital have treated 31 MF patients (24 and seven, respectively) with ruxolitinib since March 2013.
Aims: To assess the efficacy and safety of ruxolitinib in MF in the clinical setting.
Methods: Retrospective analysis of 31 MF patients treated with ruxolitinib from March 2013 to February 2016 at the James Paget University Hospital and Ipswich Hospital. Patients with greater than three months' follow-up were included.
Results: The patient group was 58% male, with a median age of 69 years (range 58–92). Ruxolitinib was first-line therapy in eight patients (26%) and second-line in 23 (74%). The indication for treatment was painful splenomegaly in 17 (55%) patients, constitutional symptoms in 13 (42%) and portal hypertension in one (3%). Fifteen patients (48%) had PMF, ten (32%) had PPV-MF, five (16%) had PET-MF and one (3%) had post-myeloproliferative disorder (unclassified)-MF. Seventeen (55%) patients had an International Prognostic Scoring System (IPSS) score of three (high risk), 13 (42%) had an IPSS score of two (intermediate risk-2), and one (3%) had an IPSS score of one (intermediate risk-1). Twenty-two patients (71%) were JAK2 V617F positive and one (3%) had a CALR mutation. The median duration of treatment was 17 months (range 5-35). Twenty-six patients (84%) had a therapeutic response. Of the 17 with painful splenomegaly, eight (47%) had >50% reduction in spleen length, six (35%) had <50%, and three (18%) had no change. Nonhaematologic adverse events (AEs) were uncommon and low grade. Weight gain was the most common, occurring in 25 (81%) patients. Minor infections, including oral candidiasis, were also common and easily treated. One patient, however, developed *Aspergillus* pneumonia after 17 months and died. The most common haematologic AEs were anaemia and thrombocytopaenia. All patients developed anaemia; nine (29%) required transfusions and 12 (39%) required erythropoietin. Nine patients (29%) developed thrombocytopaenia (grade 3/4 in three patients) which was managed by dose reduction, or drug interruption, without permanent discontinuation. Four patients (16%) progressed to acute myeloid leukaemia (AML) after a median of four months (range 3-15) and died. Another patient died after losing therapeutic response at 16 months. Our leukaemia-free survival, progression-free survival, and overall survival rate is 81% *vs* 80% in COMFORT-II at three years. Currently, 25 patients (81%) remain on treatment.
Summary/Conclusions: In our experience, overall response rate and tolerability to ruxolitinib was similar to the COMFORT-II trial. The most common non-haematologic AEs were weight gain and minor infections. Furthermore, in many cases weight gain was a desired outcome attributable to improved constitutional symptoms, spleen size, and nutritional status. Conversely, haematologic AE rates may be higher than in the trial setting. Anaemia was observed in all patients and thrombocytopaenia commonly required dose adjustments. There was also a higher than expected incidence of transformation to AML. Most AEs, however, were low grade and readily managed. The majority of patients remain on active treatment.

PB2035

CLINICAL SIGNIFICANCE OF ANISOCYTOSIS IN NEWLY-DIAGNOSED PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background: Red cell distribution width (RDW) provides a quantitative measure of anisocytosis and it is associated with the presence of subclinical systemic inflammation and a poor outcome in a variety of diseases when elevated. Anisocytosis is a feature of primary myelofibrosis (PMF) but it's prognostic role in PMF has not yet been evaluated.
Aims: To determine whether anisocytosis bears prognostic significance in patients with PMF and its relation to disease features.

Methods: 33 newly-diagnosed patients with PMF were analyzed in this study. Baseline RDW values were obtained in addition to other routine blood analyses (CRP, LDH, complete blood count and iron metabolism parameters) and *JAK2* V617F mutational status. Patients were staged according to IPSS prognostic scoring system, liver and spleen size were assessed by palpation. The Mann Whitney U test, the Pearson correlation and the X^2 test/ the Fisher test were used where appropriate. Survival analyses were performed using methods of Kaplan and Meier, the log-rank test and the Cox regression analysis. All statistical tests were two-sided and P values <0.05 were considered significant.
Results: Median RDW was 19.0% (15.2% - 22.5%). RDW correlated significantly with hemoglobin (p=0.005), CRP (p=0.031), spleen size (p=0.036) and IPSS score (p=0.003). Patients with more pronounced anisocytosis had an inferior overall survival (OS)-very-high RDW (≥19.0%) *vs* high RDW (15.1% - 18.9%) subgroup, HR 5.37, p=0.002. RDW remained significantly associated with OS (p=0.002) in a multivariate model including IPSS score, hemoglobin level and CRP.

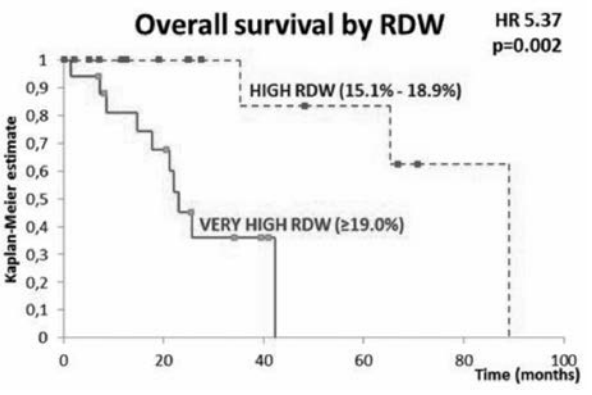

Figure 1.

Summary/Conclusions: PMF pathogenesis surpasses inflammation as only cause of anisocytosis. A higher degree of anisocytosis is associated with more advanced disease features and a decreased overall survival. RDW encompasses standard prognostic score and may help in the rapid detection of patients with an unfavorable prognosis.

PB2036

RISK FACTORS FOR THROMBOTIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Background: Myeloproliferative neoplasms (MPN) are a group of clonal hematopoietic stem cell malignancies, characterized by overgrowth of one or more blood lines with normal or nearly normal maturing of those cells in the bone marrow and extramedullary hematopoietic organs. MPN are acquired prothrombogenic conditions. The mechanism of increased predisposition to thrombosis in myeloproliferative neoplasms is not clear enough. It is thought that the mechanisms that lead to thrombosis in MPN are the following: increased blood cell mass; abnormal platelet function and the phenomenon of spontaneous aggregation. The following factors have been associated with the incidence of thrombosis: the increased level of products formed in the activation of platelets (thromboxane, p-selectin); increased microparticle formation as the part of a membrane with various cell structures of platelet origin; JAK2V617F mutation. In MPN patients an increased activity of coagulation system occurs due to resistance to the anticoagulant function of thrombomodulin.
Aims: The aim of this study is to monitor potential risk factors for the development of thrombotic complications in patients with Philadelphia-negative chronic myeloproliferative neoplasms.
Methods: During the five-year period we monitored the occurrence of thrombotic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasm. Patients were classified into the following groups: 1. Group with the polycythemia vera (PV) (61); 2. Group with essential thrombocythemia (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasm (MPNs) (25). The following possible risk factors were monitored: age, leukocyte count, platelet count, the presence of JAK2V617F mutation, cardiovascular risk factors (smoking, hypertension, diabetes mellitus, dyslipidemia). We used methods of clinical, laboratory, ultrasound and CT scans.
Results: The highest percentage of thrombotic complications (arterial and venous) was found in the group of patients with ET and MPNs (p <0,01), and then in the group with PV (p <0,05). In all three groups, the incidence of thrombotic complications in patients older than 60 years was higher (p<0,001). The leukocyte count ranged from 2,2-17,1x10^9/L and the platelet count ranged from 10,2 -1856,5x10^9/L. The highest leukocyte count was recorded in the group of patients with PV and MPNs (p <0,001) and the lowest in the group of patients with the IMF (p <0,01). The highest platelet count was found in the group of patients with ET, and the lowest in the group of patients IMF. Thrombotic complications in those groups were more frequent in percentage with patients with leukocytosis, but statistical significance was present only in the group with MPNs. No statistical significance was detected between the platelet count and thromboembolic complications in either group. Thrombotic complications were more frequent in JAK2V617F positive patients, but the statistical significance existed only in the group with PV. Considering cardiovascular risk factors only hypertension was significantly more common in the group with PV and MPNs. The largest number of patients with thrombotic complications had two or more cardiovascular risk factors (p <0,05).
Summary/Conclusions: The patients over 60 years of age, as well as the presence of two or more cardiovascular risk factors are the most important for the incidence of thrombosis. Leukocytosis and JAK2V617F may be considered as potential risk factors for thrombosis in patients with myeloproliferative neoplasms, particularly with PV, ET I MPNs. Further follow-up and a larger number of subjects are needed. The follow-up of patients with unclassified myeloproliferative neoplasms has particularly important, which showed a high prevalence of thrombotic complications, and with the aim of their further differentiation.

PB2037

JAK2, MPL, AND CALR MUTATIONS IN CHINESE HAN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA
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Background: Essential thrombocythemia (ET) is a chronic Philadelphia chromosome-negative myeloproliferative neoplasm characterized by the overproduction of circulating platelets in the periphery due to the excessive proliferation of megakaryocytes in the bone marrow [1]. The recurrent Janus kinase 2 (JAK2) V617F mutation has been an important molecular marker for myeloproliferative neoplasms (MPNs) since its discovery in 2005 [2]. However, only 50-60% of ET cases are associated with the JAK2 V617F mutation. Among the 40% of patients with ET who lack the JAK2 V617F mutation, 3-5% carry mutations at codon 515 of the gene encoding the thrombopoietin receptor, a myeloproliferative leukemia virus oncogene (MPL) [3]. In 2013, somatic mutations in calreticulin (CALR) were found in 20 to 25% of patients with ET or primary myelofibrosis (PMF) [4,5]. Like JAK2 and MPL mutations, somatic mutations of Calreticulin (CALR) have been identified as a potentially powerful diagnostic tool for patients with ET [6].
Aims: we studied a population of patients with ET and analyzed the frequency of JAK2, CALR, or MPL mutations as well as patients' hematological characteristics.
Methods: The patients and the data were selected retrospectively from the myeloproliferative neoplasm database established for scientific research at the Department of Hematology of Drum Tower Hospital. A total of 110 patients with ET were enrolled (60 females and 50 males with a mean age of 55.7 years, range 13–88 years); they had been diagnosed at the Department of Hematology between 2012 and 2015 according to the WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues (2008)[7]. The clinical and laboratory data were reviewed from medical records.
Results: Among the 110 patients tested, JAK2 V617F was the most common mutation, observed in 62 patients (56.3%), while CALR mutations were detected in 21 patients (19.1%). One patient (0.9%) carried the MPL W515L mutation. A mutation in JAK2 exon 12 was not detected in any patient. Two ET patients had both CALR and JAK2 V617F mutations. The incidence of triple-negative (negative for JAK2/MPL/CALR) patients was 25.5% (28/110).
Summary/Conclusions: In summary, we have described the mutation profile of our Chinese cohort of ET patients. CALR mutations are a useful diagnostic marker for JAK2/MPL-negative ET patients because they are typically mutually exclusive with a JAK2 mutation and are present in a relatively high frequency.

PB2038

WHAT IS THE SECRET OF THE JAK2 MUTATION ALLELE LOAD STABILITY IN SOME PATIENTS WITH MPN?
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Background: It is known that the level of JAK2 mutation allele load in patients with myeloproliferative neoplasms (MPN) in the disease dynamics can be continuously stable and not be associated with clinical symptoms or effects of hydrox-

yurea (HU) (Theocharides A et al, Haematologica, 2008; Besses C et al, Br J Haematol, 2011). Reasons and mechanisms of this kind of «homeostasis» in the level of circulating mutation JAK2 positive cells in some patients are unclear.
Aims: Evaluate the level of JAK2 mutation allele load in MPN patients in the disease dynamics and HU effects.
Methods: This study included 14 JAK2 positive (V617F or exon 12 mutations) MPN patients. The informed consents from these patients were obtained. DNA was extracted from venous blood leukocytes. Quantification of JAK2 V617F and JAK2 exon 12 mutations allele load was performed by pyrosequencing method as described in (Dunaeva E et al Klin Lab Diagn, 2014). JAK2 variance (MUT) was calculated as a measure of relative changes in allele load between the baseline and follow-up sample (Theocharides A et al, Haematologica, 2008).
Results: Following variants were identified according to the level of JAK2 mutation allele load in the disease dynamics and HU effects (Table 1): 1) The level of JAK2 mutation allele load was increased more than twice (№3 and №13). Until now this patients do not have the significant clinical MPN manifestations and do not need HU-treatment; 2) The level of JAK2 mutation allele load was found to remain stable over follow-up time of observation independently of whether patients were already or not under HU treatment at the time of first sampling; 3) The level of JAK2 mutation allele load was reduced after HU treatment. We did not find any dependency between the allele load dynamics and patient's clinical status, disease phenotype, disease duration and venous blood cellular account.

Table 1. Characteristics of the patients included in the study.

№	Age of manifestation (years)	Sex	Disease	HU	% JAK2 mutation baseline	% JAK2 mutation last sample	JAK2 variance (MUT)	Time between two assessments (mo)
JAK2 V617F mutation-positive patients								
1	67	F	ET*	No	29	27	n.s.	6
2	70	F	PV	No	34	32	n.s.	10
3	71	F	PV	No	16	35	+115	18
4	59	F	Post-PV MF	Yes	82	86	n.s.	15
5	51	F	PV	Yes	78	73	n.s.	20
6	44	F	PV	Yes	69	77	+11	12
7	59	F	PV*	Yes	42	42	n.s.	27
8	45	F	ET*	Yes	8	7	n.s.	36
9	75	F	Post-ET MF*	Yes	14	10	-38	18
10	57	F	PV*	Yes	31	22	-29	6
11	59	F	PV	Yes	81	54	-33	36
12	35	M	MF	Yes	27	20	-27	15
JAK2 exon 12 mutation-positive patients								
13	61	M	PV*	No	15	40	+166	24
14	48	M	PV*	No	11	11	n.s.	4

**The allelic load was determined at the primary address to the doctor with MPN symptoms*

Summary/Conclusions: Our data suggest the JAK2 mutation allele load can remain stable for a long time in some patients. The observed increase of the mutation allele load in untreated patients probably will be to increase to the level of its stabilization at full development of the clinical MPN picture. The differences of the allele load dynamics between individual patients may be associated with the individual features of the intracellular signaling networks and will be the subject of additional studies.

PB2039

PLATELET COUNT AS A RISK FACTOR FOR HEMORRHAGIC COMPLICATIONS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN) are a group of clonal hematopoietic stem cell malignancies, characterized by overgrowth of one or more blood lines with normal or nearly normal maturing of those cells in the bone marrow and extramedullary hematopoietic organs. Hemorrhagic syndrome is a complication that occurs in about a quarter of patients with PV and even 60% of patients with ET. Bleeding can complicate the clinical course of IMF. It has been manifested in the form of petechiae and ecchymoses, or may be life-threatening as uncontrolled esophageal bleeding. It occurs due to ineffective megakaryocytopoiesis, retention of platelets in the enlarged spleen, qualitative platelet disorders, acquired deficiency of factors V and vWF, disseminated intravascular coagulation. The increased platelet count affects the adsorption of the larger von Willebrand multimers onto the platelet membrane, thus acting to eliminate them from circulation and degradation.
Aims: The aim of this study is to monitor the platelet count as a potential risk factor for occurrence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.
Methods: During the three-year period we monitored the occurrence of hemorrhagic complications and platelet count in 120 patients of both sexes aged between 27 and 86 years, being diagnosed with Ph-myeloproliferative neoplasms. Patients were classified into the following groups: 1. Group with the polycythemia vera (PV) (51); 2. Group with essential thrombocythemia (ET) (24); 3. Group with idiopathic myelofibrosis (IMF) (20); 4. Group with unclassified myeloproliferative neoplasm (MPNs) (25). We used methods of clinical, laboratory, endoscopy, ultrasound and CT scans.
Results: Platelet count ranged from 2,2-2134,5,1x10^9/L. The highest platelet count was recorded in the group of patients with ET and MPNs ($p<0,001$) and the lowest in the group of patients with the IMF ($p<0,01$). There was no statistical significant difference detected between the groups of patients with PV and MPNs with regard to platelet count. The highest percentage of hemorrhagic complications was found in the group of patients with ET and IMF ($p<0,01$) and then in the group with MPNs. Hemorrhagic complications have been more frequent in patients with platelet count below 10x10^9/L ($p<0,05$) and in patients with platelet counts over 1000x10^9/L ($p<0,01$). Life-threatening bleeding complications were the most common in patients with platelet count below 5x10^9/L and in patients with platelet count over 1500x10^9/L. Haemorrhage was the cause of mortality in 15% of patients with MPN.
Summary/Conclusions: The platelet count can be considered as a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Further follow-up and a larger number of subjects are needed. The follow-up of patients with unclassified myeloproliferative neoplasms has particularly important, which showed a high prevalence of hemorrhagic complications, and with the aim of their further differentiation.

Non-Hodgkin & Hodgkin lymphoma - Biology

PB2040

PROLIFERATING KI67 EXPRESSING B-CELLS ASSOCIATE WITH CD4+PD1+ T-CELLS IN MARGINAL ZONE LYMPHOMA

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Background: Specific microbial antigens have been implicated in the development and maintenance of several types of marginal zone lymphoma suggesting that an abnormal immune response is essential for driving B-cell proliferation. Reasoning that T-cell stimulation, especially from CD4+ T-cells, might make important contributions to promoting B-cell proliferation we carried out a detailed analysis of infiltrating T-cells in 8 cases of nodal marginal zone lymphoma.
Aims: The aim of the study was to undertake the first detailed study of T-cell subsets in marginal zone lymphoma in order to determine the relationship of individual subsets to proliferating lymphoma B-cells.
Methods: We carried out multiplex immunohistochemistry and validated the results to show that, for the combinations of antibodies employed, there was firstly no reduction in intensity after several rounds of staining and destaining and secondly that there was no significant carry over from one round to the next. The stained slides were scanned and, utilising a custom macro written for ImageJ software, we enumerated CD4+ T-cell subsets (TH1- $CD4^+TBET^+$, Treg - $CD4^+FoxP3^+$, follicular helper (Tfh) - $CD4^+PD1^+$ and follicular regulatory (Tfr) - $CD4^+FoxP3^+PD1^+$).
Results: In all cases $CD4^+$ T-cells constituted a major portion of infiltrating T-cells, mean 39.8% (range 13.5 to 70.3%). There were, however, large differences in the $CD4^+$ T-cell subset composition; Tfh cells varied from 2.5 to 36% of all $CD4^+$ T-cells whilst Tregs accounted for 2.7 to 24.7%. We also compared architecture of T-cell infiltration across cases and found that T-cells were not homogenously distributed and that $CD4^+PD1^+$ cell clusters could show some association or no association with $CD4^+FoxP3^+$ clusters and, in one case, repulsion from $CD4^+FoxP3^+$ clusters. In order to quantitate the associations we carried out Pearson correlations. For comparison normal tonsil showed a Pearson correlation of -0.4 *i.e.* no overalp, between $CD4^+PD1^+$ and $CD4^+FoxP3^+$ cells whereas there were varying degrees of association (range 0.3 to 0.8) for the lymphoma samples. By contrast proliferating $Ki67^+$ lymphoma B-cells associated with $CD4^+PD1^+$ cell clusters (Pearson 0.1 to 0.6) whatever the relation to $CD4^+PD1^+$ cells to $CD4^+FoxP3^+$ cells. To confirm this result we used an alternative method to analyse clustering (the Morisita index). This produced similar results with normal tonsil having a Morisita index of 0.2 for $CD4^+PD1^+$ and $CD4^+FoxP3^+$ whereas lymphoma samples showed higher degrees of association (range 0.3 to 0.9). The Morisita index also confirmed association between proliferating B-cells and $CD4^+PD1^+$ cells; normal tonsil 0.8 and lymphoma (0.4 to 0.8).
Summary/Conclusions: Collectively our data suggests an unsuspected association between $CD4^+PD1^+$ T-cells and proliferating lymphoma B-cells in marginal zone lymphoma.

PB2041

CLINICAL IMPLICATIONS OF MYD88 L265P MUTATION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: It is now well known fact that recurrent mutation in the MYD88 gene (L265P MYD88) is identified in about 90% of Waldenström macroglobulinemia, in approximately one-quarter of patients (pts) with diffuse large B-cell lymphoma (DLBCL) and in a minority of cases of other lymphoproliferative disorders. Evidence is beginning to accumulate that L265P MYD88 mutation in pts with DLBCL has strong association with clinical phenotypes and outcomes.
Aims: To evaluate the clinical implications of MYD88 L265P mutation in pts with DLBCL.
Methods: We analyzed the data of 75 pts (median age 50 years, range 18-77, 33/42 female/male ratio, 39 extranodal and 36 nodal disease) with de novo DLBCL diagnosed between 1999 to 2015 at National Research Center for Hematology. Pts were classified as germinal center B-cell-like (GCB) or non-GCB DLBCL using the Hans algorithm. For molecular analysis DNA was extracted from 56 cryopreserved and 19 formalin-fixed paraffin-embedded tumor tissue. The mutational status of a sample was determined by real-time PCR (RT-PCR).
Results: MYD88 L265P mutation was detected in 14(18,7%) out of 75 pts. Half of pts with MYD88-positive MYD88 L265P mutation DLBCL are older than 60 years compared with only 24,6% of those pts with MYD88 unmutated DLBCL. In terms of sex ratio the data show a double preponderance of males at MYD88 L265P positive DLBCL (9:5) as compared with MYD88 wild type DLBCL with the same number of males and females (33:28). There was equal distribution of pts across high-intermediate and high risk groups of the international prognostic index both in MYD88 mutated and MYD88 unmutated pts (71,7% *vs* 63,9%). The majority of pts in both groups had elevated serum lactate dehydrogenase levels, 12/14 (85,7%) and 47/61 (77%) respectively. Tumors with high Ki-67>80% expression were found in 13(92,8%) of pts MYD88 mutation *versus* 43(70,5%) pts with MYD88 wild type DLBCL. All 14(100%) pts with MYD88 L265P DLBCL were non-GCB DLBCL as compared to 42/61(72%) pts with MYD88 unmutated DLBCL. Eleven (78,6%) out of 14 pts with MYD88 mutation had extranodal lesions (p<0,05). Five (45,4%) of 11 cases MYD88 L265P extranodal DLBCL were present in immune-privileged site DLBCL (central nervous system, testis) *versus* 4(14,2%) out of 28 pts with MYD88 wild type extranodal DLBCL (p<0,05). The table summarizes baseline characteristics pts.

Table 1.

Patients Characteristics n=75 (%)		
	MYD88 mutation	MYD88 wild type
Number of patients	14 (18,7)	61 (81,3)
Sex (male:female)	9:5	33:28
Age at diagnosis, years.		
Median	58	49
Range	18-70	20-77
Over 60years old	7 (50)	16 (26,2)
Serum LDG		
Level > normal	12(85,7)	47(77)
Ki67 > 80%	13(92,8)	43(70,5)
International Prognostic Index		
Low	-	7 (11,5)
Low-intermediate	4 (28,6)	15 (24,6)
High-intermediate	3 (21,4)	20 (32,8)
High	7 (50)	19 (31,1)
Lymph node involvement	3 (21.4)	33 (54,1)
Extranodal sites:	11 (78.6)	28 (45,9)
Bone	2	8
Skin	2	4
Gastrointestinal tract	1	10
Adrenal	1	-
Breast	-	2
Immune-privileged site		
Testis	2	2
Central nervous system	3	2
Bone marrow	3 (21.4)	14 (22,9)

LDG: lactate dehydrogenase

Summary/Conclusions: It is apparent from the present study that MYD88 L265P mutation was significantly associated with extranodal DLBCL (78,6%) and prevalent in immune-privileged site DLBCL. Detection of MYD88 L265P mutation by RT-PCR could improve diagnosis non-GCB DLBCL as a complement to immunohistochemistry.

PB2042

QUINACRINE SYNERGIZES WITH DOXORUBICIN IN INHIBITING THE SURVIVAL OF DLBCL CELL BY SUPPRESSING NF-KB AND ALTERING CELL-CYCLE PROGRESSION

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Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive B-cell non-Hodgkin lymphoma that affects patients of all ages with a wide range of clinical presentations. Although DLBCL is curable even in advanced stages, up to one-third of patients will not achieve cure with initial therapy. To test drug combinations that could improve the efficacy of chemotherapeutics used currently. We combined doxorubicin (dox) with quinacrine (qc), which inhibits the FACT complex that is required for NF- κB transcriptional activity.

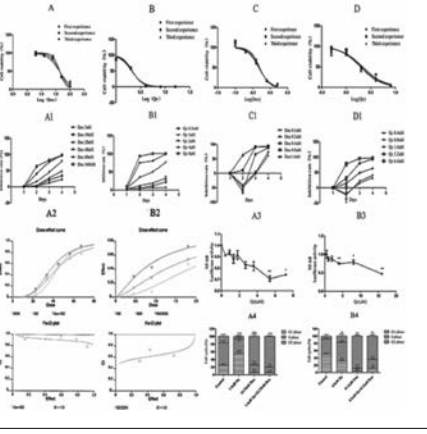

Figure 1.

Aims: The aim of this study was to test drug combinations that could improve the efficacy of chemotherapeutics currently used, such as dox, to Provide new and better drugs for clinical treatment of DLBCL.
Methods: Cells were maintained in RPMI-1640 medium supplemented with 10% FBS. All cells were kept at 37°C in a humidified atmosphere with 5% CO_2. Cell viability was determined by the MTT assay. The combination index (CI) was assessed by using Calcusyn software. Cells stained with propidium iodide (PI), the cell-cycle distribution was assessed by FACScan (BD) Biosciences analysis. Cells were infected with the NF-κB-luciferase Lentiviral construct and stably selected with puromycin. The influence of Qc on the activity of NF-κB was tested using luciferase assay system.
Results: Qc synergizes with dox in inhibiting the proliferation of DLBCL cell lines; Qc has the ability to suppressing NF-κB-dependent luciferase activity, which means qc inhibits the activity of NF-κB, as well as alrer the cell cycle of DLBCL cell lines.
Summary/Conclusions: Qc can inhibit the growth of DLBCL tumor cells, enhance the curative effect of chemotherapy drugs such as dox, its possible mechanism is qc could inhibit the NF-kB activity which plays key roles in DLBCL cell survival and chemotheraputic resistance, as well as alter the cell cycle progression.

PB2043

VALIDATION OF LST EUROFLOW® PROTOCOL BY MULTIPARAMETRIC FLOW CYTOMETRY IN SCREENING OF LYMPHOPROLIFERATIVE NEOPLASTIC DISEASE IN CHILE

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Background: Immunophenotype analysis by multiparametric flow cytometry (MFC) is an important tool, together with pathology, cytogenetical and molecular analysis, for the diagnosis, classification and treatment monitoring of hematological malignancies. The accurate identification of abnormal lymphocytes by MFC requires a unique standardized combination of fluorochrome-conjugated antibodies. The international consortium of standardization, Euroflow®, elaborated a lymphoproliferative disease screening panel (LST) aiming the identification of normal or aberrant B, T or NK lymphocytes in different tissues.
Aims: Confirm that the screening of lympholiferative disease using MFC analysis with LST Euroflow® panel, in an accurate method to recognize, quantify and characterize abnormal lymphocyte populations in lymphoma positive tissue biopsies in Chilean population.
Methods: Retrospective analysis of tissue and/or bone marrow samples sent simultaneously to both the pathology laboratory for biopsy (reviewed by only one expert hematopathology of our institution) and to immunology section of Clinica Alemana de Santiago for immunophenotyping analysis, since the implementation of LST Euroflow® protocol for lymphoproliferative disease screening. All tissues were processed in 8 colors FACSCanto cytometer by BD Bioscience II. The panels and fluorochromes used in every analysis were: CD4 and CD20 in PacB, CD45 in AmCyan, CD8 and SmIgl in FITC, CD56 and SmIgk in PE, CD5 in PerCPCy5.5, CD19 and TCRgd in PECy7, SmCD3 in APC and CD38 in APCCy7.
Results: One hundred and seventy-four samples with suspect of lymphoproliferative disease and concomitant pathology report in our institution were revised from June 2013 to December 2015. Exclusion criteria included Hodgkin Lymphoma and non-hematological neoplastic diagnosis by histology (14 and 11 tissues samples respectively). Fifty tissues were positive for lymphoproliferative disease by biopsy, including 16 follicular lymphomas, 12 diffuse large B cell lymphomas, 7 SLL/CLL, 5 mantle cell lymphomas, 3 lymphoplasmocytic lymphomas, 3 marginal zone lymphomas, 2 Hairy cell leukemia, 1 T gamma-delta lymphoma and 1 multiple myeloma. The screening analysis by MFC using LST panel showed abnormal and/or clonal population in 100% of positive lymphoproliferative disease biopsies. In the analysis of the non-neoplastic histological biopsies (87 samples), MFC detected subclinical clonal or abnormal lymphocytic population in 10 cases, being both negatives in 77. The sensitivity, specificity, positive predictive value and negative predictive value of LST for diagnosis of lymphoproliferative disease was 100%, 89%, 86% and 100% respectively.
Summary/Conclusions: Lymphoproliferative panel LST is an effective screening tool for lymphoma diagnosis in Chilean population in different tissues including bone marrow.

PB2044

IMPACT OF THE NAGASAKI ATOMIC BOMB ON THE INCIDENCE RATE AND OUTCOME OF FOLLICULAR LYMPHOMA

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Background: In Nagasaki and Hiroshima, approximately 75,000 and 120,000 citizens, respectively, died within 3 months of the 1945 atomic bomb explosions. Furthermore, 80,000 and 140,000 citizens, respectively, survived exposure. During the following 70 years, high-dose radiation exposure has induced various cancers, including leukemia; thyroid, lung, gastric, and colon cancers; and myelodysplastic syndromes (MDS). Additionally, approximately 10% of cancer-carrying patients of Nagasaki atomic bomb survivors have multiple primary cancers in various tissues. This high-dose radiation exposure not only increased the risk of developing various types of cancer, but also worsened the overall survival (OS) rate. We recently reported significantly shorter OS among Nagasaki atomic bomb survivors with MDS *versus* patients without such radiation exposure (Jo et al., Anticancer Research 2015).
Aims: We investigated whether high-dose radiation exposure would similarly affect the incidence rates and outcomes of follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL).
Methods: The youngest atomic bomb survivors are aged approximately 70 years, presenting a challenge to perform prospective cohort studies. Therefore, we conducted a retrospective cohort study of the incidence rates and outcomes of FL and DLBCL in cases diagnosed at our hospital between January 2004 and December 2013. The primary objectives were to compare the incidence rates of FL and DLBCL in atomic bomb survivors (A-Bomb) and non-A-Bomb patients, and determine the OS rates.
Results: Table 1 lists the number of patients. Seven and 51 A-Bomb patients and 49 and 91 non-A-Bomb patients had FL and DLBCL, respectively. The incidence rate of FL *versus* DLBCL was significantly less among A-Bomb patients than among non-A-Bomb patients (P=0.0009). There was no significant difference in OS between A-Bomb and non-A-Bomb patients with DLBCL (P=0.3065, hazard ration [HR]: 1.311, 95% confidence interval [CI]: 0.7813–2.198). In contrast to DLBCL, the OS rate was significantly lower in A-Bomb patients with FL than in non-A-Bomb patients (P=0.0092, HR: 5.425, 95% CI: 2.151–220.1). In both FL and DLBCL patients, there were no significant differences of background factors except for the median age between A-Bomb and non-A-Bomb patients. The respective median ages of A-Bomb and non-A-Bomb patients with DLBCL differed significantly at 75 (range, 62–91) and 73 (range, 17–93) years (P=0.0038). The respective median ages of A-Bomb and non-A-Bomb patients with FL also differed significantly at 80 (range, 63–90) and 60 (range, 28–80) years (P <0.0001). The age difference had no impact on the OS rate of DLBCL. Therefore, the shorter OS of A-Bomb patients with FL cannot be accounted only for the age difference between A-Bomb and non-A-Bomb patients. Some other unknown factors caused by high-dose radiation exposure of the Nagasaki Atomic Bomb could be linked to the OS difference.

Table 1.

Table 1. Numbers of atomic bomb (A-Bomb) and non-A-Bomb survivors with follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL)

	FL	DLBCL	Total	P-value*
A-Bomb	7	51	58	
Non-A-Bomb	49	91	140	
Total	56	142	198	0.0009

*Fisher's exact test

Summary/Conclusions: Compared with non-A-Bomb patients, A-Bomb patients had a significantly lower FL incidence rate and significantly shorter OS. We previously reported significantly reduced OS among MDS patients exposed to the Nagasaki atomic bomb. These data suggest an influence of atomic bomb exposure and warrant further study for etiological clarification.

PB2045

CD39 EXPRESSION ON CD4 T INFILTRATING LYMHOCYTES IN THE TUMOR MICROENVIRONMENT OF LYMPHOMAS

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Background: In tumor microenvironment (TME) a variety of mechanisms favor a tumor-protection, one of them is the accumulation of extracellular adenosine (ADO) by up-regulating activity of ectoenzime CD39. The generation of ADO from ATP metabolism is recognized as a mechanism for immunosuppressive function of regulatoty T cell (Treg), a component of the CD4+ T lymphocytes expressing variable functional molecules and involved in tumor escape.
Aims: It has been well-documented the increase of Treg in various solid tumor and the higher CD39 expression in some types of human cancer. We have evaluated the CD39 and the CD26 (an adenosine deaminase) on CD4+T infiltrating lymphocytes in lymph node with lymphoma to verify the expression of two markers and whether they could be an attractive features for analysis of TME of a non Hodgkin lymphoma (NHL).
Methods: We analyzed by flow cytometry (FC) immunophenotyping non-neoplastic (10) and B-NHL (48) lymph node samples. and we measured the CD26 and CD39 on CD4+ T lymphocytes in all tissues. Among B-NHL were 14 diffuse large B cell lymphoma (DLBCL) and 3 relapsed.

Results: Compared to non-neoplastic samples, CD4+T cells of NHL showed statistically significant difference on CD26 (67% *vs* 39.5%; *p* <0.001) and on CD39 (6% *vs* 34%; *p* <0.001). In NHL, compared to other entities the value of CD26 on DLBCL (39.5% *vs* 34%) and on the relapsed (39.5% *vs* 29%) was not significantly different; while CD39 on both groups, DLBCL (34% *vs* 54%; p <0.005) and relapsed (34% *vs* 74%; p <0.001), resulted significantly increased.

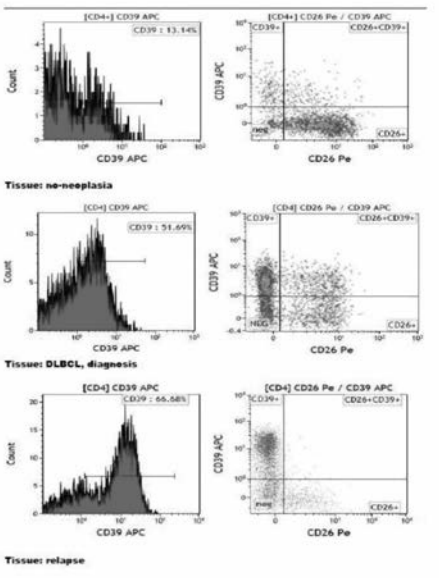

Figure 1.

Summary/Conclusions: Although the regulatory activity of immune system can employ a diversity of molecular mechanisms, the presence of enzymatically-competent CD39 on surface of infiltrating CD4+T suggest that the CD39 pathway can be a distinct mechanism utilized by Treg in TME of lymphomas. Furthermore, the tendency to the reduction (heightened in the relapsed) of CD26, connectable to a lower activity of adenosine deaminase, beside the up-regulated expression of CD39, could underlies an increased accumulation of extracellular ADO and its important role in Treg-mediated immune response suppression. Data of DLBCL, resembling the relapsed, tend to differentiate from other entities for a more suppressive functional profile. Together, these simple and fast assays obtained by FC support the finding that ATP-ectonucleotidase-adenosine system may have a role in mediating Treg activity also in lymphomas suggesting that CD39 represents a possible target for cancer immunotherapy.

PB2046

SERUM PROTEOMIC PROFILES OF HODGKIN'S LYMPHOMA PATIENTS SHOW DIFFERENTIAL EXPRESSION ACCORDING TO EPSTEIN-BARR VIRUS STATUS

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Background: Despite the advances in understanding of Hodgkin's lymphoma's molecular pathogenesis, its association with EBV remains unclear. In this study, we assessed serum protein profiles from EBV associated and non-associated Hodgkin's lymphoma patients and identified three differentially expressed proteins that are candidate biomarkers.
Aims: The aim of the present study was to identify and analyze differentially expressed proteins in pooled sera from patients with HL according to EBV status.
Methods: Blood samples were obtained from 16 patients with recently diagnosed HL before treatment. Blood samples from 10 healthy volunteers were also included. The inclusion criteria used for EBV non-associated HL were the negativity of EBV in buffy coat, plasma, and lymph node. Conversely, patients with EBV associated HL should have EBV detected in buffy coat or plasma, and lymph node. MALDI-MS was performed using a 4700 Proteomics Analyzer® (Applied Biosystems).
Results: The patients' median age at diagnosis was 26 years (range 15-56) and 38% were men. Our analysis revealed about 265 spots with a mean of 86 (35%) identified spots on each gel. Five spots were indicated as having different normalized volumes when compared to each other (p<0.05) (Figure1). Two up-regulated proteins were detected in patients with EBV non-associated HL, and identified as inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) and haptoglobin (HP). Only one protein, fibrinogen beta chain, was down-regulated in the serum from EBV-associated HL patients.

Table 1.

Protein	Ip	MM	Corresponding gene	Localization	EBV-negative vs EBV-positive	P value
Inter-alpha-trypsin inhibitor heavy chain H4	6.51	103489	*ITIH4*	3p21-p14	+2.85	0.025
Haptoglobin (isoforms)	6.13	45861	*HP*	16q22.3	+3.99	0.024
					+5.12	0.027
					+5.86	0.027
Fibrinogen beta chain	8.54	56577	*FGB*	4q28	-2.69	0.004

Table 2 - Identified proteins with differential expression higher than 2-fold change in Hodgkin's lymphoma patients according to EBV status

Ip= isoelectric point; MM= molecular mass

Figure 1 - 3D view representing the volume profile of 3 haptoglobin protein spots differentially expressed between all groups. A, B: control group; C, D: EBV associated HL group; E, F: EBV non-associated HL group.

Summary/Conclusions: We identified three serum proteins that may serve as potential biomarkers in HL, according to EBV status.The biological function of ITIH4 is still unknown. A possible role is the modulation of cell migration and proliferation during the development of the acute-phase response. These findings may suggest that EBV presence could inhibit ITIH4 expression. Serum HP levels in the lymphoma patients have also been described to be significantly higher than in the control group and have been correlated to response to treatment. These results suggest HP as a new tumor marker for lymphoma. Here HP is not increased in patients with advanced stage disease, but in absence of systemic EBV infection, which is in line with prior observations: a recent study described some phenotypes with a higher HP concentration (HP 1-1 and HP 2-1) are less prone to positive EBV serology. In spite of FGB has been described as a serum acute phase reactant protein, their levels were decreased in patients diagnosed with EBV associated HL. The cytokines IL-4, IL-10 and IL-13 have a protective effect against vascular injury leading to atherosclerosis, dose dependently down regulate the biosynthesis of fibrinogen, and some of these cytokines are known involved in the HL pathophysiology. In conclusion, the present study represents an initial and necessary step needed to identify proteins that are differentially expressed between patients with EBV associated and non-associated HL. We demonstrated that protein profiling of serum significantly, accurately and reproducibility distinguished EBV associated and non-associated HL patients. This is the first report combining analysis of serum proteomics of HL patients, according to EBV status, and the pooled sera approach as an appropriate initial approach. Following the identification of spots, future studies with more samples are still required to validate our results. Besides such validation could be performed in large scale by other techniques (e.g., ELISA-based methods).

PB2047

EVALUATION OF NEUTROPHIL-LYMPHOCYTE RATIO IN PATIENTS WITH MYCOSIS FUNGOIDES

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Background: Neutrophil lymphocyte ratio (NLR), an indicator of inflammation, has been lately demonstrated as a prognostic factor in various lymphoproliferative disorders. However, the effects of NLR has not been investigated in mycosis fungoides (MF) patients yet.
Aims: The aim of this study is to investigate the relationship between the neutrophil-lymphocyte ratio (NLR) and treatment demand (systemic PUVA and/or chemotherapy), time to treatment, progression in stage, time to progression in stage in MF patients.
Methods: The data of 117 patients, who were followed with the diagnosis of MF at Department of Dermatology in Istanbul Training and Research Hospital between April 2006 and January 2016, were reviewed retrospectively. Neutrophil-lymphocyte ratio was calculated from complete blood count of patients at the time of diagnosis. The cut-off score for NLR was determined as 2 according to the median NLR level which was 1.96. Statistical evaluation was made by SPSS 15 package program. x^2,Fisher's exact test was used for evaluating categorical values and Mann Whitney U test for continuous values in patient groups.
Results: At the time of diagnosis, the median age of patients was 54 years (range, 21-90). 62 (53%) of the patients were female and 55 (47%) were male. 60 (51.3%) patients had stage Ia, 18 (15.4%) had stage Ib, 35 (29.9%) had stage IIa, 1 (0.9%) had stage IIIa and 3 (2.6%) had stage IVa disease. 77 (65.8%) patients required treatment during follow up. The median time from diagnosis to treatment was 2 (range, 0-64) months. 63 (53.8%) patients showed progression in disease stage. The median time from diagnosis to progression in stage was 23 (1-108) months. There was no significant difference in treatment neces-

sity, time to treatment, progression in stage, time to progression in stage in patients with a NLR≥2 and NLR<2 (p=0.331, 0.987, 0.065, 0.119 respectively).
Summary/Conclusions: Although NLR has been demonstrated to have prognostic role in various lymphoporliferative disorders, it seems that there is no association between the NLR and treatment demand (systemic PUVA and/or chemotherapy), time to treatment, progression in stage, time to progression in stage in mycosis fungoides (MF) patients.

PB2048

TO DOSE OR NOT TO DOSE: ARE IL-10 AND IL-10:IL-6 RATIO ACCURATE BIOMARKERS TO DETECT LEPTOMENINGEAL INVOLVEMENT IN SMALL B-CELL LYMPHOPROLIFERATIONS?

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Background: Identifying the etiology of neurological symptoms in blood malignancies is still a challenging issue. Lymphomatous meningitis (LM) is mainly described in aggressive B-cell lymphomas (diffuse large B-cell (DLBCL) and Burkitt lymphomas). Leptomeningeal involvement in small B-cell lymphoproliferations is a rare, poorly described condition, mentioned only in a few case reports. The diagnosis is suspected in patients with non-specific central nervous system (CNS) symptoms and non-specific results on medical imaging. The diagnosis is confirmed by cytology (detection of tumor cells) in the cerebrospinal fluid (CSF). However, its sensitivity is low due to the scanty amounts of CSF and the rapid cell death. During the past decade, new complementary approaches have been proposed including flow cytometry (FCM) and quantifications of soluble biomarkers. More recently, interleukin-10 (IL-10) has been described as a candidate to diagnose primary central nervous system lymphoma (PCNSL) and systemic DLBCL with CNS involvement. Dosages can be processed using multiplex techniques with the possibility to undergo simultaneous multiple dosages on small volumes.
Aims: The aim of our study was to evaluate the diagnostic value of interleukin IL-10 and IL-6 quantifications combined with the IL-10:IL-6 ratio in CSF of patients suffering from LM secondary to systemic small B-cell lymphoproliferations.
Methods: We carried out a retrospective monocentric study over 4 years on 23 patients suffering from small B-cell LM: 5 chronic lymphocytic leukemias, 3 mantle-cell lymphomas, 13 Waldenström macroglobulinemias (WM) and 2 unclassified B-cell lymphomas. All patients presented CNS symptoms and documented LM (revealed either by cytology or FCM) at the diagnostic stage or before intrathecal chemotherapy. IL-10 and IL-6 quantifications were performed in CSF using the *Cytometric Bead Array*® technique (BD Biosciences™) on a FACSCanto II flow cytometer (BD Biosciences™), with a limit of detection of 2.5pg/ml.
Results: As there is no well-defined IL-10 cutoff in the literature, we applied the IL-10:IL-6 ratio with a threshold set at 1 used to diagnose intra-ocular lymphoma. More than half of the patients (n=14) had an undetectable level of IL-10 along with low levels of IL-6, leading to an IL-10:IL-6 ratio <1 or *undeterminable* (group n°1). Patients in group n°2 also had ratios ≤1 but with detectable levels of IL-10. All patients with ratios≤1 (groups n°1 and 2) presented small B-cell lymphoproliferations with LM but no evidence of aggressive B-cell lymphoma. Patients in group n°3 had ratios >1. Two patients had PCNSL simultaneously diagnosed in the CSF and one patient had a WM transformed into DLBCL during follow-up. These 3 patients presented an increased IL-10 level (≥10 pg/ml). Thus, IL-10 and IL-10:IL-6 ratios in small B-cell lymphoproliferations unexpectedly differed from those observed in DLBCL. Our findings demonstrate the need of IL-10 and IL-6 quantification with the use of IL-10:IL-6 ratio in small B-cell lymphoproliferations to exclude any other aggressive B-cell malignancy. Interestingly, in WM patients with L, also called Bing Neel syndrome, IL-6 levels seemed higher (median: 10 pg/ml) than in other patients.
Summary/Conclusions: In conclusion, we describe for the first time that CSF IL-10 is not increased in small B-cell lymphoproliferations with LM. However, we report its usefulness in revealing more aggressive lymphomas in the context of either a transformation or when associated with another "hidden" lymphoma such as PCNSL. Supplementary data will be prospectively collected to confirm our results.

PB2049

PROGRESS IN THE DIAGNOSIS OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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Background: Central nervous system lymphomas are aggressive tumors requiring a prompt diagnosis for successful treatment. Stereotactic brain biopsy remains the standard procedure, but the time needed for histopathology is usually over 2 days.
Aims: During stereotactic brain biopsy, the trocar is rinsed with saline solution and we evaluated the contribution of cytomorphology and flow cytometry of this rinse fluid usually removed.
Methods: Eighteen patients with suspected localized brain lymphoma underwent stereotactic brain biopsy. Brain biopsy tissue sample and/or brain biopsy rinse fluid were analyzed by cytomorphology combined with flow cytometry. Eleven patients had both types of sample, four patients had only brain biopsy tissue sample, and three patients had only brain biopsy rinse fluid. Histopathology was used as a reference.
Results: Histopathology characterized ten diffuse large B-cell lymphomas and eight other diseases: three glioblastomas, one necrosis, one Erdheim-Chester disease, one cerebral vasculitis, one stroke and one anaplastic astrocytoma. Cytomorphology and flow cytometry showed lymphoma cells in nine out of the ten lymphomas. Three cytomorphology or flow cytometry negative results were reported for lymphomas in tissue samples due to low cellularity and biopsy sample conditioning. No lymphomatous cells were found by cytomorphology or flow cytometry in the eight other diseases. Rinse fluid results were consistent with histology in all cases studied (sensitivity and specificity, 100%). All lymphomas characterized by flow cytometry were: CD5-, CD20+, Ki67 high and CD10 was expressed in six out of the nine cases as in histopathology. CD44, CD184, BCL2, and BCL6 showed variable expression. The median time to result was 4.5 days (range, 2-10) for histopathology, while 5 h (range, 3-20) were required for both cytomorphology and flow cytometry.
Summary/Conclusions: Brain biopsy rinse fluid alleviates problems of tissue sample distribution compared to tissue sample. Its analysis performs diagnosis of B-cell lymphoma in a few hours and, associated with histopathology, allows a multidisciplinary diagnosis. This study shows that cytomorphology combined with flow cytometry on brain biospy rinse fluid is a new, fast, and useful strategy.

PB2050

CSF IGH REARRANGEMENT IN HIGH-RISK DLBCL PATIENTS TREATED WITH INTRATHECAL PROPHYLAXIS

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Background: Central nervous system (CNS) involvement is one of the most serious and devastating complications for patients with diffuse large B-cell lymphoma (DLBCL). Patients with risk factors for secondary CNS localization as specific extranodal sites, bone marrow localization and high IPI score, are candidates for intrathecal prophylaxis. Cerebrospinal fluid (CSF) is routinely analyzed by cytology for the presence of lymphoma cells, while the role of the IgH rearrangement assay on CSF has not been studied in high-risk DLBCL patients treated with intrathecal prophylaxis.
Aims: To evaluate the incidence of IgH clonal rearrangement at diagnosis in high-risk DLBCL treated with intrathecal prophylaxis.
Methods: Patient population included 55 patients with aggressive B-cell lymphoma without CNS involvement, including 51 pts with DLBCL and 4 patients with unclassifiable lymphoma with intermediate features between DLBCL and Burkitt lymphoma. All patients were treated with R-CHOP-like regimens associated to intrathecal methotrexate prophylaxis given during the first 4 cycles of systemic therapy. Twenty-two patients were consolidated with ASCT. CSF samples at the first lumbar puncture were analyzed for IgH rearrangement. After CSF centrifugation, DNA was extracted through the EZ1 DNA Tissue Kit (Qiagen) using the BioRobot EZ1 (Qiagen). VH FR2-JH and VH FR3-JH rearrangement were amplified in two semi-nested PCRs by using the B-cell lymphoma kit-FL (Experteam), and analyzed by Gene Scanning on the ABI 3100 Genetic Analyzer (Applied Biosystem).
Results: A clonal IgH rearrangement in the liquor was detected in 16 of 55 patients (29%), while it was polyclonal or absent in remaining 39 patients. In 4/55 (7%) patients, the number of cells in the CSF was elevated. In the 4 patients with elevated CSF cells, IgH analysis showed a monoclonal band in 1 patient and a polyclonal rearrangement in 3 patients. The presence of clonal IgH rearrangement was associated with elevated (>40 mg/dl) protein levels in the liquor (*p=0.001*). In particular, only 3/30 (10%) patients with a normal CSF protein level showed a clonal IgH, while a clonal IgH rearrangement was present in 13/25 (60%) patients with elevated CSF protein levels. No association was found between detection of clonal IgH in CSF and patient characteristics, as age, stage, bone marrow or extranodal involvement, LDH and international prognostic index IPI. Eight patients with clonal IgH on CSF were serially analyzed at sequential lumbar punctures: the clonal IgH peak disappeared in 7 patients and persisted only in one patient. Three patients developed CNS relapse (5.5%), one of 16 patients with clonal IgH on CSF (6%) and 2 of 39 patients with polyclonal IgH on CSF (5%).
Summary/Conclusions: Clonal IgH rearrangements are frequently present in the CSF of high-risk DLBCL patients and tend to disappear during repeated CNS-directed prophylaxis. No association between detection of clonal IgH

rearrangement and risk of CNS relapse was found in patients treated with intrathecal methotrexate.

PB2051

HODGKIN'S LYMPHOMA AND AUTOIMMUNE DISEASES

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Background: Hodgkin's lymphoma (HL) is a tumor disease of the lymphoid tissue characterized by presence of Reed-Sternberg cells. Autoimmune phenomena are associated with HL. They may precede the clinical presentation of HL, occur concurrently or later, either spontaneously or following treatment. The relationship between HL and autoimmune diseases has been reported as bi-directional, however there is a few data in general population.
Aims: Evaluation of the percentage of autoimmune diseases associated with HL on a retrospective study of 80 cases.
Methods: A retrospective study in the hematology department, conducted over a period of 5 years (from 2008 to 2012), which concerned 80 cases of HL.
The diagnosis is histological completed with immunohistochemistry using anti CD15 and anti CD30 anibody. Patients are classified according to the classification of Ann Arbor.We used prognostic classifications: the EORTC for localized stages and IPS for advanced stages.A clinical assessment is performed and includes: blood glucose, renal assessment, liver function tests, viral serology, a respiratory functional exploration, electrocardiogram and echocardiography. Patients are treated with ABVD chemotherapy associated with mantle radiotherapy.In front of presence of warning signs (palpitations, dyspnea, asthenia, bone pain, thrombosis) an immunological assessment is carried out in search of lupus anticoagulant, anticardiolipin, anti-histone antibodies, anti-nuclear factor, thyroid function tests (TSH, FT3, FT4, ATG, ATPO), cervical ultrasound. Biopsies are sometimes carried out in the context and the type of autoimmune disease and sought by the existence of clinically detectable lesions (biopsy of the salivary glands, skin...).
Results: Eighty cases were included, 5 (6,2%) of which, had an autoimmune disease diagnosed previously, simultaneously or after lymphoma. There are 4 women and 1 man. Autoimmune diseases were: lupus erythematosus, thyroiditis autoimmune, celiac disease, sarcoidosis. The diagnosis was made after lymphoma in 4 patients and before in 1 patient,no deaths were registered.

Table 1.

Patient	Antecedents	Histological type	Stage	Treatment	Outcome	Auto-immune disease type	Delays occured	Treatment	Decline
1	Thyroiditis autoimmune (hypothyroidism 3 years before diagnosis in Levothyrox)	1	II Aa	3 ABVD+ Radiotherapy	Complete remission	lupus erythematosus: Lupus anticoagulant +, anti histone +, cardiolipin +	3 months after diagnosis	Steroids	5 years
2	Absent	2	I Aa	3 ABVD + Radiotherapy	Complete remission	thyroiditis autoimmune: TSH collapsed low FT4 high ATPO high ACTG	10 months after RxT	Levothyrox	3 years
3	Absent	2	I Aa	4 ABVD + Radiotherapy	Complete remission	thyroiditis autoimmune: low TSH high-TPO	2 months after RxT	Levothyrox	1 year
4	Celiac disease since the age of 6 years	2	IIIBb	4 ABVD + Radiotherapy	Complete remission	Celiac disease	17 years before diagnosis	diet without gluten	6 years
5	Absent	2	II Ba	3 ABVD + Radiotherapy	Complete remission	Sarcoidosis: converting enzyme / CT mediastinal biopsy of the salivary glands and skin	25 months after diagnosis	Corticosteroid (restrictive pattern)	4 year

Summary/Conclusions: HL association with an autoimmune disease is estimated at 6.2%. There was an important prevalence of autoimmune diseases in girls with HL.The contribution of the activity of the autoimmune disease and immunosuppressive drugs in the development of HL is still not understood. It might be interesting to try to define a population at risk, by personal or family history of autoimmunity and regular and rigorous monitoring of patients during and after treatment with unusual clinical and biological signs.

PB2052

EFFECTS OF MICRORNA-21 ON APOPTOSIS BY REGULATING THE EXPRESSION OF PTEN IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy and the most common subtype of non-Hodgkin's lymphoma in China. We found that plasma miR-21 level was significantly higher in B-cell lymphoma. However, the exact contribution of miR-21 in DLBCL remains unknown.
Aims: To determine the function and mechanism of miR-21 in DLBCL.
Methods: miR-21 and phosphatase and tensin homolog (PTEN) expressions were examined through real-time PCR and immunohistochemical methods. Moreover, the effects of antisense oligonucleotide (ASO) targeting miR-21 (ASO-21) were observed in DLCBL cell line.
Results: Results showed that miR-21 expressions in cell line and tissues of patients were significantly higher than those in normal controls, which were inversely correlated with PTEN expression. MiR-21 expression was significantly higher in stage III/IV patients than in stage I/II patients. After downregulating the miR-21 expression, apoptosis of DLBCL cells increased and PTEN protein was upregulated.

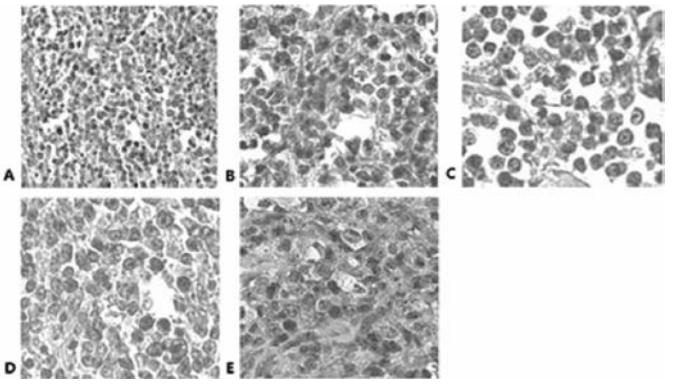

Figure 1.

Summary/Conclusions: These results suggested that miR-21 affects apoptosis of lymphoma cells by regulating the expression of PTEN in DLBCL, which may be associated with increased poor prognosis for DLBCL patients and represents a useful approach for DLBCL treatment.

PB2053

PROSPECTIVE COMPARATIVE STUDY BETWEEN CYTOLOGY COUPLED TO THE FLOW CYTOMETRY BY FINE NEEDLE ASPIRATION AND HISTOLOGY IN THE DIAGNOSIS AND CLASSIFICATION OF LARGE B CELL LYMPHOMA

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Background: Lymphoma B cells can be studied by flow cytometry (FCM) using fine needle aspiration (FNA) on suspensions of lymph node or extra nodal samples. The cytological analysis and the use of special immunophenotypic markers allows orientation of diagnosis.
Aims: Importance of the FCM coupled to morphology in ambulatory diagnosis of B-cell lymphoma.
Methods: Cases of NHL that were diagnosed primarily by FNA at our institution between january 2010 and december 2015. 180 samples were studied (140 lymph nodes and 40 extra nodal samples). 134 samples from the 121 evaluable patients referred for suspicion of lymphoma were studied: these patients were, a cytological study and a fine needle aspiration coupled with FCM. 10 antibodies were tested. Large B cell lymphoma was diagnosed in 37 patients (28%) of 33 lymph nodes, 2 pleural fluids and 2 subcutaneous masses. Histological examination with immunohistochemistry were performed in 31 patients: 23 lymph nodes, 1 bone marrow, 2 tonsillectomies, 2 vaginal, 1 skin, 1 bronchial and 1 nasal mass.
Results: In our study the mean age was 60 years, with a male predominance (Ratio =1.8). On the cytological analysis: all smears were monomorphic in most cases, with large cells appearance, regular nucleus and basophile cytoplasm. On CMF analysis, the diagnosis was de novo in 31 patients and at relapse in 6 patients. lymph node involvement in 33 cases (89%), extra nodel in 4 cases (11%). The markers (CD20, CD22, CD79b) positive in 100% of cases; CD5 positive in 45% with low expression. CD23 and CD10 were negatives in 90% of cases. Isotype restriction was lambda in 83%. Histological analysis: 26 cases Diffuse Large B-cell lymphoma (DLBCL), 3 cases B small-cell NHL, 1 case reactive hyperplasia (RH) and 1 case adenocarcinoma. Immunohistochemistry: CD20 was positive in all DLBCL. The correlation between the FCM and histology in the diagnosis of de novo large B cells NHL was 84%.
Summary/Conclusions: FCM enhances diagnostic ability of FNA cytology, playing a crucial role in a rapid and accurate differential diagnosis between RH, B-NHL and T-NHL. In addition, immunophenotyping of FNA samples contributes to a more precise sub classification of B-NHL when combined with histopathology.

PB2054

THE DURATION OF FIRST COMPLETE REMISSION OF DIFFUSE LARGE B CELL LYMPHOMA COMPARED TO NONGCB, MUM1/IRF4, AND EXPRESSION OF BCLXL AND KI67 IN IMMUNOCHEMOTHERAPY ERA

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Background: The cell has developed mechanisms for maintaining haemostasis. Apoptosis with its internal and external signalling pathways is one of those mechanisms.

Aims: To determine the impact of immune phenotype and oncogene characteristics on the duration of first complete remission with DLBCL
Methods: Study was retrospective-prospective. 60 patients were analysed with *de novo* DLBCL were treated and followed in Clinical Center University of Sarajevo. Follow up median was 47 months (3-91). Patients were divided into two groups: GCB and nonGCB. In the first line of treatment all patients received immunochemotherapy R-CHOP. Biopsy material was analysed for: CD20, bcl-6, CD10, MUM1/IRF4, bcl2, bclxl, bax, bad and bid. Statistics: spearman's analysis was used where p<0.05 was considered significant.
Results: Significantly positive correlation in the group IPI>2 was found with respect to bclxl expression p=0.044 and IPI>2 with respect to expression of Ki67 ≥50% p=0.035. Clinical stage III/IV has a significant negative impact on the duration of CR1 in the total group, p=0.005 and the group Ki67 ≥50% p=0.009. Duration of CR1 in the entire group had negative correlation when compared to MUM/IRF4 with p=0.024 and bclxl with p=0.013. Duration of CR1 is in negative correlation to the group nonGCB p=0.03 and Ki67 ≥50%, p=0.001.
Summary/Conclusions: found a significant negative correlation between the duration of first complete remission of DLBCL compared to nonGCB expression MUM1/IRF4, bclxl and Ki67.

Non-malignant hematopoietic disorders

PB2055

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: EXPERIENCE OF AN HOSPITAL IN NORTHEN SPAIN IN THE LAST 22 YEARS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a very unusual and disastrous dysfunction of the immune system leading to an uncontrolled cytokine storm that has become more recognized over the past decade. HLH was first described in 1939 by Scott and Robb-Smith. This disease is characterized by lengthy and overwhelming activation of antigen-presenting cells (macrophages, histiocytes) and CD8+ T and NK cells. All this scenario produces an important hyperinflammatory condition and organ damage including fever, splenomegaly, cytopenias, coagulophaty and/or hypertrigliceridemia. Histiocyte Society (HS) criteria have been extensively applied for diagnosing HLH, however not all of them are usually showed at the initial presentation. HLH could be displayed in two different contexts: primary (genetic, usually in children, known as familial form) and secondary (acquired), caused by malignancy, infections, metabolic conditions or autoimmune disorders. This disorder is a life-threatening disease which should be suspected and treated as soon as possible.
Aims: Analyzing the casuistry of hemophagocytic syndromes, diagnostic criteria, treatment applied and evolution in our hospital and making a comparison with the current literature.
Methods: A retrospective analysis was carried out through the medical records of all patients with suspected diagnosis of HLH between 1994 and 2016 in one hospital. Age, clinical features, diagnostic criteria proposed by the HS, etiology, treatment and evolution were analyzed. In our study only 17 out of 49 patients met the requested criteria.
Results: The number of cases was 17 (M7/F10). The median age at diagnosis was 67 years, ranged between 4 and 84 years. Clinical development was fast in almost all patients, except in two of them with long-standing pancytopenia. The most frequent causes of consultation were fever and general syndrome. All of them met 5 or more of the criteria necessary for the diagnosis. sIL-2R level and NK activity were underestimated because they were not tested in more than half of the cases. All of them were secondary (11 malignancies, 4 infections, 1 autoimmune disease), unable to clarify the cause in 1 of them. Only 8 patients survived HLH (47%), although 6 died for other complications. 94/04 HLH treatment according to protocol was established in 5 of them. In the rest of them the triggering cause was treated. The most frequent causes of death were liver failure, infectious complications and bleeding. Test results were: hyperferritinemia (100%), hypertriglyceridemia (87.5%), liver enzyme alteration (52.9%), hypofibrinogenemia (33.3%), esplenomegaly (70.6%), hemophagocytosis in bone marrow (94.1%), 100% of tested patients presented high sIL-2R level.

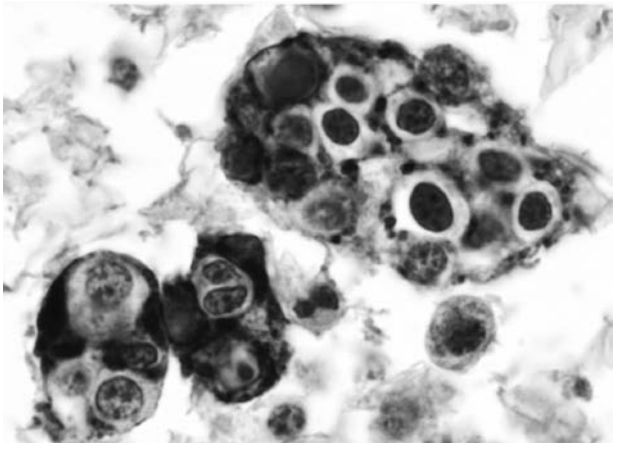

Figure 1.

Summary/Conclusions: Despite being a serious disease and with rapidly torpid course it remains underdiagnosed, obtaining the diagnosis, in most of the cases after hemophagocytosis phenomena is visualized in bone marrow. According to the literature, the main reasons for consultation and hospitalization are similar to those presented in our hospital. The response to treatment is difficult to compare because most of patients has been treated with different therapeutic regimens. In conclusion, it is always needed considering the possibility of a HLH diagnosis when we have a patient with fever and pancytopenia not clearly explained, and the importance of requesting simple test with high profitability such as ferritin and triglycerides in all suggestive picture.

PB2056

SPLEEN ENLARGEMENT AS ONLY MANIFESTATION OF NIEMANN PICK DISEASE TYPE C

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Background: Niemann-Pick disease type C (NPC) is a rare autosomal recessive lysosomal storage disorder (LSD) characterized by multiple lipid storage triggering intracellular impairment trafficking. NPC shows a wide phenotypic spectrum (visceral, neurological and psychiatric manifestations), the most of patients develop symptoms in early childhood; however there are a subtype mainly identified in adults and characterized by aisle spleen enlargement and cytopenias raising the differential diagnosis with a hematologic malignancy.
Aims: The main objective of this study is report probands state NPC only-visceral phenotype and its characterization. Secondary aims are identifying if plasma lysosomal biomarkers can facilitate the differential diagnosis between LSD and haematological malignancies and if that only-visceral phenotype presented genetic differences with neurological phenotypes
Methods: Firstly, plasma lysosomal biomarkers as Chitotriosidase activity (ChT), CCL18/PARC and 7-Ketocholesterol (7-KC) concentrations were assessed trough biochemistry approaches ChT activity was evaluated through an artificial substract, CCL18/PARC concentration using ELISA technique and 7-KC employing liquid chromatography and tandem mass spectrometry. Lastly, genetic characterization of *NPC1* and *NPC2* genes were carried out. Clinical/analytical records were gathered from the information provided by physicians.
Results: In the last five years, 363 probands were recruited. 51 of them were identified at least one genetic variation reported as pathogenic or unknown significance (VUS) in NPC, 10/51 showed only-visceral symptoms related to NPC. Focused in only visceral cases, they were 35.0 (13.4-41.5) [median (Interquartil range)] years old and a ratio male/female 6/4. None developed neurological symptoms during following-up. Splenomegaly was 16.5 (9.2-18) cm under costal rib in all cases with size information and 1 case presented neonatal jaundice. Hemoglobin 14.5 (12.7-15.3) g/dL, platelets 121.5 (106-231.5) $x10^9$/L. Increased biomarkers: ChT activity 119.0 (69.0-266.0) nmol/mL/h, CCL18/PARC and 7-KC concentration were 156.0 (151.0-158.0) ng/mL and 190.3 (103.7-355.9) ng/mL respectively. Four patients had been splenectomized. The histological study showed numerous histiocytic cells, loaded with small vacuoles and lipid appearance ("foamy cells"). These cells were CD68+ and some of them were stained with hard Giemsa blue and PAS positive, diastase resistant, while others showed a slight positivity. Molecular variants identified in *NPC1*, reported as pathogenic or VUS, were: p.C177T, p.N222S, p.V664M, p.Q775P, p.N916S, p.P1007A, p.D1097N, p.A1151T, p.L1241*, p.R1274T and c.1998+8C>G. *NPC2* gene does not show any variations. All genetic variations were found in heterozygosity, 6/10 subjects presented two variants.
Summary/Conclusions: The biomarkers CCL18/PARC or 7-KC are increased in all cases that can be analyzed and they constitute good indicators of lysosomal storage disease that might facilitate the differential diagnosis with hematologic malignancies. None of the genetic variants found in *NPC1* were exclusively for this only-visceral phenotype.

PB2057

THROMBOPOIETIN MIMETICS AS "A BRIDGE TO RECOVERY". SINGLE CENTER EXPERIENCE IN FOUR PATIENTS WITH CHRONIC PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: The thrombopoietin (TPO) mimetics are approved for the treatment of chronic primary immune thrombocytopenia (ITP) and have the potential for use in several additional clinical settings. Treatment is usually indicated in patients with platelet counts <$30x10^9$/L and those with counts between 30 and $50x10^9$/L with bleeding or at risk of bleeding (*e.g.* planned surgery, dental extraction, parturition, active peptic ulcer). Although most ITP patients require initial or second line treatment, sometimes platelet levels or hemorrhagic manifestations are not clinically relevant and observation may represent an acceptable clinical strategy. Unfortunately, clinical management of chronic ITP may change over time because of the onset of new clinical condition that may require a temporary higher platelets number for a safe clinical management. Surgery, cancer chemotherapy, dangerous activities may temporarily change the therapeutic strategy of patients with chronic ITP. In these patients there is a significant probability of obtaining some improvement or remission but "on demand treatment" with short courses of therapy (often with corticosteroids, IVIg or TPO mimetics) although warranted to manage bleeding or high-risk of bleeding, are poorly investigated and not sufficiently addressed in the current guidelines.
Aims: In particular some patients with a otherwise "safe" platelet levels may require a temporary treatment to overcome the onset of new clinical conditions. Here, we report on four patients with refractory ITP who received TPO-mimetics as a bridge to recovery from surgical procedures or chemotherapy treatment for diagnosis of cancer.
Methods: Four patients with isolated ITP were given TPO mimetics to increase the platelet count prior to initiation their medical o surgical treatment. We reviewed their baseline clinical characteristics, and clinical course.
Results: We found that a short course of Romiplostim or Eltrombopag may increase the platelet counts enough to enable surgery or to complete chemotherapy. All 4 patients achieved sustained remissions requiring no further treatment. No rebound thrombocytopenia was observed after stopping Romiplostim and none of the patients had bleeding or thrombotic complications.
Summary/Conclusions: Our clinical experience shows that TPO mimetics treatment, in certain patients with milder chronic ITP, may be chosen as intermittent treatment to temporarily raise platelet counts to overcome upcoming surgery, chemotherapy treatment or other clinical conditions requiring a "safer" platelet level.

PB2058

EVALUATION OF INTRACRANIAL CEREBRAL BLOOD FLOW VELOCITIES IN SPLENECTOMIZED AND NON-SPLENECTOMIZED B-THALASSEMIA INTERMEDIA PATIENTS USING TRANSCRANIAL DOPPLER SONOGRAPHY

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Background: Beta-thalassemia intermedia (βTI) is a congenital hemolytic anemia with a widely variable clinical phenotype. A high incidence of clinically silent cerebral ischemic events (SCI) has been reported in splenectomized β-TI patients. These could be due to cerebral large-vessel disease.
Aims: Based on the example of sickle cell disease, we applied transcranial Doppler sonography (TCD) to evaluate cerebral vessels velocity as a possible indicator of cerebral vasculopathy in these patients.
Methods: We included in our study 30 patients with βTI, 17 patients with splenectomy (group A) and 13 non splenectomised ones (group B), aged between 13 and 63 years-old (median: 34, mean: 40). Non imaging TCD was performed in all patients and the TAMV values in the anterior (ACA), middle (MCA), posterior (PCA) cerebral and the basilar (BA) artery were measured. Nine of the patients have been also examined by brain MRI/MRA (7 patients from group A and 2 patients from group B).
Results: None of the patients had a TAMV value ≥200cm /sec. In group A the maximum TAMV of the examined vessels were 104 cm/sec MCA R, 139 cm/sec MCA L, 89 cm /sec ACA R, 93 cm/ sec ACA L, 59 cm /sec PCA R, 64 cm/sec PCA L, 75 cm/sec BA. In the same group the mean and median values were 72.4/ 75 cm/sec MCA R, 72/72.5 cm/sec MCA L, 66.3/67 cm /sec ACA R, 59/57 cm/sec ACA L, 43.5/43 cm/ sec PCA R, 43.5/ 41 cm /sec PCA L, 55.5/ 55 cm /sec BA respectively. In group B the maximum TAMV were 88 cm/sec MCA R, 93 cm/sec MCA L, 98 cm /sec ACA R, 93 cm/ sec ACA L, 55 cm /sec PCA R, 59 cm/sec PCA L, 68 cm/sec BA. In the same group the mean and median values were 69.6/ 66 cm/sec MCA R, 76.5/78 cm/sec MCA L, 69.7/69 cm /sec ACA R, 56.7/57 cm/sec ACA L, 38.5/37 cm/ sec PCA R, 43.6/44 cm /sec PCA L, 47.1/ 46.5 cm /sec BA respectively. We found no statistically significant difference ($p>0.05$) in the TAMV values for all examined vessels between the two groups. No statistically significant difference was found between the two groups concerning age, gender, hemoglobin and hematocrit levels. There was a statistically significant difference in PLT blood count that was elevated in splenectomised β-TI patients ($p<0.01$) as it was expected. From the patients who were also examined by MRI only one had a SCI lesion on MRI, but without any abnormality on TOF-MR angiography.
Summary/Conclusions: Our results do not show increased TAMV velocities and do not support the presence of large vessels vasculopathy in splenectomised β-TI patients. These findings agree with recent published data according to which SCIs in these patients might be due to microangiopathy or venous thromboembolism.

PB2059

FAT EMBOLISM SYNDROME IN 4 PATIENTS WITH NON-HB SS SICKLE CELL DISEASE

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Background: Fat embolism secondary to bone marrow necrosis may occur in patients with sickle cell disease giving rise to respiratory, neurological and haematological complications - the so-called fat embolism syndrome (FES). FES is more common in non-SS genotypes.
Aims: We present 4 recent cases of suspected or confirmed FES in adult patients with Hb SC seen in our hospitals and review the clinical, radiological and histological findings of this under-recognised and potentially devastating complication of 'milder' forms of sickle cell disease.
Methods: Case 1. A 53 year old man with Hb SC was admitted with malaise, fevers and rigors. The following day he dropped his GCS and was transferred to ITU. MRI brain showed widespread small infarcts. He became pancytopenic. Bone marrow examination showed widespread necrosis. Follow-up MRI showed multiple microhaemorrhages. Despite supportive care including top up transfusions he retained a major neurological deficit. Case 2. A 50 year old woman with Hb SC was admitted with abdominal and joint pain. That evening, she deteriorated with hypoxia, pyrexia and tachycardia. CT showed no major PE. She was given antibiotics and transferred to ITU where exchange transfusion was initiated. A drop in the platelet count was noted. She suffered a cardiac arrested and died. At post mortem pulmonary fat embolism was found. Case 3. A 56 year old man with Hb SC was admitted with body pain and breathing difficulties and deteriorated within hours. Chest x-ray showed bilateral haziness. Despite antibiotics he deteriorated further with a fall in GCS and acute kidney injury and was transferred to ITU before being exchange transfused. CT showed cerebral oedema. An MRI performed a few days later showed numerous widespread small haemorrhagic foci. GCS remained low despite removal of sedation. Case 4. A 30 year old man with Hb SC was admitted with leg and arm pain. Over the following days he complained of back pain of an unusual severity. On the third day of admission, he suddenly deteriorated with hypoxia and confusion. He developed acute renal failure, deranged liver function tests and coagulopathy. He had a rapid drop in haemoglobin and platelets. He was admitted to the intensive care where an exchange transfusion was performed. MRI brain showed numerous microhaemorrhages. An MRI of his lumbosacral region showed extensive bone marrow change in keeping with the suspected diagnosis. He had a stormy few days on ITU but slowly improved and was discharged from hospital alive but with subtle cognitive issues.
Results: Fat embolism is an under-recognised phenomenon associated with major morbidity and mortality in sickle cell disease. The presentation may mimic a thromboembolic complication but is often characterized by a combination of venous and arterial infarction. It has a characteristic radiological appearance when involving the brain. Pancytopenia with high numbers of nucleated red cells is typical. LDH is often very high.
Summary/Conclusions: A high index of suspicion is required to make the diagnosis of fat embolism syndrome ante-mortem. In case reviews, a survival advantage has been reported in patients managed with exchange transfusions although this has not been verified by prospective studies.

PB2060

RESULTS OF STANDARD TREATMENT FOR APLASTIC ANEMIA IN CHILDREN

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Background: Acquired aplastic anemia (AA) is a disorder characterized by pancytopenia, hypocellular bone marrow and an absence of infiltrative disease of the bone marrow. The incidence of AA in Russian Federation is 2-6 cases among 1 million children per year. The hematopoietic stem cell transplantation (HSCT) is the most effective treatment for AA. Immunosuppressive therapy (IST) including antilymphocyte globulin, cyclosporine is the standard therapy for patients who do not have a HLA-matched sibling donor.
Aims: Evaluation of results of treatment of children with acquired aplastic anemia in 2005-2015.
Methods: The study included 23 children with acquired aplastic anemia at the age from 3 to 18 years (median age - 11 years): 11 boys and 12 girls. Very severe disease was diagnosed in 12 patients, severe AA - in 8, non-severe AA- in three patients. The hemorrhagic syndrome with uterine, nasal and intestinal bleeding was observed in 25% of patients.
Results: Remission was achieved after the first course of IST in 12 patients. The second course of IST was held in 5 patients with very severe AA, including as a therapy for relapse in three patients. Early hematologic relapse was diagnosed in 2 patients, the later relapse - in 1 patient. HSCT from an related donor carried two children after the first course of ICT. Unrelated donor HSCT was performed in two patients after the second course of IST. Five patients at this time continue to receive cyclosporin therapy, two of these patients are dependent on transfusional support. One child was killed in a month from the beginning of ICT. Cause of death - sepsis, severe hemorrhagic syndrome. On 01.01.2016, 17 patients (77%) are in complete clinical remission.
Summary/Conclusions: Five year overall survival rate 95,6%. Event-free five year survival rate 80,3%.

PB2061

THE OUTCOME OF HIGH RISK LANGERHANS CELL HISTIOCYTOSIS (LCH) IN EGYPTIAN CHILDREN. A SINGLE CENTER EXPERIENCE

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Background: High risk multi-system organs (RO+) Langerhans cell histiocytosis (LCH) including hemopoietic cytopenias, liver and spleen disease, have the highest mortality if failing first line treatment. Methotrexate (MTX) association with Vinblastine (VBL) and Prednisone (PRED) has been tried upfront and a second line cladribine (2cda) has been proposed in RO+LCH.
Aims: To present the outcome of RO+ LCH in a pediatric single centre population.
Methods: A total of 50 RO+ LCH patients, M/F 26/24, median age 1.7 years (0.19-10.1) treated between August 2007 and June 2015 were retrospectively analyzed. A first line VBL, PRED with MTX without a different second line was adopted till 2012 where methotrexate was omitted and 2cda was introduced as a salvage line.
Results: The 5 year overall survival (OS) is 69% and the event free survival (EFS) is 17%. At week 6 and 12, "better' response was obtained in 37(74%) and 33 (66%) patients respectively. Seventeen (34%) and 15 (30%) patients progressed and reactivated their disease respectively. At last follow up, 34 and 2 patients were in 'better' and 'worse' status respectively and 14 patients died. In univariate analysis, the factors that affected OS were hemopoietic cytopenias with 33%, 76% and 62% in tri, bi and monocytopenia respectively p0.017, hepatic affection with 25%, 93% and 61% in hepatic dysfunction, hepatomegaly and both respectively p 0.027, combined RO+ with 47%, 68% and 100%, in tri, bi and mono organ(s) respectively p 0.045, number of induction: 47% and 76% in one cycle and 2cycles respectively p0.026, failure of 1st line treatment: 27%, 76% and 50% in progression, reactivation and both respectively p <0.001,Salvage with 2cda 56%, and 45% without p0.001, progression salvage with 2cda 57% and 10% without p0.008. The factors that affected EFS were cytopenias with 0%, 19%, and 34% in tri, bi and monocytopenia respectively p0.015, bony CNS risk sites with 9% *versus* 27% if none p 0.06, MTX 26% with and 13% without p0.005. The factors that affected reactivation were: male gender p0.014, season autumn/winter p<0.01, lung cysts p0.043. The factors that affected disease progression were tricytopenia p <0.01, hepatic dysfunction p<0.01, hepatic dysfunction and hepatomegaly combination p0.04.
Summary/Conclusions: The outcome of RO+LCH in pediatric egyptian population remains satisfactory despite a high proportion of reactivation. Disease progression post induction carries a dismal prognosis without a salvage line. Hemopoietic tricytopenias and hepatic dysfunction carry the worst prognosis. Methotrexate as a 1st line and 2cda as salvage line are efficient in improving the outcome.

PB2062

GENDER DISPARITIES IN CARDIO-RESPIRATORY FITNESS AMONG A COMORBIDITY FREE ANEMIC POPULATION

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Background: Anemia is a common hematologic disorder characterized by reduced absolute number of circulating red blood cells. It has been established as a poor prognostic factor in a variety of medical disorders. Nonetheless, the functional impact of anemia on cardiorespiratory fitness (CRF) in healthy individuals has mostly been explored in small interventional studies, simulating acute rather than chronic states.
Aims: Using differing criteria for anemia diagnosis, while analyzing males and females separately, the primary and secondary objectives were to evaluate the effect of anemia on CRF and overall survival, respectively, in apparently healthy adults.
Methods: We retrospectively analyzed 16,332 self-referred subjects undergoing exercise stress testing as part of a routine screening program. Subjects were non-smoking, free of diabetes, ischemic heart disease, or chronic kidney disease. Anemia was defined by either the World Health Organization (WHO) criteria (Hemoglobin (Hb) less than 13 g/dl and 12 g/dl for males and females, respectively), or Beutler and Waalens' (BW) criteria (*Blood, 2006*) (less than 13.7 g/dL and 13.2 g/dL for males younger and older than 60 years, respectively, and 12.2 for females). Fitness was categorized into age- and sex-specific quintiles according to Bruce protocol treadmill time. Multivariate logistic regression was used to evaluate the association between anemia and low CRF in the

baseline visit as well as during multiple visits. A multivariate Cox proportional hazards regression model was constructed for survival analysis.
Results: The mean age of the cohort was 46±10 years and 70% were men. Median follow-up was 10 years. Mean Hb levels were 13.09±0.96 and 15.05±0.96 among women and men, respectively (p<0.001), with higher proportion of anemia among women (10.8% *versus* 1.6% according to WHO criteria, and 15% *versus* 6.1% according to BW criteria, respectively; p<0.001 for both). Baseline visit anemia, according the WHO and BW criteria, was associated with a risk adjusted increase of 33% (1.06-1.66, 95% confidence interval (CI)) and 24% (1.01-1.52, 95% CI) for low CRF, respectively (p<0.05 for both). However, no association was observed in males, regardless of anemia threshold. These findings were corroborated in a repeated measures analysis of 71,200 sequential visits (Figure 1), where only in females, anemia resulted in a 38% (1.17-1.63, 95% CI) and 22% (1.05-1.40, 95% CI) increase in risk for low CRF (p<0.01 for both). Lack of regular physical activity, elevated body mass index, total cholesterol and fasting glucose levels were also independently associated with low CRF in the multivariate model, regardless of gender. With respect to survival, only anemia in men, as opposed to women, was associated with an increased risk for mortality, especially when adopting a more stringent criteria for diagnosis (hazard ratio of 3.06 (1.65-5.68, 95% CI) and 1.83 (1.06-3.17, 95% CI), using the WHO criteria and BW criteria respectively, p<0.05 for both).

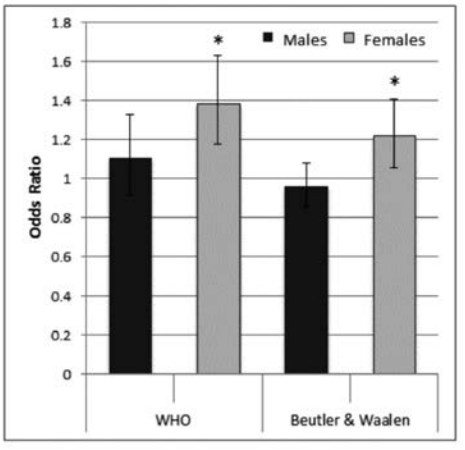

Figure 1 – Risk for low cardiorespiratory fitness, across sequential visits, in anemic versus non-anemic subjects. Bars represent 95% confidence intervals.
* p < 0.01 for incorporation in a logistic regression model.

Figure 1.

Summary/Conclusions: Anemia's functional and prognostic significance differs between genders in a cohort of comorbidity free subjects. Anemia, regardless of diagnostic criteria, was associated with low fitness only in females, while increased mortality was solely observed in males. Taken together our findings indicate differing anemia mechanisms and physiological responses between genders. Moreover, we suggest a functional and prognostic, rather than an epidemiologic approach, for setting anemia diagnosis thresholds.

PB2063

ASSESSMENT OF A COHORT OF BETA THALASSEMIA PATIENTS: NEW CHALLENGES

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Background: The combination of transfusion and chelation therapy has dramatically extended the life expectancy of Beta-thalassemic patients who can now survive into their fourth and fifth decades of life. The goal of long-term transfusional support is to maintain the patient's hemoglobin level at 9-10 g/dl. However, complications are still frequent and affect the patients' quality of life, particularly in countries with limited resources.
Aims: The main objective of this study is to determine the prevalence of prominent complications in a cohort of Algerian thalassemia patients.
Methods: This study was conducted to assess 81 polytransfused Beta-thalassemic patients, mean age 16 years (6-31), sex ratio: 37M/44G: We assess blood transfusion regime, pre-transfusion hemoglobin, iron overload, by serum ferritin level, hepatic iron concentration. Bone mineral density (BMD) measurements at lumbar and femoral regions have been done using dual x-ray absorptiometry. We assess Growth and pubertal maturation (Taner stage), and dosing gonadic hormone dosage, calcium, vitamin D, Parathyroid, and thyroid hormone.
Results: The pretransfusion hemoglobin level was maintained between 6 and 9 g/dl in 90% of patients. Additionally, 5% had levels less than 6 g/dL, 5% from 9 to 11 g/dL. Fifty-five percent of patients had undergone splenectomy at a median age of 9 years (range, 1-31 years). Ferritin levels ranged: 147 to 13500 ng/mL (median, 4992 ng/mL). Among 44 patients assessed, 77% had values of 15 mg/g dry weight or higher, 50% LCI>43 mg/gdw, 20% had moderate LCI 15 to 7 mg/gdw, only 1 patient <7mg/gdw. Hepatic biopsy, performed in 26 patients (assessed for bone morrow transplantation), all had hepatic fibrosis moderate to severe (Metavir score). BMD performed in 49 patients, prevalence of lumbar osteoporosis and osteopenia were 38% and 62%. Dosage of vitamin D performed in 34 patients:5 <10ng/ml, 17 [10-20ng/ml], 8 [20-30ng ml], 4 normal >30ng/ml. 12 patients receive Biphosphonate treatment. Short stature was seen in 65% of our patients. Hypogonadism was diagnosed in 22,9% of 74 patients who had reached pubertal age: 50% of hypogonadic females and males were receiving hormonal substitution. No patient had Hypoparathyroidism and primary hypothyroidism. 3 cases of primary amenorrhea, and 2 diabetes aged 17 and 31 years. Despite high ferritine serum no patients had heart disease requiring medication. The goal of long-term hypertransfusional support is to maintain the patient's hemoglobin level at 9-10 g/d. This threefold is rarely reached in our patients, for mixture of reasons (compliance, limited blood pack). Iron chelation has been formerly limited, reason of the high prevalence of hemochromatosis in the majority of our patients. Intensification of iron therapy is now assessed to obtain reversal tissues lesions. Well treated 90% of thalassemics patients reached normal puberty; in contrast, in our group of patients, only 20% achieved normal pubertal status after 16 years. Poor pubertal growth and impaired sexual maturation, and endocrine abnormalities in children, adolescents and young adults have been observed because of conventional treatment deficiency, substitutive hormonal therapy is indicated. BMD is a good index of bone status in patients with Thalassemia and should be done in these patients annual.
Summary/Conclusions: High prevalence of complications among our thalassemics signifies the importance of more detailed studies along with therapeutic interventions. The survival of patients with thalassemia major improving, but the prevalence of severe complications is still high.

PB2064

MYCOPLASMA PNEUMONIAE AS A CAUSE FOR NEUTROPENIA IN CHILDREN

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Background: Extrapulmonary manifestations of *Mycoplasma pneumoniae* infection are uncommon and include hematologic, gastrointestinal, musculoskeletal, dermatologic, and neurologic complications. Neutropenia on the other hand can be caused by a variety of causes including infections. The microorganisms that are commonly associated with neutropenia are: viruses (Epstein-Barr virus, Cytomegalovirus, hepatitis viruses, human immunodeficiency virus, parvovirus B19, influenza, measles), bacteria (Salmonella, Brucella, Bordetella pertussis, Rickettsia), mycobacteria and rarely fungi (disseminated histoplasmosis).
Aims: Based on the observation that some of our patients that were evaluated for neutropenia had serologic evidence of recent Mycoplasma infection we hypothesised that *Mycoplasma pneumoniae* might also *be* a cause of neutropenia in children.
Methods: To this aim we retrospectively studied the medical records of all patients that had been hospitalized in a tertiary pediatric department during a 17-month period because of a serologically proven *Mycoplasma pneumonia* infection (positive IgM antibody titers). The demographic characteristics (age, gender), the clinical presentation, medications and other laboratory parameters were also recorded. Neutropenia was defined as an absolute neutrophil count below the 3rd percentile for age and gender. Every patient was matched for age and gender with another patient with similar clinical presentation but in whom antibodies against *Mycoplasma pneumoniae* were not detected. Student's t-test and chi-square test were used for statistical analyses as appropriate.
Results: In total 290 patient were tested for Mycoplasma. Ninety patients (50 females) with positive IgM antibodies againt Mycoplasma pneumoniae were identified. Amongst them 18 patients (20%) were also found to have neutropenia (four severe, four moderate, and 10 with mild neutropenia). Mean age (±SD) was 7.11 (±3.67) years. Mean neutrophil count was 1045/mm^3. Thirteen patients (72%) presented with a respiratory tract infection, two were investigated because of fever of unknown origin, one for ITP, one for parotitis and one for cervical lymphadenopathy. All patients recovered from their neutropenia. This group was then compared with the patients that had negative antobodies for Mycoplasma, in which 7 cases of neutropenia were recorded (7.8%). Comparison with chi-square test based on a two by two table showed that this difference is statistically significant (p<0.05).
Summary/Conclusions: Infection with *Mycoplasma pneumoniae* is usually associated with hemolytic anemia. There are only a few reports of other hematologic toxicities in humans. In this study we showed that Mycoplasma infection

might be associated with a form of a transient post-infectious neutropenia in 20% of the study group. Given the fact that evaluation for neutropenia maybe time consuming and expensive, it is important for practitioners to be aware of this rare complication of Mycoplasma infection. Moreover, should this observation be confirmed, the administration of an appropriate antibiotic might be beneficial and shorten the duration of the neutropenia.

PB2065

EVANS SYNDROME: A RETROSPECTIVE STUDY OF 30 CASES

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Background: Evans syndrome is a very rare autoimmune disorder in which an individual's antibodies attack their own red blood cells and platelets. Both of these events may occur simultaneously or one may follow on from the other. Usually idiopathic, it is also associated with lymphoproliferative disorders and autoimmune diseases.
Aims: Study the epidemiological, clinical, biological, etiological and therapeutic aspects of Evans syndrome.
Methods: This is a retrospective and analytic study about 30 cases of Evans syndrome observed in the hematology department of Sousse, over a period of 14 years.
Results: There were 18 men and 12 women (sex ratio=1,5) with a median age of 40 years. The circumstances of discovery were an anemic syndrome in all patients, mainly due to paleness and asthenia. Concerning biology, regenerative anemia was normocytic in 20 cases and macrocytic in 10 cases, thrombocytopenia below 100000/mm^3 was observed in all patients. There were also biological signs of hemolysis: hyperbilirubinemia, high LDH rate in all patients. Direct Coombs test was positive for Ig G + Complement, IgG and complement in 13, 8 and 9 cases, respectively. Evans syndrome was idiopathic in 20 cases and secondary to lymphoproliferative disorders in 5 cases, autoimmune disorders in 5 cases. All patients received corticosteroid treatment in addition to folic acid therapy and etiological treatment in the non idiopathic cases with complete remission in 10 cases. Immunosuppressive therapy (endoxan) was prescribed in 10 patients with complete remission in 5 patients. Anti-CD20 monoclonal antibody was prescribed in 8 patients with complete remission in 4 cases.
Summary/Conclusions: Glucocorticoids and/or intravenous immunoglobulins are the mainstay of the treatment in the majority of patients with Evans syndrome. When these treatments fail, patients often require cytotoxic drugs or splenectomy.

PB2066

THE STUDY OF THE SPECTRUM OF THALASSEMIC MUTATIONS AT THE NORTH-WEST REGION OF RUSSIA

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Background: Beta-thalassemia - a hereditary disorders of hemoglobin synthesis, which are transmitted in an autosomal recessive inheritance. The main stage of the prevention of thalassemia is the molecular diagnosis of thalassemic mutations in the fetus conducted at 1st trimester of pregnancy. The possibility of this diagnosis is determined by the availability of data on the molecular defects of beta-globin gene in this region.
Aims: To examine the incidence of mutations of beta-globin gene in the North-West region for the development of measures for the prevention of beta-thalassemia.
Methods: Molecular diagnostic was performed at 65 patients (men - 31, women - 34, from 2 to 58 years) with a diagnosis of beta-thalassemia (minor and major), were examined and treated at The Consultative and Diagnostic Center for Children and The Russian Research Institue of Hematology and Transfusiology (Saint-Petersburg). All patients living in St. Petersburg, but have a different ethnic composition: 40% - Azerbaijanis; 33.8% - Russian, who (according to them) havn't Caucasian or Mediterranean roots; 11.3% - Bulgarians, Cypriots; 8.7% - Dagestan; 6.2% - patients of mixed families (one parent - Russian, the other - from the Caucasus or Mediterranean). Diagnos thalassemia set on the basis of red blood cells parameters (microcytosis - the MCV <80 fl, hypochromia - MCH <27 pg, Mentzer index <11.4) and hemoglobin fractions (Hb A2>3% and/or Hb F>1%). The material for the study served as the venous blood. Red blood parameters were determined on the hematology analyzer Sysmex XT-4000i (Sysmex, Japan), fractions of hemoglobin investigated by capillary electrophoresis (MINICAP, Sebia, France). Mutations of the beta-globin gene was determined by reverse- hybridization of method of biotinylated multiplex-PCR products (β-GlobinStripAssay, ViennaLabDiagnostics, Austria).
Results: In examined patients 5 homozygous (large form of thalassemia), 2 compound heterozygous and 58 heterozygous (small form) were applied. A total of 10 thalassemic mutation was found: codon 8 (-AA) - 38%; IVS 1.110 (G>A) - 27%; 5 codon (-CT) - 7%; IVS 1.6 (T>C) and IVS 2.1 (G>A) - 6%; IVS 2.745 (C>G), IVS 1.1 (G>A) iIVS 1.5 (G>C) - 4%; codon 8 \ 9 - 3%; -101 (C>T) - 1%.
Summary/Conclusions: The most frequent mutations of the beta-globin gene in St. Petersburg were codon 8 (-AA) - 38% and IVS 1.110 (G>A) - 27%.

PB2067

THE ETHNIC DIFFERENCE IN RBC-RELATED INDICES BETWEEN KOREAN AND UAE AND THE MUTATION PROFILE IN THALASSEMIA OF UAE: ONE-YEAR EXPERIENCE IN UAE AS THE KOREAN PHYSICIANS

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Background: Sheikh Khalifa Specialty Hospital (SKSH) of the United Arab Emirates (UAE) has been operating by Seoul National University Hospital of the Korea since February 2015. The rules of three [3 x red blood cells (RBC)=Hemoglobin (Hb), 3xHb=hematocrit (Hct)] has been used to evaluate RBC-related indices of patient samples. We empirically found out that Arab patients are showing the discrepancy in these ratios due to relatively low mean corpuscular volume (MCV) in comparison with Korean patients, although they are in the non-anemic state. Also, regarding the thalassemia, characterized by the microcytosis, the prevalence of thalassemia in Korea is much lower than those in UAE. Therefore, the microcytic feature of UAE patients and thalassemia have been the unique experiences to Korean physicians.
Aims: First, we analyzed the RBC-related indices of different ethnic groups (Korean *vs* Arab) and adult patients showing microcytic anemia, such as iron-deficiency anemia (IDA) and thalassemia. Second, we reported the mutation profile showing in thalassemia that diagnosed in our hospital during one-year.
Methods: We collected the additional blood sample for the complete blood cell (CBC) count from the SKSH staff who received a medical check-up and gave written consent to the study. Among the data obtained, we selected data from Korean and Arab staff who were in the non-anemic state according to the WHO criteria (Hb ≥13 g/dL in male and Hb ≥12 g/dL in female). RBC indices, such as the MCV, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were measured. We also retrieved the CBC data from the IDA patients showing the ferritin level of lower than 10 ng/mL, and from the patients diagnosed as thalassemia through the Hb electrophoresis or *HBA1/HBA2* and *HBB* gene analysis.
Results: The data from 48 Korean staff, 53 Arab staff, 69 IDA patients, and 21 thalassemia patients were enrolled. The RBC-related indices are following according to the groups; MCV, 92.2±3.5 fL (range, 85.3-102.0 fL), 86.9±4.2 fL (73.7-94.4 fL), 72.0±7.1 fL (58.1-90.0 fL), 70.4±5.5 fL (61.1-82.5 fL), respectively; MCH, 30.8±1.4 pg, 28.2±1.9 pg, 21.3±3.3 pg, 21.6±2.5 pg, respectively; MCHC, 33.4±1.1 g/dL, 32.5±1.2 g/dL, 29.3±2.4 g/dL, 30.5±1.5 g/dL, respectively. The MCV, MCH, and MCHC of Korean are significantly higher than those of Arab (P <0.001, P <0.001, P=0.001, respectively). There are no significant differences in RBC-related indices between IDA and thalassemia patients in our study. For mutation profile of thalassemia, we analyzed total 22 adult and children thalassemia patients confirmed by gene analysis. Among 22 patients, 12 were α-thalassemia, seven were β-thalassemia, two were diagnosed with both α- and β-thalassemia, and one was diagnosed with both α-thalassemia and sickle cell anemia. The most common α-thalassemia was α$^+$-thalassemia trait due to 3.7-kb and deletion of *HBA1/HBA2* gene (7/14, 50.0%) and the most common mutation of β-thalassemia was c.92+5G>C mutation of *HBB* gene (5/9, 55.6%). Distinctively, the homozygous or heterozygous c.1-?_429+?del in the *HBA2* gene were detected in four α-thalassemia patients, which has not been reported previously.
Summary/Conclusions: There is the difference in the RBC size between Korean and Arab. We found out the unique deletion mutation of alpha-thalassemia. This is the report by one-year experience in UAE as Korean physicians. We expected to report the characteristics of RBC size and thalassemia mutation profile in UAE with accumulated experience in the future.

PB2068

Abstract withdrawn.

PB2069

THE INCIDENCE OF CD36 DEFICIENT MONOCYTE AND PLATELET AND ITS CORRELATION WITH DIABETES IN SOUTH KOREA

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Background: CD36, a synonymous term for platelet glycoprotein IV, GP (glycoprotein) IIIb, is an 88-kD glycoprotein that is expressed in such various cells as platelets, monocytes, macrophages, erythroblasts, capillary endothelial cells, adipocytes, epithelial cells in the kidney and cardiac myocytes. Hematologically,

it is used as a representative antigen of platelets and monocytes in flow cytometric differential cell counting. Various reports prove the existence of racial variations with regard to CD36 deficiency: 0.3% in Caucasians, 3-11% in Japan, 0.5% in Type 1 and 1.3% in Type II for Chinese, and 0.3% of the U.S. population. And other studies show decreased insulin sensitivity and postprandial hypertriglyceridemia as the metabolic manifestations of CD36-deficient subjects. However, there has been no report on the nature of CD36 deficiency for Koreans.
Aims: The authors aim to find out CD36 deficiency incidence of South Korea and any potential correlations between diabetes patients by studying blood samples from diabetes patients, leukopenic samples, and normal samples.
Methods: We collected data from 757 individuals including 521 leukopenic patients, 105 diabetes patients, and 131 normal subjects at Seoul St. Mary's Hospital, Seoul, Korea between August 2013 and March 2014. Routine CBC with WBC differential count with an automatic blood cell analyzer (DxH800) was performed in conjunction with analysis by flow cytometry by using Hematoflow with Cytodiff. The 5-color/6-marker reagents (Cytodiff panel) were CD36-FITC, CD2-PE, CD294-PE, CD19-ECD, CD16-PC5, and CD45-PC7 antibodies. To confirm the platelets, CD41a-PE/CD36-FITC dual staining was added. To confirm the monocytes, CD11c-PE/CD36-FITC dual staining and peripheral blood smear slide reviews were used. We strictly adhered to the manufacturer's manual for analysis: 100 uL of blood samples was mixed with 10 uL of Cytodiff reagent, and incubated for 20 min at room temperature. Lysing solution (Versalyse solution; Beckman Coulter) was used for 15 min to break down red blood cells. After washing, a flow cytometer (FC500) was used to collect 10,000 cells. The analysis software, self-gating and separating populations by automatic logic pathways, analyzed the Cytodiff results automatically. The gates were adjusted only in case of large debris contamination or incomplete separation of basophils and myeloblasts. Samples were all analyzed in duplicate.
Results: Of the 757 cases, 22 cases (2.91%) were either of type I (9 cases, 1.19%), or of type II (13 cases, 1.72%). Of 131 normal subjects, 4 cases (3.05%) were either of type I (1 case, 0.76%), or of type II (3 cases, 2.29%). Of 105 diabetes cases, 3 cases (2.86%) were of type II. And finally, of 521 leukopenic cases, 15 cases (2.88%) were either of type I (8 cases, 1.54%), or of type II (7 cases, 1.34%). Our t-tests showed no significant differences among the groups, and no correlation was found between diabetes and CD36 incidence, or between leucopenia and CD36 incidences (chi-square test and Fisher's exact test), with the odd ratios of 0.97 and 0.98, respectively. No significant difference was found between the 22 cases and the remaining 735 cases in terms of sex, age, previous illness, hemoglobin level, WBC count, monocyte count, and platelet count.
Summary/Conclusions: The overall incidence of CD36 deficient monocytes and platelets based on 757 Korean subjects was 2.91%, manifesting no significant difference from the Japanese or Chinese studies, and no evident correlation with diabetes.

Platelets disorders

PB2070

OSELTAMIVIR IN THE TREATMENT OF PRIMARY IMMUNE THROMBOCYTOPENIA

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Background: Primary immune thrombocytopenia (ITP) is an immune bleeding disorder with an increased destruction of autologous platelets. Autoantibody specificity is usually against GPIIb/IIIa in 70-80% of patients or GPIb in 20% of them. Firts-line treatment includes immunosuppressive and immunomodulatory agents, that is corticosteroids (CS) or intravenous immunoglobulin (IVIG). Splenectomy is considered for patients with persistent lack of response. However, about 20% patients are inexplicably refractory to both therapies. Recent studies in refractory ITP patients suggest an association between anti-GPIb autoantibody and a poor response to conventional therapies, as well as platelet desialylation. This leads to platelet elimination by hepatocyte Ashwell-Morell receptors, especially in the absence of macrophages. Therefore, it may be a role for sialidase inhibitors in ITP treatment. Oseltamivir phosphate conventionally serves as anti-influenza agent. It inhibits viral neuraminidase as well as glycolipids sialylation on human T cells surface. We report a case of an adult ITP patient who was resistant to multiple therapeutic approaches but was successfully treated with Oseltamivir phosphate.
Methods: A 64-year-old female was diagnosed with ITP. She was initially treated with high dose CS and IVIG, with a complete response (CR) that lasted for 15 years. She relapsed with severe thrombocytopenia and minor bleeding. Her platelet counts remained low despite using CS and IGIV, so she initiated recombinant human thrombopoietin (TPO) agonist reaching maximum dose without response, so we added danazol and rituximab, either with non response. During this period, she presented with lower gastrointestinal bleeding so she underwent a splenectomy. She continued with TPO agonist and Danazol to maximum doses. Three months after splenectomy she reached secure but fluctuating platelet counts, presenting a massive portal and mesenteric thrombosis. She started therapeutical LMWH with close monitoring of platelets count, and suspended TPO agonist as well as danazol. Two months later, she lost platelet response and had to iniciate different immunosuppressive drugs: Vincristine, Azathioprine, Mycophenolate Mofetil and Cyclosporin, without response. Patient's platelet was then assessed by flow cytometry and revealed a loss of terminal sialic acids in platelets surface as well as neuraminidase activity in plasma, data compatible with a platelet destruction mechanism independent of immune receptors that could justify refractoriness to prior treatments. An alternative therapeutic regimen with Oseltamivir phosphate, as a last resort for the management of this patient, was initiated. She was informed and consented. It was given orally 75mg twice daily for 5 days. Then, a new platelet assessment was done, demonstrating normalization of sialic acids expression. But thrombocytopenia persisted, so Azathioprine was added looking for a synergic effect. Nowadays, she continues treatment with Azathioprine twice a day and remains in CR for seven months
Summary/Conclusions: In this case, treatment with oseltamivir could restore platelet membrane, avoiding its destruction by the hepatic rethiculoendothelial system and allowing immunosuppressive drugs (Azathioprine) to control the immune destruction mechanism. At the present time Oseltamivir is not still approved for ITP. However, this case highlights the need for prospective studies to verify the effectiveness of this new approach and the possibility of new indications for this drug.

PB2071

MULTIPLE PHENOTYPIC EXPRESSION OF HARRIS PLATELET SYNDROME

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Background: Harris platelet syndrome (HPS) is an autosomal dominant macrothrombocytopenia with mild to moderately severe thrombocytopenia and normal platelet function. HPS has been reported from healthy blood donors of the Indian subcontinent, particularly from the northeastern part of India including West Bengal.
Aims: A prospective study was done to characterize the different phenotypes of HPS among blood donors from West Bengal.
Methods: HPS is defined as healthy blood donors having a mean platelet volume (MPV) greater than 12 fL (normal <10 fL) with or without concomitant thrombocytopenia in the absence of any significant past history of bleeding disorder in either themselves or their first degree relatives. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) and analyzed using impedancemethod (Coulter SYSMEX X100, Tokyo, Japan).

Results: A total of 326 eligible blood donors were included in this study. Two hundred (61%) of the blood donors fulfilled criteria for a diagnosis of HPS. Three distinct phenotypes were observed. Fourteen (4%) was noted to have varying thrombocytopenia with normal MPV; 166 (51%) had anormal platelet count with giant platelets and 20 (6%) donors had classic HPS characterized by thrombocytopenia with giant platelets. HPS donors with low platelet count had a lower median platelet bio-mass compared with their normal counterparts, (13.62 *vs* 29.38, p<0.001).In comparing peripheral smears between controls (i.e normal platelet count and normal MPV) and the variants of HPS, no obvious difference was noted in the red blood cell morphology.

Table 1.

Blood Indices	Giant platelets with Normal Platelet Count (N=166)	Giant platelets with thrombovytopenia (N=20)	Normal MPV with thrombocytopenia (N=14)
Haemoglobin	13.74 ± 2.102	13.07 ± 2.250	12.84 ± 2.213
MCV	89.47 ± 6.720	88.45 ± 8.703	89.43 ± 6.915
Platelet Count	2.37 ± 0.609	1.02 ± 0.282	1.01 ± 0.278
MPV	13.52 ± 1.137	13.22 ± 0.879	10.61 ± 0.896
PDW	20.77 ± 3.001	20.48 ± 2.726	18.09 ± 1.889
Platelet Biomass	29.38 ± 6.761	13.62 ± 4.061	11.02 ± 3.494

Summary/Conclusions: A prospective population based study will be ideal to further explore the clinico-pathological significance of these variants of HPS.This study shows that HPS may manifest with isolated thrombocytopenia without giant platelets. It is important to reccognize this variant to avoid unnecessary investigations and treatment.

PB2072

FREQUENCY OF PLATELET FUNCTION DISORDERS IN PATIENTS PRESENTING WITH BLEEDING

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Background: Inherited platelet function disorders may present with bleeding of variable severity and unknown frequency and may be frequently missed. They are increasingly recognized as an important cause of bleeding, particularly in adolescent girls with menorrhagia. The prevalence of congenital platelet disorders in our population has not been established, however, these disorders are relatively more common in communities where consanguineous marriages are more frequent like in the Middle East and India, but very limited information is available in developing countries like Pakistan about their prevalence so we attempted to assess the frequency of these disorders in our population.
Aims: Considering the higher prevalence of consanguineous marriages in Pakistani population, it is assumed that the incidence of platelet disorder must be higher. Therefore, the purpose of this study is to determine the frequency of platelet function disorders in patients presenting with bleeding. Previous studies conducted in Pakistan observed all causes of bleeding disorders like Haemoglobinopathies, von willebrand disease, Hemophilia & others, but our study specifically focused on platelet function disorders because these can lead to severe bleeding and are frequently missed.
Methods: Cross sectional study done between March to October 2014 at section of Haematology Aga Khan University Hospital. 5mlof whole blood sample in sodium citrate tube was collected for detection of platelet functions disorders. Platelet rich plasma was made by centrifugation and was used for testing. Platelet function studies were performed by using aggregation platelet aggregometer (Chrono-Log aggregometer model 700). ADP, Collagen, Epinephrine and Ristocitin were used as agonists.
Results: 32 patients fulfilled inclusion criteria 18 females, 14 males with age range 3 years to 22 years. Out of these 32 patients 13 patients (40.62%) had platelet function disorder with female predominance (8 females, 5 males).Age ranges between 6 years to 22 years. Out of these 13 patients, 7 had Glanzmann's- thrombasthenia, 4 had Bernard-Soulier syndrome & 2 patients were labeled with Quebec platelet disorder.
Summary/Conclusions: 40% of these 32 patients had platelet function disorders. This is an ongoing study further data will be added on completion of specific period.

PB2073

ELTROMBOPAG USE IN SEVERE ITP AND BEYOND, A SINGLE CENTRE COHORT

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Background: Thrombopoietin receptor (TPO) agonists are licensed for immune thrombocytopenia purpura ITP). Potential for use in other thrombocytopenic conditions is being explored.
Aims: Our aim was to demonstrate the efficacy of eltrombopag in ITP -whether for severe disease or bridging therapy for surgery or chemotherapy. We also examined the use of eltrombopag in malignancy-associated thrombocytopenia, whether from disease or chemotherapy.
Methods: We retrospectively identified all patients who had received eltrombopag from a single institution. Patients were identified who had received eltrombopag as part of standard treatment pathways, as well as those for whom special consideration was sought (ie refractory thrombocytopenia in advanced malignancy). Eltrombopag was commenced from between March 2012 (as the first documented prescription time point) to January 2016. For ITP, we included those deemed to have a 'severe' phenotype according to the international consensus guidelines. All patients fulfilled these criteria, warranting second-line treatment beyond steroids and IVIg. Many had failed multiple previous lines or had comorbidities limiting options. For non-ITP thrombocytopenic patients, indication for eltrombopag was examined on a case-by-case basis, according to benefit *versus* risk, since prescription was off-label. The primary indication was to enable chemotherapy, based on oncology requirements for platelet counts >$100x10^9$/L.
Results: Our total cohort of 62 patients (36 males and 26 females) was treated over 8 years. This included 50 ITP patients requiring treatment for severe/refractory disease (n= 36), or bridging therapy (n=14). In the refractory group, 25 patients had primary ITP and 11 secondary ITP; including HIV (n=5), viral (n=2) malignancy (n=1) and other autoimmune aetiologies (n=3). Follow up was 1 to 85 months (median 12.5 months), with median 4 previous lines of therapy. Median time between ITP diagnosis and eltrombopag commencement was 24 months in the refractory ITP group. The delay was partially due to eltrombopag availability. In the overall ITP cohort (n=50), complete response (CR) was achieved in 28 patients (56%), partial response (PR) in 19 (38%) and no response (NR) in 3 patients (6%). Median time to response was 3 weeks (1 to 44 weeks). At follow up, 17 patients were in CR (34%) and 29 patients in PR (58%). Eltrombopag dose ranged from 25 to 100mg daily, with a median of 25mg (n=12). The difference in response at follow up was due to dose reduction, whilst maintaining haemostatic platelet counts. The malignancy-associated group included 12 patients. Eltrombopag use achieved CR in 6 patients (50%), PR in 3 (25%) and NR in 3 (25%).
Summary/Conclusions: In conclusion, eltrombopag use in the severe ITP setting achieved a response in 94% cases. For severe or refractory disease, dose reduction was possible once response achieved. Although not CR by strict definition, a safe platelet count could be maintained- typically >$50x10^9$/L- without bleeding sequelae. In the bridging cohort, eltrombopag proved a reliable means of improving platelet counts for intervention, without the problems associated with steroids or IVIg. Beyond ITP, eltrombopag may be of practical benefit during chemotherapy and other myelosuppressive treatment, to ensure optimal dosing of these therapies via platelet count support. Although our non-ITP cohort was small, CR was achieved in half of these patients. Further trials are needed to demonstrate which patients may benefit from eltrombopag during treatment for malignancy-associated thrombocytopenia.

PB2074

CHANGES IN PLATELET AGGREGATION DURING PREGNANCY AND THE IMMEDIATE POSTPARTUM PERIOD

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Background: Platelet dysfunction is implicated in uteroplacental disorders. During the early stages of gestation platelets have important roles in the process of placentation. Platelet function contributes to enhanced haemostasis at delivery. However, there is limited data on the changes of platelet function during normal pregnancy. Understanding physiological changes of platelet aggregation during different stages of pregnancy is helpful for better understanding of pathophysiology of abnormal placentation.
Aims: To assess platelet aggregation during three trimesters of pregnancy and immediate postnatal period in normal healthy women compared to control non-pregnant group.
Methods: Cross-sectional cohort study including a total of 46 women: 10 participants for each trimester, 10 postnatal cases and 6 control non-pregnant women. Case selection was based on specific inclusion criteria. 30mL of venous blood was obtained from each participant following consent. Light transmission aggregometry was performed with Dual channel Payton 600B aggregometer using six platelet aggregating agonist (epinephrine, adenosine triphosphate, collagen, ristocetin, arachidonic acid and U46619).
Results: The findings included reduced secondary aggregation curve appearance in pregnant and postnatal women when compared to control group, which was most apparent in the third trimester. Compared to non-pregnant controls, platelet aggregation induced by ADP and collagen were reduced during third trimester while epinephrine induced aggregation was reduced during the first trimester.
Summary/Conclusions: Reduced platelet reactivity in response to epinephrine during early pregnancy can be considered as a mechanism to reduce thrombosis and allow normal placentation while diminished ADP and collagen induced aggregation in third trimester could be a compensatory mechanism since pregnancy associated with hyper-coagulation particularly in late stages.

PB2075

PLATELET DISTRIBUTION WIDTH IS ELEVATED IN IMMUNE THROMBOCYTOPENIC PURPURA, BUT THE VALUES REPORTED IN XN-3000 AND ADVIA2120I WERE NOT COMPATIBLE

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Background: Platelet distribution width (PDW) value as well as its unit is reported through different algorithms in different hematologic analyzers, and the correlation between PDW values in different hematologic analyzers has never been explored. Recently, PDW is indicated as a marker estimating the status of platelet production.
Aims: As the clinical significance of PDW is increasing, but not yet established, we analyzed the correlation between PDW values reported in Advia 2120i and XN-3000. We also compared the PDW values in immune thrombocytopenic purpura (ITP) and essential thrombocythemia (ET).
Methods: PDW was measured in 153 healthy individuals as a control group using both instruments. Twenty-three ITPs and 15 ITPs were tested using Advia 2120i and in XN-3000, respectively. ET group consists of 15 in Advia 2120i and 18 in XN-3000.
Results: In the control group, PDW did not correlated with platelet count, mean platelet volume (MPV), and age in Advia 2120i. In XN-3000, PDW did not correlated with platelet count or age, either, however, PDW significantly correlated with MPV ($y=2.034x-9.111$, $r^2=0.916$, $p<0.001$). PDW values reported in the two instruments did not correlate with each other ($y=0.184x+2.592$, $r^2=0.474$, $p<0.001$). The reference value was 40.0% ~ 64.2% in Advia 2120i, and 9.0 fL ~ 16.0fL in XN-3000. PDW was elevated in ITP in both instruments compared with control group (median PDW 63.1% *vs* 50.5% in Advia 2120i; 14.9 fL *vs* 11.9fL in XN-3000) with statistical significance ($p<0.001$). PDW was elevated in ET in Advia 2120i (59.1%, $p<0.0001$), but decreased in XN-3000 (10.0fL, $p<0.001$).
Summary/Conclusions: We analyzed the correlation of PDW values reported in Advia 2120i and XN-3000 for the first time, and showed that they are not compatible. PDW was affected by MPV in XN-3000, probably due to the calculation algorithm. PDW was elevated in ITP, suggesting the high proportion of large young platelets in this disease. However, the changes in PDW in ET were opposite in the two instruments, which requires further investigation.

PB2076

INTERLEUKIN 31 AS A MARKER OF ALLERGY IN IMMUNE THROMBOCYTOPENIA IN CHILDREN

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Background: Allergy and autoimmunity are two potential outcomes of dysregulated immunity both are characterized by inflammatory reaction that leads to the injury of target tissues. Interleukin-31 (IL31) is a recently discovered cytokine expressed in many human tissues, predominantly by activated CD4(+) T cells and has integral role in allergy pathogenesis.
Aims: to evaluate the relation between ITP and allergy in children by questionnaire for allergic manifestations and measuring serum IL31 and serum IgE..
Methods: 62 ITP patients aged 1-18 years (median 6 years) were included, 36 had chronic ITP and 26 acute ITP, male to female ratio was 4:1, and were compared to 30 age and sex matched controls.All were subjected to: determination of allergy score by questionnaire, measuring IgE level (RAST method) and ELISA serum IL31 level assessment.
Results: Compared to controls, ITP patients had signinificantly higher allergy score ($p<0.001$), higher IL31 level ($p=0.000$), but non significant difference in serum Ig E levels.There was statistically significant higher IL31 level in allergy positive score ITP patients compared to allergy negative score patients ($p=0.000$), and statistically significant positive correlation between IL31 level and score of allergy ($r=0.646$, $p=0.000$).There was no correlation between serum IgE level and score of allergy,and no significant difference between patients and controls regarding serum IgE level. There was no significant difference between acute and chronic ITP in the score of allergy, IgE level or IL31levels, and there was no difference in these parameters between steroid responsive and steroid resistant ITP patients.
Summary/Conclusions: This preliminary study reveals that children with allergic manifestations are at higher risk to develop ITP, but this will not probably affect the clinical presentation, treatment outcome or prognosis of immune thrombocytopenia.

PB2077

SHORT AND LONG-TERM OUTCOMES OF SPLENECTOMY AND SURGICAL COMPLICATIONS IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP) IN A SINGLE CENTER IN ALGERIA

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Background: Among several treatment of immune thrombocytopenic purpura, glucocorticoides are the first one resulting in a high rate of response; unfortunately most patients (pts) responding to this treatment fail to maintain durable response. Splenectomy, the second line treatment produces an important rate of durable responses, about 60-80%.
Aims: To report and evaluate the short and the long-term outcomes and surgical complications in adults and children with ITP who underwent splenectomy.
Methods: We rewieved the medical records of 142 pts who underwent splenectomy for ITP from 1987-2014. Response was defined as: complete response (CR): platelet count>100000/µl ; partial response (PR): platelet count >50000/µl, no response (NR) or failure: platelet count <50000/µl, relapse: loss of CR.142 patients were splenectomized: 44 males (31%) and 98 females (69%),Sex ratio=0,45.Median âge at diagnosis was 26,8 years (2-71); there were 101 adults and 41children .Median âge at splenectomy was 28,8 years (5-72). At the time of splenectomy, 31 pts were children (29%) and 111 were adults. 71 (50%) were splenectomized because they were corticostreroid dependency and 50% because they failed to corticosteroids. The median time from diagnosis to splenectomy was 25,1 months (2-156) The median platelet count at diagnosis was 27000/µl and the median preoperative platelet count was 60000/µl.
Results: Before splenectomy,134 pts(94,3%) received only steroids at initial treatment. 139 pts were vaccinated (pneumococcal vaccine) before splenectomy and after it. In pts with severe thrombocytopenia intravenous corticosteroids were done in 33 cases(23,2%).Laparoscopic splenectomy was performed in 54 pts and open splenectomy in 88 cases.In the post operative period, we noted wound infection in one case; 3 cases requiring open surgery because intra abdominal hemorrhage, one death resulting from neurological disorder related to adrenal insufficiency. Accessory spleen was removed in one patient. Prophylactic antibiotic therapy was systematic during the 2 first years. For 136 informative pts: the median follow up after splenectomy was 80,1 months.Good responses (CR+PR) was achieved at month 1 for 127 (89,4%) of pts: CR: 118 (83%),PR: 09 (6,3%) whereas the remaining (09) were refractory to splenectomy. After 1 year: 125 pts followed: 95 CR, 4 PR, 9 NR. After 2 years: 114 pts followed: 89 CR, 2 PR, 7 NR. Relapses are encountered in 24 (16,9%) pts with an median time of 28,6 months. 21 of these pts were treated, resulting of 12 CR, 03 PR and 06 steroids dependency (one was reoperated for an accessory spleen for relapse 4 years after the first splenectomy). Among the failures, a multiple myeloma apparead 43 months after the ITP ; In one case, the histological study revealed a non hodgkin lymphoma; the ITP has preceded an autoimmune disease in 4 cases (an autoimmune hemolytic anemia after 62 months, 2 cases of systemic lupus erythematous respectively after 42 and 100 months and 1 case of Biermer's anemia 128 months after the ITP) ; 2 cases of family ITP (one pt splenectomized and her daughter followed for ITP that preceded rheumatoid arthritis,2 sisters splenectomized have died, one in operative period by fatal h.emorrhage and the second 151 months after splenectomy after relapse as Evans syndrome.The median total follow up of the 142 patients was 103 months.
Summary/Conclusions: Our study documents that splenectomy si an effective treatment for ITP by the frequency of RC, the excellent outcomes of this procedure for patients with ITP and the mortality rate decreased. However predictive factors of success remain to be clarified.

PB2078

THROMBOPOIETIN RECEPTOR AGONIST SWITCH IN ADULT PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) PATIENTS: A RETROSPECTIVE COLLABORATIVE SURVEY FROM 4 SPANISH CENTERS

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Background: Thrombopoietin receptor agonists (TRA), romiplostim (ROM) and eltrombopag (ELT), have changed in the last years the treatment of ITP showing that there is both a increased platelet destruction and a suboptimal platelet production in ITP. Although both drugs activate the same receptor, mechanism of action is different and they don't activate the same intracellular signalling pathways. That explains why some patients respond to one and not to the other TRA and also the different side effect profile. There are few data evaualting the TRA switch in ITP.
Aims: To describe the reasons and the result of TRA switching in adult ITP patients of the four main centers of our region (Canary Islands) in Spain.
Methods: We retrospectively analized a total of 26 adult patients with ITP who were switched from ROM to ELT or *vice versa*. Clinical and biological parameters were recorded.

Results: Our series comprise 10 men and 16 women with a mean age of 55 years (range 16-84) when they received the first TRA. All the patients received steroids as the first line treatment. Before starting the first TRA, 9 patients (35%) received Rituximab, 7 patients (27%) received anti-D immunoglobulin, 5 patients (19%) received azatioprine, and only 8 patients (31%) had splenectomy performed. The mean number of previous lines before the first TRA was 2.7 (range 1-4), and 9 patients (35%) received a TRA as second line. ROM was the first TRA in 17 patients who switched to ELT with a median time of 8,8 months. In 9 patients ELT was the first TRA and median time to switch to ROM was 3,4 months. The main reasons for switching were lack of efficacy (n=10), patient's preference (n=8), side effects (n=5) and platelet-count fluctuation (n=3). The following table shows what happened in each of these situations. When switch was due to inefficacy, all the cases who received ROM first responded to ELT but only 66% of the patients who received ELT first reponded to ROM. Conspicuously the average of ROM maximum dose was only 4,5 µg/kg/week, very far from the maximum recomended dose (MRD), while the average of ELT maximum dose was 70 mg/day, very near to the MRD. All the changes due to patient preference were from ROM to ELT because of the route of administration. All the patients who switched due to side effects responded to both TRA. Three patients switched from ROM to ELT because platelet-count fluctuation with poor outcome: one did not respond and two responded to ELT but count fluctuation persisted. Six responders patients to the second TRA are now without treatment: four patients achieved a complete response with the second TRA and two patients underwent splenectomy after second TRA and they also achieved a complete response.

Table 1.

Reason for change	Group	First TRA	Second TRA response
Inefficacy 10 patients	4 ROM →ELT	ROM average dose 4,5 µg/kg/week	100% resp. ELT
	6 ELT → ROM	ELT average dose 70 mg/d	66% resp. ROM
Pat. preference 8 patients	8 ROM → ELT	100% resp. ROM	100% resp. ELT
Side effect 5 patients	2 ROM → ELT	-Headache	100% resp. ELT
	2 ELT → ROM	-Dermatitis -Diarrhea -Transaminase elev.	100% resp. ROM
Count fluctuation 3 patients	3 ROM → ELT	-33% no response ELT -66% resp. ELT with count fluctuation	

Summary/Conclusions: As previously reported, in our series we can observe that: When the patient respond to the first TRA and the switch is not due to inefficacy there is a high probability of responding to the second TRA (100% in our series). When the switch is motivated by lack of efficacy, the patient could respond to the second TRA specially if the first TRA dose is bellow the MRD.

PB2079

THROMBOPOIETIN-RECEPTOR AGONISTS (TPO-RAS) IN IMMUNE THROMBOCYTOPENIA (ITP): EXPERIENCE IN OUR CENTER

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Background: Thrombopoietin-receptor agonists (TPO-RAs), Romiplostim (ROM) and Eltrombopag (ELT) constitute an effective therapeutic option for patients with immune thrombocytopenia (ITP). However, several questions remain unclear concerning their use.
Aims: Our aim is to describe our experience with the use of TPO-RAs in ITP with particular reference to sequential treatment with both drugs, their use prior to invasive procedures and the prognostic factors for response.
Methods: We reviewed all patients with ITP who received treatment with TPO-RAs at standard doses in our department. The following variables were evaluated: age, gender, platelet count, previous treatments (including splenectomy), time of follow- up from diagnosis and response to TPO-RAs. Response was defined by the ITP International Working Group response criteria. Comparisons between categorical variables were made with χ^2 or Fisher exact test and Mann-Whitney U test was used for quantitative variables. Logistic regression was used to identify factors associated with response.
Results: Between October 2009 and December 2015, 49 patients were treated with TPO-RAs, 17 men and 32 women, with a median age of 67 years. 36 patients received treatment with ROM (32 as first line and 4 as second line TPO-RAs) and 34 with ELT (17 1st line, 17 2nd line), with a median platelet count of 19.000 (range 2000-78000) before starting treatment. The overall response with ROM was 83% (30/36) and 74% (25/34) for ELT. 10 patients were treated with TPO-RAs previous to invasive procedures, 6 with one dose of ROM and 4 with ELT for a month. 6 patients achieved response, 4 with ROM and 2 with ELT. 21 patients with ITP were treated with both ROM and ELT sequentially. Response was observed in 13 of 17 patients switched from ROM to ELT, including 2 of 3 non-responders to ROM. Three of 4 patients switched from ELT to ROM responded to treatment. Response rate for patients switched because of relapse after transient response was observed in 5 of 9 patients; in contrast, 8 patients switching because of poor tolerance or personal preference achieved response. There was no statistical difference between responders and refractory patients in terms of age, gender, platelet count before therapy, splenectomy or number of previous treatments (table 1). Patients who initially responded to corticoids had a higher response rate to TPO-RAs. In logistic regression only initial response to corticoids showed prognostic impact with a hazard ratio of 10,5 (p=0,005).

Table 1.

		Response	No Response	p
Gender	Male	14	3	p=1
	Female	26	6	
Age (median) years		66.5y (26-88y)	68y (34-76y)	p=0,638
Initial platelet count		19,500 (2000-74.000)	13,000 (4-78,000)	p=0,998
Splenectomy	Yes	12 (80%)	3 (20%)	p=1
	No	28 (82%)	6 (18%)	
Nº previous treatments	(1-2)	32 (82%)	7 (18%)	p=0,848
	(3-4)	8 (80%)	2 (20%)	
Initial response to corticosteroids	Yes	30 (75%)	10 (25%)	p=0,005

Summary/Conclusions: TPO-RAs are an effective salvage therapy for refractory ITP. Switching from one TPO-RAs to the other is an effective option. TPO-RAs could be a useful treatment option when aiming a rapid platelet count increase. Initial response to corticosteroids is the only predictive factor of response in our study.

PB2080

THROMBOCYTOPENIA AND FOLATE DEFICIENCY IN PREGNANT WOMEN

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Background: Micronutrient deficiency among pregnant women is common, folate deficiency is seen in the developments ways countries in parturients having a low socioeconomic level in blood count it may be noted a thrombocytopenia.
Aims: To evaluate the thrombocytopenia observed in parturients deficient in vitamin B9.
Methods: this is a study of cases of thrombocytopenia observed in a cohort of 375 pregnant women with a B9 deficiency, followed antenatal care in the first trimester(T1), second (T2) and third (T3).
Results: the average age of pregnant women was 30.11±6.9 years. The average platelet in our sample is 223 + 89.38 10^3/ml, the average with extreme depending on the quarters is as follows: T1= 223 10^3/ml (34000-512000)/ml, T2= 226 10^3,(4500-512000) T3= 221,10^3/ml (31000-414000). The average serum folate is 7.36 + 3.19 ng/ml: T1= 7.6 (2.3 to 20.0) ng/ml, T2= 7.07 (2.2 to 14.2) ng/ml and T3=7.3 (2.0 to 18.0) ng/ml. The average red cell folate is 201 + 61.74 103ng/ml; T1=206,9 (65.0 to 397.0), T2=195,5(65-321) and T3= 202.6 (66-417). The correlation between red cell folate: and platelets is r=0.284 ** p=2,06.10-8 Regression thrombocytopenia was noted during treatment (B9 orally) during the first month in all cases.
Summary/Conclusions: This study shows that it is necessary to know this association because thrombocytopenia associated with deficiencies of vitamin B9 in pregnant women are frequent and rapid response to treatment of deficiency of vitamin B9.

PB2081

SAFE BUT FLUCTUATING RESPONSE TO TREATMENT WITH TPO MIMETICS IN PREGNANT PATIENT WITH REFRACTORY ITP

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Background: Maternal platelet count less than 20x10^9/L has been associated with risk of spontaneous bleeding, postpartum haemorrhage, and placental abruption. 5% of newborns will be born with platelets less than 20x10^9/L and less than 1% will have bleeding complications. Primary treatment options for maternal ITP are similar to those of non pregnant adult patients: corticosteroids and IV-Ig are the first-line treatments.
Aims: Describe a case of use of TPO-mimetic during pregnancy in refractory ITP.
Methods: We report a case of a 25 years old Moldovan woman with refractory chronic primary immune thrombocytopenia (ITP), diagnosed on 2002. On 2006 the patient underwent splenectomy with a partial response and low dose of prednisone (2.5 mg/die) obtaining a stable remission (platelets 150 x10^9/L). During the follow-up the patient underwent abdominal CT scan, which was negative for an accessory spleen. In November 2013, the ITP recurred with petechiae and platelets were 6 x10^9/L; platelet associated autoantibodies against GpIIb/IIIa (HPA 1a/3a) and GpIb (HPA 5b/5a) were positives. Since the patient was refractory to full doses of prednisone (1 mg/kg) and IV-Ig (1g/kg for 2 days), she was treated with increasing doses of romiplostim, (NPLATE®) (1 mcg/kg up to 3,5mcg/kg) once a week for 26 weeks. The response to TPO-mimetic was fluctuating with both severe thrombocytosis and symptomatic thrombocy-

topenia needing platelet transfusions. The patient was then treated on April 2014 with Rituximab (375mg/m x 4 doses) with complete response.
Results: The ITP remission continued until April 2015, when at 21st week of gestation, relapsed (Figure 1). She was treated again with prednisone and two IV-Ig courses with transitory response. After an unsuccessful course with dexamethasone (40mg/kg) we obtained the patient informed consent for the use of romiplostim (1mcg/kg up to 10 mcg/kg) once a week for 3 weeks before delivery, without response. The elective caesarean delivery was performed on 26 week of pregnancy with the infusion of platelet concentrates due to a severe thrombocytopenia (platelets 3x10^9/L. Major bleeding complications did not occur, and baby's platelets were normal. After delivery, romiplostin was administered for other 10 weeks with fluctuating positive response (Figure 1). Because of the discontinuous response to romiplostim, we shifted to oral eltrombopag (Revolade® 50 mg/day) waiting for a new course with rituximab. Due to a severe thrombocytosis (platelet 1985x10^9/L) the TPO mimetic has been withdrawal after only one week. The response to rituximab was complete and stable, steroid was gradually tapered until suspension and the ITP remitted without any therapy so far.

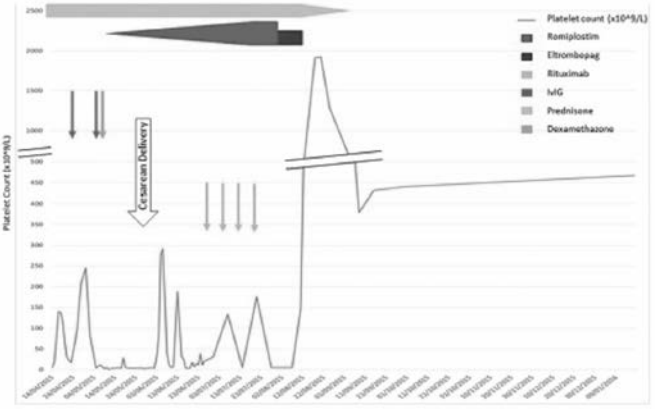

Figure 1.

Summary/Conclusions: There are few case report on ITP treated with romiplostim during pregnancy, and no one showing fetal complications. Although TPO mimetics have not been indicated in pregnant ITP, it may represent an important alternative treatment choice for refractory cases because its speed of activity and an high rate of success. In our patient, we obtained a positive response to romiplostim during the first recurrence of ITP and after delivery but both responses were discontinuous without finding the lowest effective dose. Therefore we tried a switch to eltrombopag after delivery, but the response to the standard initial dose was characterized by an extreme thrombocytosis. We can speculate that TPO mimetic are safe during pregnancy in ITP but they may require more customized dosages mostly after delivery. Instead, using rituximab we obtained a complete and longstanding response showing the effectiveness of the combination of drugs that act by different but complementary mechanisms.

PB2082

HIGH-DOSE DEXAMETHASONE IN TREATMENT OF CHILDREN WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA
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Background: Chronic idiopathic thrombocytopenic purpura (ITP) is a big dilemma in hematology. About one fifth of cases diagnosed as acute ITP takes a chronic course which known as thrombocytopenia that persists after twelve months after onset. Cellular immunity plays a central role in thrombocytopenia. Alterations in T cell subsets and decreased numbers and activity of regulatory T cells are common. Cytotoxic T cells may also mediate toxicity against platelets and megakaryocytes. Treatment strategy includes first line therapy as corticosteroids, IVIG, anti-Rh (D), and second line therapy as high dose dexamethasone, anti-CD20 (rituximab), thrombopoietin receptors agonist and splenectomy.
Aims: to evaluate the therapeutic response of a high dose dexamethasone in a series of Egyptian children with chronic ITP.
Methods: The study included 27 children with chronic ITP comprising 12 males (44.4%) and 15 females (55.6%). One child was withdrawn from the study due to acquired diabetes and hypertension with 26 patients completing the treatment course. Dexamethasone should be administrated by intravenous infusion in a dose of 40 mg/m^2/day on two divided doses for four consecutive days and repeated every 4 weeks for 6 cycles. DXA scan should be done as a baseline and after finishing therapy to evaluate bone mineral density of the patients. Repeated CBC, blood pressure, and blood glucose levels were evaluated through infusion days.
Results: Complete remission (CR) was achieved in 3 patients (11.1%) after the 1st dose, in 5 patients (18.5%) after the 2nd dose, in 5 patients (18.5%) after the 3rd dose, in 6 patients (22.2%) after the 4th dose, in 7 patients (26.9%) after the 5th dose and in 7 patients (26.9%) in the 6th dose. Assessment of the overall response after 1 months of course completion revealed that CR was achieved in 7 patients (26.9%) while remission (R) was achieved in 8 patients (30.8%) and 11 patients (42.3%) had no response. In the current study, on binary logistic regression, older age was the only significant predictor of treatment response.
Summary/Conclusions: High dose dexamethasone can be proposed as a first-line treatment for children with chronic ITP with tolerable side effects. Repeated cycles of therapy seem to be more efficient than only one cycle. Statistical significance of remission in older children, age can be considered as a predictor of treatment response.

PB2083

GAUCHER DISEASE WITH IMMUNE THROMBOCYTOPENIA (ITP): A CASE REPORT AND TREATMENT WITH ELTROMBOPAG
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Background: Gaucher disease (GD) is a rare multi-systemic metabolic disorder due to the accumulation of glucocerebroside in macrophages. The non-neuronopathic type is characterized by enlargment of liver and/or spleen, skeletal abnormalities, pancytopenia. Thrombocytopenia is usually related to hypersplenism and infiltration of bone marrow by lipid-laden macrophages namely Gaucher cells. Enzyme replacement therapy (ERT) restores the hemoglobin and platelet count in GD patients.
Aims: In GD ERT treated patients, immune thrombocytopenia (ITP) should be considered if persistent low platelet counts is found. Traditional treatmant regiments with steroid and splenectomy should be used with caution. Splenectomy may worsen bone lesions and steroids may induce osteopenia and joints avascular necrosis. Thrombopoietin receptor analogues (romiplostim and eltrombopag) are therapeutic option in ITP patients. The use of romiplostim is reported in 2 cases of GD patients. We report the beneficial use of eltrombopag in one patient who suffered from GD.
Methods: 18 YO female patient was diagnosed with Gaucher disease at age 14 (N307S/D399U) and she received imiglucerase. At age 21 she developed purpura and ecchymosis. The platelet count was 0/microL. Bone marrow biopsy (BM) showed a normal erytropoiesis and myelopoiesis and a large number of megakaryocytes. Autoantibodies were negative. A concomitant diagnosis of immune thrombocytopenia was achieved. 1 mg/Kg/die Prednisone and immunoglobulin were given without any response on platelet counts. Splenectomy was not considered due to known bone complication risk in splenectomised GD patients. The patient developed also a severe anemia due to metrorrhagia. Rituximab was given without any results on platelet count. Eltrombopag (dose 50 mg) was initiated raising platelet count from 3,000/μL to 25,000/μL. The dose was increased to 75 mg raising patelet count of 60,000/μL after 5 we3eks. Same dose Romiplostim is maintained for the last 6 months with platelet counts between 40,000 and 80,000/μL without any bleeding events. Repeated BMB showed slight increase of fibrosis and marked hyperplasia of atypical megakaryocytes.
Results: Thrombocytopenia is often present in GD and may be severe in approximately 15% of the patients. Persistent cytopenias may be caused by other underlying pathologies such as autoimmune disorders and it's important to recognize other causes. Before ERT era GD patients with hypersplenism and severe cytopenia were splenectomised. Risks of splenectomy include serious bacterial infection and vascular complications limiting its use in chronic refractory ITP. Splenectomy is avoided in Gaucher patients, because of risk of increasing of skeletal complications (bone infarcts, avascular necrosis). Stable bone marrow results regarding fibrosis in our patients are consistent with data from a recent 2-year follow-up of 100 ITP patients receiving Romiplostim treatment with no evidence of BM fibrosis.
Summary/Conclusions: For patients with type I Gaucher disease and concomitant ITP, adjunctive treatment with Eltrombopag was successful in maintaining haemostatic platelet counts without adverse effects. Traditional treatment based on corticosteroids and splenectomy should be used with caution or avoided in GD patients due to possible risk of Gaucher skeletal disease, osteopenia and avascular necrosis, usually determining increased morbidity in this cohort of patients. Use of TPO-RA should be considered in GD patients with ITP.

PB2084

IDIOPATHIC THROMBOCYTOPENIC PURPURA: DESCRIPTIVE AND RETROSPECTIVE STUDY OF 151 PATIENTS
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Background: Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease characterized by a variable intensity of thrombocytopenia related to the

presence of anti-platelet antibody originally of a destruction of platelets by the spleen macrophages associated with production Medullar quantitatively or qualitativement inadequate. The ITP treatment todate remains poorly standardized.
Aims: We report the results of a clinical study, biology and therapeutic study realized from 151 patients.
Methods: This is a retrospective and monocentric study of 151 patients with ITP (a platelet count <100 10 3/mm 3) collected in the department of hematology hospital Farhat Hached Sousse over a period of 10 years (2004-2014).
Results: The data of 151 patients were included in our study. The average age was 40 years (15-85 years). The sex ratio was to 0.42 (m/f). The average rate of platelets at diagnosis was 20 000 G/L (0-50000). Thrombocytopenia was discovered incidentally in blood cell in 21.2% of cases. In addition 78.8% of patients experienced a hemorrhagic syndrome of varying intensity but not binding prognosis in any case. Eight percent of these patients received platelet concentrate transfusions because of active bleeding. A functional anemic syndrome was present in 17.8% of patients. Regarding treatment, corticosteroid was prescribed first line in 92.1% of cases and only 7.9% of patients received immunoglobulin. The response rate was 83.4% with an complete responce estimated at 62.9%. A relapse occurred in 29.8% of patients and ITP evolves to chronicity in 47% of cases. Only 3 patients (2%) were splenectomized with a normalization of the number of platelets in a patient. 23 patients (15.2%) received treatment with rituximab, the response rate was 39%. After a mean follow-up 38 months, only one death was occurred and it wasn't related to PTI.
Summary/Conclusions: ITP is a rare disease that affects a middle aged population. It evaluate to a chronic mode, the prognosis is good and only a minority patient progresses to a severe form of the disease. Indeed, the rate of relapse or non-response is important and imposed using several therapeutic lines. Place of new treatments receptor agonists such as thrombopoietin (TPO) or new anti lymphocyte B therapies remain to be defined.

PB2085

SUSTAINED RESPONSES FOLLOWING STOPPED TREATMENT WITH ROMIPLOSTIM IN IMMUNE THROMBOCYTOPENIA: A SINGLE-CENTRE EXPERIENCE

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Background: Thrombopoietin-receptor agonists (Tpo-RAs) are highly effective in immune thrombocytopenia (ITP). In the last years, cases of durable remission after Tpo-RA discontinuation in adults' ITP have been reported. This observation raises the possibility that these agents may restore immune tolerance to Tpo-RAs in some patients and supports the practice of down titrating the dose. In this moment, there are no studies that show association with the remission and the variables studied.
Aims: Our objective is to remark the results about the remission and the discontinuation to thrombopoietin-receptor agonists in our population.
Methods: We analyzed 28 patients in treatment with romiplostin from Dr. Negrin Hospital from 2009 to nowadays. Five of them were Primary ITP and 3 had a Secondary ITP (SLE, Crohn's Disease, and hypothyroidism). The average of previously received treatments before the use of romiplostin was either 1 or 2. 8 out of 28 patients, maintained the platelet response in spite of having discontinued therapy with Romiplostim. The discontinued therapy was performed in different ways: 1 patient's drug was decreased weekly to 1 µg/kg and then stopped. In another patient we maintained the dose but increased the interval between treatments to every 3 weeks until off treatment, and as to the remaining 6 patients, the minimal dose was performed every 2-3 weeks until suspension. All of them have maintained the platelet count without therapy.
Results: The median dose of romiplostim was 2 µg/kg weekly (range 1–10 µg/kg). The median duration of romiplostim treatment was 21 months (range 8-46 months). 8 patients (28%) responded (platelet count >100x109 /L without concomitant ITP therapy). In seven patients (26.3%) remission was sustained for longer than 6 months after discontinuing romiplostim therapy. Characteristics of these patients are outlined in Table 2.

Table 1.

N°	Median dose	Max dose	Platelets in the end of treatment	Median duration of treatment
1	1,5mcg/kg	2mcg/kg	146000/ul	12
2	1,5mcg/kg	4mcg/kg	366000/ul	1º: 24 2º: 15
3	<1mcg/kg	1mcg/kg	279000/UL	8
4	3mcg/kg	4mcg/kg	215000/ul	11
5	1mcg/kg	3mcg/kg	284000/ul	46
6	1 mcg/kg	1.5mcg/kg	176000/ul	30
7	1mcg/kg	4mcg/kg	269000/ul	34
8	6mcg/kg	6,5mcg/kg	220000/ul	9

Summary/Conclusions: There is more than one possible mechanism which might explain the lasting responses seen in our patients. For example, natural remission or a change in immune regulation through an impact on T regulatory cells with restoration of immune tolerance. In our retrospective study, and in the other reported studies, we found no relationship between the different variables (age and number of previous lines) or the response pattern (early or late) and the possibility of discontinuance. In addition, we observed that the use of demand doses does not predict the loss of long-term response. The thrombopoietin receptor agonists could be used for short term treatment to cover invasive procedures and to treat patients who are at risk of bleeding and are refractory to other therapies but prospective studies should be set up to confirm the observation of sustained response off therapy and to identify potential predictive factors of response.

PB2086

NEONATAL OUTCOMES OF PREGNANCY WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background: Neonates born to mothers with idiopathic thrombocytopenic purpura (ITP) have an increased risk for neonatal thrombocytopenia and hemorrhagic complications.
Aims: The aim of this study was to determine the maternal and neonatal outcomes of pregnancies with ITP and also to identify risk factors that predicts neonatal thrombocytopenia
Methods: We performed a retrospective analysis of 40 pregnancies with ITP and their 40 neonates.
Results: Among the 40 neonates, thrombocytopenia (platelet count of less than 150×10^9/L) was detected in 15 neonates (37,5%) whom 8 of them had severe thrombocytopenia (platelet count of less than 50×10^9/L). Ten of the 15 neonates with thrombocytopenia required treatment to increase the platelet counts. There was statistically significant association between neonatal thrombocytopenia and maternal splenectomy history and maternal duration of thrombocytopenia. There was no statistically significant correlation between maternal platelet count and neonatal platelet count.
Summary/Conclusions: Clinicians should pay special attention in these neonates because of risk for development of neonatal thrombocytopenia. Maternal and neonatal outcomes in patients with idiopathic thrombocytopenic purpura is generally good.

PB2087

SAFETY AND EFFICACY OF RITUXIMAB IN ADULT IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenia is characterized by immune-mediated destruction and suboptimum production of platelets. Despite the absence of supporting evidence, the anti-CD20 chimeric monoclonal antibody rituximab has been effectively used off-label in the treatment of patients with primary immune thrombocytopenia (pITP).
Aims: The aim of this analysis is to describe our experience with Rituximab in patients affected by chronic ITP.
Methods: In this monocentric analysis we retrospectively evaluated 43 adult patients affected by chronic ITP resistant to 2 or more lines of therapy that were treated with four weekly infusions of 375 mg/mq rituximab to asses safety and efficacy.
Results: Of 43 patients treated with Rituximab, 39 were retrospectively evaluated. 20 F, 19 M. Median age was 60 years (range 29-91 years) and median platelets value at start treatment was 16.000/µl (range 5.000-40.000). 24/39 (62%) showed an initial response, 17/39 (44%) patients obtained complete response (CR) and 7/39 (18%) showed partial response (R). Of those achieving an overall response 4 (17%) patients relapsed, median time to relapse was 30 months (range 8-45). Of these 4 patients relapsed, 3 received re-treatment with four weekly infusions of 375 mg/mq rituximab and 2 patients achieved a complete response, one was no-responder. With a median follow-up of 22 months (range 2-95 months), 17/24 patients (71%) showed a lasting response out of treatment, of these 15 patients maintained a complete response with a median platelets count of 156.000/µl (100.000-362.000/µl) and 2 patients was in partial response. 7 patients underwent further line of therapy (6 treated with TPO-mimetics and 1 with splenectomy) During the follow-up, no opportunistic or severe infectious complications were observed.
Summary/Conclusions: In our limited experience these data confirm, over a long period of observation, the efficacy and safety of Rituximab treatment in the management of patients with resistant ITP and Rituximab used off-label may remain a valid option for treating persistent or chronic ITP in adults. Further investigations and specific clinical trials are warrented.

PB2088

EFFICACY OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA

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Background: Immune thrombocytopenic purpura (ITP) is an autoimmune disorder in which antibodies are produced to circulating platelets. The isolation of TPO and better understanding of its role in thrombopoiesis has led to the development of new highly effective tTPO analogs had some successes in treating highly refractory ITP patients but were taken out of development due to TPO-antibody induction.
Aims: The aim of this study is to describe our experience with thrombopoietin receptor agonists (TPO-RA).
Methods: From November 2008 and April 2015 54 patients (29 M; 25 F) were treated with TPO-mimetics: 38 underwent therapy with Romiplostim and 16 to Eltrombopag. Median age was 71 years (range 39-94 years). 8/54 (14%) patients received both of therapies: 5 (4 F; 1 M) switched from Romiplostim to Eltrombopag and 3 (3 M) switched from Eltrombopag to Romiplostim.
Results: In the group of patients treated with Romiplostim, 16/38 had already received more than 4 lines of therapy, while 10 were at the 3rd line of therapy, and 5 were at 2nd line. Only 2/16 patients who received Eltrombopag were at the 2nd line of therapy, and the others were at least at the 3rd line. The median platelet count was 17.000/µl at the start of Romiplostim and 10.000/µl in patients treated with Eltrombopag. With median follow-up of 36 months (4-74), we observed 33 responses (86%) with Romiplostim (19 complete response, 14 partial response) and 5 no responders; we had also 3 loss of response. In our study 18 (33%) patients stopped Romiplostim after a median time of 23 months (1-56): 6 for stable response; 2 for adverse events; 3 for loss of response; 2 underwent splenectomy; 5 for no response. The median platelet count at suspension of Romiplostim was 94.000/µl (2.000-739.000); the patients discontinued Romiplostim after a median time of 26,6 months. Now 7 patients are out of treatment with a stable platelet count (median time after discontinuation: 25 months). In patients treated with Eltrombopag 13 (81%) achieved a response (9 complete response, 4 partial response), 3 were no responders. 11 (69%) patients stopped Eltrombopag after a median time of 10 months (1-16): 5 for adverse events; 3 for no response; 2 for stable response; 1 for splenectomy. The median platelet count at suspension was 74.000/µl (2.000-739.000); the patients discontinued Eltrombopag after a median time of 11,1 months and now are out of treatment from 3 months.
Summary/Conclusions: Several studies reported Romiplostim and Eltrombopag to be highly effective against chronic ITP, with average immediate responses exceeding 80% in our study. TPO-mimetics have proved efficacy in patients with ITP and their use can be applied in several conditions: bridge to splenectomy, sustained response, switch and discontinuation.

PB2089

NOVEL PERSPECTIVES IN PATIENTS WITH REFRACTORY CHRONIC IMMUNE THROMBOCYTOPENIA FOLLOWING ELTROMBOPAG TREATMENT

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Background: Primary immune thrombocytopenia (ITP) is characterized by an antibody-mediated accelerated destruction and inadequate production of platelets. Therapeutic strategies in ITP aim not only to increase and maintain platelet counts in safe levels without bleeding episodes but also to improve quality of life in this chronically ill patient population. Eltrombopag is the first oral thrombopoietin agonist approved for patients with ITP refractory to at least one line of treatment.
Aims: The present study aims to evaluate the safety and efficacy of etrombopag in clinical practice.
Methods: Eltrombopag was administered in 13 patients, 5 male: 8 female with a median age of 46 years (range 19-48) for a median 6.4 months (0.5-32.4). Patients had received 1 to 7 lines of treatment (median=1), including corticosteroids (13 patients), immunoglobulins (4 patients), rituximab (1 patient), vincristine (1 patient), cyclosporine (1 patient), romiplostim (1 patient), danazol (1 patient) and splenectomy (1 patient). In accordance with previous clinical trials, complete response to treatment was defined as a platelet count of ≥100x10^9/L.
Results: At initiation of eltrombopag treatment, the majority of patients (7/13) showed thrombocytopenia (WHO grade 4, <25x10^9/L). Initial dose was 50 mg, increased to 75 mg daily in 3 patients for optimal treatment. In 10/13 patients eltrombopag was administered in combination with corticosteroids that were gradually tapered by the fifth week of eltrombopag administration. Median platelet value by the second week of administration was 125x10^9/L (5-450x10^9/L); whereas, by the fourth week platelets increased to 185x10^9/L (16-500x10^9/L). At the end of follow-up, median platelet count was 132x10^9/L (60-400x10^9/L). All patients achieved complete response to treatment (platelet count≥100x10^9/L), except for one patient with a platelet count of 60x10^9/L at the end of follow-up. Regarding adverse events, one patient developed grade 2 hepatobiliary abnormalities and one patient grade 1 hemolytic anemia. Both adverse events resolved when the drug was temporarily discontinued. Only one patient switched to another treatment due to pulmonary embolism during the first month of treatment.
Summary/Conclusions: In the daily clinical practice eltrombopag is safe, well tolerated and highly effective in maintaining a safe platelet count when administered in ITP patients refractory to at least one line of treatment. Eltrombopag role in other subgroups of ITP patients remains to be investigated. In addition, larger studies with longer follow-up are needed to determine the incidence, predisposing factors and prophylactic measures to prevent severe adverse events.

PB2090

HIGH-DOSE DEXAMETHASONE AND ELTROMBOPAG IN CHRONIC IMMUNE THROMBOCYTOPENIA: A SINGLE INSTITUTION EXPERIENCE

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Background: Eltrombopag is a thrombopoietin (TPO) nonpeptide mimetic that has been shown to raise the platelet count in both continued long-term administration and in a repeated short-term administration in chronic immune thrombocytopenia (ITP). However, information dealing with the concomitant use of eltrombopag and dexamethasone in patients with chronic ITP are lacking.
Aims: The purpose of this study was to assess the efficacy of eltrombopag in combination with dexamethasone in a consecutive, unselected series of patients with chronic ITP followed up in a single institution in the period May 2014- December 2015.
Methods: Eleven patients (6 F/ 5 M) with median age 53 years (range, 29-70) who had at least a 6-month history of ITP (median 26 months; range 6-220 months) were considered eligible for the present analysis. All patients had a platelet count lower than 30x10^9/Land bleeding manifestations were present in 7 out of 11. No patient had an active infection, drug-associated thrombocytopenia, positive serology for HIV, hepatitis B or hepatitis C, malignant diseases or was pregnant. After initiating eltrombopag (50 mg once a day)doses were adjusted to achieve and maintain a platelet count ≥50,000/µl as necessary to reduce the risk for bleeding. Eltrombopag dose did not exceed 75 mg/day. Dexamethasone 40 mg/day was given for 4 days every 28 days. Patients with glucose intolerance or diabetes needing therapy received an mitigated dose of dexamethasone (20 mg/day). Response and complete response (CR) were defined as an increase in platelets ≥30 <100x10^9/L and ≥100x10^9/L, respectively.
Results: All patients achieved a responseduring the treatment while a CR was obtained in 10 of 11 patients. Maximum response was reached after a median time of 12 weeks (range, 3-39). After a median follow-up time of 29 weeks (range, 8-89) response was still maintained in all patients while 5 patients lost CR. Four patients who lost CR were receiving maintainence therapy with eltrombopag (25 mg/day from a period ranging between 2 and 14 weeks). In the fifth patient CR was lost 7 weeks after treatment was interrupted because of pregnancy. Finally, the median probability of maintaining CR-free survival was 42 weeks.
Summary/Conclusions: Results of this study although limited by the small sample sizeand the lack of a comparative randomized design suggest that dexamethasone in combination with a thrombopoiesis stimulating agent led to a long-lasting remission of ITP. High-dose dexamethasone may modify the immunological milieu, resulting in an enhanced response to the thrombopoietin receptor agonist.

PB2091

THROMBOPOIETIN RECEPTOR AGONISTS IN THE TREATMENT OF PRIMARY ITP: EXPERIENCE OF APPLICATION IN CLINICAL PRACTICE ONE MEDICAL CENTER

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Background: Therapy of thrombopoietin receptor agonists(aTPO-r) is a new approach to the treatment of patients with resistant primary ITP.Data on the effectiveness and safety of aTPO-r outside of controlled clinical trials is limited.
Aims: To study the efficacy and safety of prolonged use of aTPO-r in routine clinical practice in patients with chronic ITP in case of loss or inadequate response to at least one line of therapy. To evaluate the possibility of achieving a sustained response after the cessation of the aTPO-r therapy.
Methods: A retrospective analysis of aTPO-r threatment results in chronic ITP patients in the outpatient department in the period from 2010 to 2015.Romiplostim therapy received 45 patients(group 1) and eltrombopag therapy-15 patients(group 2).Efficacy was evaluated by levels of platelet response and the percentage of patients who managed to cancel or reduce the dose of drugs for concomitant ITP therapy.The main indicators of safety were incidence of adverse events,including thrombotic complications,bleeding and abnormalities in laboratory parameters.
Results: Twenty-one patients (47%) from 1 group and four (28%) from group 2 received two or more lines ITP therapy, nine of them underwent splenectomy.

Platelet response was achieved in 88% patients treated with romiplostim and in 93% eltrombopag. During aTPO-r therapy the majority of patients (92% from group 1 and 100% group 2) were able to completely cancel the previous long-term steroid therapy. Resistant to aTPO-r therapy were 8 patients-5(11%) from romiplostim group and 1(7%) eltrombopag group),three of them were splenectomised in future. The most frequent adverse events in romiplostim group were headache,arthralgia and dermatitis. This events were minimal and not require change in therapy.In eltrombopag group noted shot-term hepatotoxicity with the need for correction or therapy(dose reduction or treatment interruption)without additional hepatotropic therapy. Cancel aTpo-r therapy was required in connection with the development of thrombotic complications: 4% patients romiplostim group and 6% eltrombopag. Five patients (4 from the romiplostim group and 1 from eltrombopag) maintained a stable remission after discontinuation of aTPO-r therapy without any treatment.
Summary/Conclusions: Presented results confirm high efficiency,favorable safety profile aTPO-r,importance of this drugs in the modern ITP therapy algorithms,which was reflected in the inclusion of aTPO-r in national treatment standarts of ITP therapy.

PB2092

ELTROMBOPAG FOR CHRONIC IMMUNE THROMBOCYTOPENIA: A SINGLE CENTER EXPERIENCE

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder that leads to peripheral destruction, as well as a decreased production of platelets. The incidence of ITP in adults is around 3-4 per 100000 people per year. The probability of spontaneous remission in adults is low. Up to 90% of cases become chronic, and 10% do not respond to standard therapy. Eltrombopag is a non-peptide thrombopoietin receptor agonist that has been approved for second-line treatment of ITP.
Aims: The primary aims of the study were to determine the efficacy and safety of long-term eltrombopag treatment in their own clinical practice.
Methods: Fifty adult chronic ITP patients who were refractory to previous ITP treatments (platelet count <30,000 cells/μL) were enrolled in the study in the period from October 2012 to December 2015. Treatment was started in all patients with 50 mg/d oral eltrombopag. Response was assessed at 1, 2, 3 and 12 months. The target platelet response was defined as an increase in the platelet count ≥50,000 cells/μL. The analysis is performed at the time of Februare 2016.
Results: The median age was 57.4 years (range, 18.2-87.1 years), and 35 patients were women. The median number of prior ITP treatments was 2 (range, 0-4), including 9 (18%) patients who had undergone a splenectomy and 14 (28%) - rituximab. The median ITP duration before eltrombopag treatment was 3.1 (range, 0.6-37.9) years. The median platelet count at baseline was 12,000 (range, 1,000-27,000) cells/μL. After 1 month 25 (50%) achieved the target platelet count (>50,000 cells/μL). The median platelet count reached 50,000 (range, 9,000-334,000) cells/μL. After 3 month 35 (70%) achieved the target platelet count with median platelet count is 84,000 (range, 9,000-334,000) cells/μL. After 12 month 37 (74%) achieved the target platelet count with median platelet count is 107,000 (range, 22,000-330,000) cells/μL. The current median duration of eltrombopag treatment was 14.8 (range, 1.1-40.3) months. 43 (86%) patients continued therapy, and 7 (14%) patients who have not achieved a response were excluded from the study for 2-3 months. On the likelihood of achieving a platelet response at 3 months do not affect gender, age, previous therapy (rituximab, splenectomy) and an initial platelet counts of less than or more 15,000 cells/μL (p>0.05). Twelve patients had bleeding episodes of mild to moderate severity. Thromboembolic events were not reported. Eltrombopag is a well-tolerated treatment. Most reported adverse effects have been mild-moderate and have not led to cessation of treatment.
Summary/Conclusions: Eltrombopag is well tolerated and effectively achieves target platelet counts in refractory adult chronic ITP patients.

PB2093

MANAGEMENT OF CHRONIC IMMUNE THROMBOCYTOPENIA, SINGLE CENTRE EXPERIENCE

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Background: The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely.
Aims: To evaluate the treatment of ITP patients in Departament of Hematology, County Hospital, Timisoara during 10 years (I.2000-XII.2014).
Methods: A retrospective study for 325 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient's medical charts for the 12 months prior to their most recent visit.
Results: The average age was 43.2 years, with 57% women and 43% men. Median time from the diagnosis of ITP to the start of the observational period was 20 months. Prior to the observational period, 34% of patients have been splenectomized and the most used treatment was corticosteroids. During the observational period, 76% of the patients were treated. The most frequent reasons given for treatment was low platelet count (67%), followed by bleeding symptoms (53%). Corticosteroids represented 64% of treatments, followed by IVIg (20%), azathioprine (8%), rituximab (8%). Only a few patients (six) were treated with Nplate (Romiplostim). Splenectomies (15% of patients) and platelet transfusions (32% of patients) were performed during the observational period. For monitoring the platelet levels, 78% of patients visited their hematologist 1 to 10 times during the observation. Main reasons for a visit were low platelet count (46% of visits) and bleeding (37% of visits). Overall, 39% of patients required hospitalization. Mean duration of hospitalization was10,5 days.
Summary/Conclusions: The retrospective study of 325 patients provides therapeutic outcomes resulting from treatment methods from our department. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP, but lifethreatening bleeding rarely occur. Corticosteroids and splenectomy represent the most used treatments from our department.

Quality of life, palliative care, ethics and health economics

PB2094

PREGNANCY IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: OUTCOMES DEPENDING ON THE THERAPEUTIC APPROACH

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Background: The management of paroxysmal nocturnal hemoglobinuria (PNH) during pregnancy recently has been challenging because of a high risk of severe maternal and fetal complications. Now pregnancy planning and eculizumab treatment changed the prognosis and made it possible to minimize complications associated with pregnancy in PNH.
Aims: To analyze the pregnancy outcomes in PNH depending on the therapeutic approach- using eculizumab or symptomatic treatment only. Establishment of effective and safe algorithms for the management of pregnancy, delivery and postpartum period in PNH patients is crucial to their quality of life.
Methods: From 1999 to 2015, we have analyzed 17 pregnancies in eight women with PNH, treated in our centers. Only three patients had planned the pregnancy, most of cases- 14 (82,4%) were unplanned. The median age at the start of pregnancy - 25 years (21-34). All of them were diagnosed with PNH following treatment for aplastic anemia (AA) with antithymocyte globulin, cyclosporine A and splenectomy in two cases before pregnancy. Most patients were in partial remission of AA at the time of pregnancy- 8 (47,1%), complete remission was achieved in 6 (35,3%). Three patients (17,6%) from 2013 exposed to eculizumab: two- started the treatment before conceiving, one-received eculizumab from third trimester. Other women received only symptomatic therapy (82,3%), such as anticoagulation with low molecular weight heparin in 29,4%, erythrocytes or platelets transfusion -35,3%, immunosupressive therapy -17,6%.
Results: The median of PNH granulocyte clone at the start of pregnancy was 74,7% (17,8-94,1). Progression of aplasia observed during 23,5% pregnancies, but it was not severe and special treatment delayed until the completion of pregnancy. No thrombotic events during pregnancy and postpartum have been observed. There were pregnancy complications: abortion threat 76,5%, fetal growth retardation syndrome 3/9, preeclampsia 2/9. Pregnancies resulted in the birth of healthy infants in 9 (52,9%) cases - three girls and six boys. There were no adverse effects in the newborns from PNH patients both on eculizumab and without it. Newborn health status differed from the norm only in the group of patients without the targeted therapy due to the presence of complications, mainly related to prematurity. Successful outcomes were in 3/3 pregnancies on eculizumab treatment and in 6 (42,9%) cases without the drug. Caesarean sections were performed in all of births, early surgical delivery (30-34 weeks)- in 4/9 cases (preeclampsia-2, placenta previa- 1, breakthrough hemolysis-1). Adverse pregnancy outcomes occurred only in patients not receiving eculizumab and amounted to 8/14 (57,1%). 28,6% cases of pregnancy in the midst of illness required the abortion for medical reasons. Spontaneous miscarriage was registered in 3 (21,4%) patients, fetal death on 27th gestation week- in one case (7,1%). Transfusion requirements increased in two pregnancies (14,3%) with symptomatic therapy, but did not increase on eculizumab. PNH granulocyte clone size decreased in 2/3 cases of eculizumab treatment during pregnancy.
Summary/Conclusions: Pregnancy in PNH with symptomatic treatment with a high probability ends adversely. The risk of complications during pregnancy and postpartum in PNH may be minimized by pregnancy planning and applying the management algorithm with eculizumab treatment. Despite the small number of observations, we can conclude that pregnancy outcomes in PNH patients with eculizumab are better than with symptomatic therapy. Our experience confirms that eculizumab can be safely used in PNH during pregnancy. This therapeutic approach may result in improved quality of life in PNH.

PB2095

EVALUATION OF THE IMPLEMENTATION OF A NURSE CASE-MANAGER FOR THE DIAGNOSIS AND FOLLOW UP OF PATIENTS WITH SYMPTOMATIC MULTIPLE MYELOMA

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Background: An early diagnosis and prompt treatment of patients with symptomatic multiple myeloma (SMM) is essential. Hence, the reduction in the timing of diagnostic tests is a key element that allows to initiate early treatment and to avoid secondary complications. The nurse case-manager (NCM) has a crucial role reducing the time needed for the completion of tests and results as well as in the optimization of visits in order to start therapy as soon as possible. In addition, the NCM may improve the follow-up of patients by reducing admissions and random visits, thus potentially decreasing costs.
Aims: To assess the intervention of the NCM in relation to: 1) the time needed to establish the diagnosis and start treatment and 2) the follow-up process.
Methods: From September 2014 to January 2016 a prospective, single center study in patients with suspected SMM (interventional group; IG) was started. Results were compared to a control group (CG) of patients diagnosed of SMM in the two years previous to the study. Primary end-points were: 1. Time from referral to first visit in the Hematology Dept., 2. Time from referral to completion of diagnostic tests, and 3. Time from referral to initiation of treatment. Secondary objective was to assess hospital admission incidence during the first year of follow-up in both groups. The effect of the NCM in time to event variables was investigated using a multiple linear regression (MLR), using the corresponding time variable as the dependent variable (Log10 transformed due to left skewed distributions) and admission at diagnostic period as a confounder. Fisher exact test was used for assessing incidence ratios. In IG, a 11-item patient satisfaction questionnaire (PSQ) was obtained and a log of phone calls (PC) was recorded. This log collects all contacts to the NCM made by IG patients. Bilateral p value of 0.05 was considered statistically significant.
Results: During this period, 22 patients were studied in the IG and compared to a CG of 37 patients. Table 1 shows the results of the effect of the NCM in the three main end-points.The median value of PSQ was of 8,5/10, highlighting a significant patient satisfaction. A reduction in the incidence of admissions was observed (odds ratio: 1.20 p<0.05). During the study, 262 PC were collected and divided according to the discussed topic. 48.2% of PC were due to symptoms control: 85,7% were resolved by NCM or Physician and 14,3% were attended at the hospital. 26.7% of PC were related to treatment information: 86,6% were resolved by NCM or Physician and only 13.3% needed to attend the site. Finally, 25.1% of PC were related to schedule information and, of note, 98.4% were resolved during the call.

Table 1.

	control*	intervention*	p value[c]
Time referral, first visit (days)	16,6	7,6	0,022
Time first visit, completion of last test (days)	38,2	16,3	0,037
Time completion of last test-initiation of treatment (days)	45,0	24,4	0,044

*5% trimmed mean;
[c]p value of CM effect in MLR.

Summary/Conclusions: The incorporation of a NCM for the diagnostic and follow up of patients with SMM has allowed to reduce time from referral to visit, completion of tests and initiation therapy in the IG when compared with CG. Data collected during follow-up suggests that NCM involvement may lead to a decrease in hospital costs, with a significant patient perceived satisfaction. Further observation is warranted to confirm our preliminary findings.

PB2096

RELATIONS BETWEEN FAMILY FUNCTIONING AND HEALTH STATE OF PATIENTS WITH CUTANEOUS CHRONIC GRAFT-VERSUS-HOST DISEASE

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Background: Chronic graft-versus-host disease(cGVHD) is a common complication after allogeneic hematopoietic stem cell transplantation(allo-HSCT). Patients with cutaneous cGVHD always changed much in appearance and many of them were in the bad health of psychology.
Aims: The aim of this study was to explore the relations between family functioning and health state of patients with cutaneous cGVHD.
Methods: A questionnaire survey was undertaken among patients with cutaneous cGVHD by the way of Family APGAR and COOP/WONGA. According to the scores gained from family APGAR index questionnaire, the patients were divided into two groups: family dysfunction group with scores between 0 and 6 and better family function group with scores between 7 and 10. The scores of each item gained from COOP/WONGA were further compared between the two groups and the correlations of the results were analyzed.
Results: 38.7 percent of the patients had good functioning families and 61.3 percent of them had defective functioning families.The differences between the two type families in the total healthy state, emotion, social communication, daily activity and pain had statistical significance (P<0.05). The family functioning had positive relation with the health state in patients with cutaneous cGVHD(r=0.901, P<0.01).The adaptability, cooperation, growth, emotion and intimation in Family APGAR questionnaire survey and ten items in COOP/WONGA had positive relations (r=0.711 ~ 0.925, P<0.05, P<0.01).
Summary/Conclusions: The relation between the family functioning and the health state of patients with cutaneous cGVHD is close. Medical workers should pay attention to the desirable effects of the good family functioning on these patients.

PB2097

REAL-WORLD CHARACTERISTICS, TREATMENT PATHWAYS AND HEALTHCARE RESOURCE USE IN PATIENTS TREATED FOR RELAPSED REFRACTORY MULTIPLE MYELOMA IN SPAIN: PRELIMINARY RESULTS FROM THE PREMIERE STUDY

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Background: The management of patients (pts) with multiple myeloma (MM) has changed considerably in recent years. Pts' life expectancy has significantly increased, and in some cases MM is now considered a chronic disease, with pts receiving multiple lines of therapies. Real-world data on health and resource-related outcomes of different treatment patterns may help promote more efficient resource allocation within the National Health System.
Aims: To describe baseline characteristics, treatment patterns and healthcare resource utilization (HCRU) in a cohort of pts treated for relapsed refractory (RR) MM in Spain.
Methods: The PREMIERE study is a retrospective chart review study conducted in a representative cohort of pts with RRMM in Spain. Enrolled pts were aged ≥18 years, had ≥1 prior treatment for MM, and began a new line of therapy (index therapy) during the recruitment period of 1st July 2010–30th June 2012 due to relapsing or refractory disease. Pts were followed from initiation of index therapy (index date) for 3 years, or until death, loss to follow-up, or enrollment into a clinical trial for MM. Using an interim data extraction from November 30th 2015, descriptive statistics of pt characteristics at initiation of index therapy were calculated, and treatment patterns and HCRU in pts with complete data at the end of the 3-year follow-up estimated.
Results: Overall, 100 pts were included in this interim analysis; 91.0% had received only 1 prior treatment for MM. 61.0% were male, 69.0% were aged ≥65 years; median weight was 70 kg (Q1–Q3: 62–80 kg). Median time from MM diagnosis to enrollment was 2.2 years (range: 0.1–15.5 years). Most pts had relapsed (74.0%) or refractory (13.0%) MM. The majority of pts were in MM ISS stage II (31.3%) or III (29.3%). Most common previous therapies were bortezomib (74.0%) and melphalan (68.0%). 28.0% of pts had received a stem cell transplant (all autologous), 96.4% (27 pts) were single transplantations, 2.6% (1 pt) was a double transplant. 56 pts completed the 3-year follow-up period. During the follow-up period, 50.0% (28 pts) initiated a subsequent 2nd line of therapy after index therapy, 23.2% (13 pts) initiated a 3rd RR line, and 7.1% (4 pts) initiated a 4th. In 2nd, 3rd, and 4th lines, the most commonly prescribed treatments were RevDex or bortezomib-based combination therapies. The most commonly prescribed classes of medications for index therapy were immunomodulators (24 pts, 42.9%; of whom 18 pts received RevDex), and proteasome inhibitors (17 pts, 30.4%; of whom 13 pts received VelDex). 11 pts (19.6%) received chemotherapeutic agents. Time from the start of index therapy to the next line differed between RevDex and VelDex, with longer treatment durations on RevDex (16.2 months) compared to VelDex (12.5 months; Table). However, duration of subsequent lines (after progression on index therapy) was longer for pts treated with VelDex as the 1st RR line (RevDex: 9.3 months; VelDex: 15.1 months; Table). HCRU during the time spent on-therapy with the index therapy during the 3-year follow up period showed overall higher resource use for VelDex treated pts, especially for hospital consultations (Table).

Table 1.

Table. Index therapies and healthcare resource use in patients with RRMM in Spain

	Index therapies (N=56)		
	RevDex [a] (n=18, 32.1%)	VelDex [b] (n=13, 23.2%)	Other [c] (n=25, 44.6%)
Line of treatment duration, months, mean (SD)			
Time from start of 1st line after enrollment to next line	16.2 (11.5)	12.5 (10.7)	11.2 (9.5)
Therapy duration	13.9 (10.1)	7.5 (7.1)	9.3 (8.5)
Subsequent therapy duration	9.3 (8.1)	15.1 (9.8)	9.2 (8.2)
Healthcare resource use during 1st line after enrollment, mean (SD)			
Hospital admissions	0.7 (0.7)	1 (0.8)	1.4 (1.3)
Clinical visits			
Hematologist visits	15.8 (8.8)	14.1 (15.3)	11.8 (14.7)
GP visits	2.2 (3.4)	5.8 (15.4)	2.2 (5.3)
Hospital outpatients' consultations	7.4 (10.0)	14.5 (19.8)	9.7 (19.0)

[a] RevDex: lenolidamide (oral) + dexamethasone (oral)
[b] VelDex: bortezomib (IV/SC) + dexamethasone (oral)
[c] Other: IMiDs other than RevDex (n=4), PIs other than VelDex (n=1), chemotherapy (n=11), PI+chemotherapy (n=3); PI+IMiD (n=1); chemotherapy+IMiD (n=2); PI+IMiD+chemotherapy (n=2); other (n=1)

Summary/Conclusions: These are the first data published describing treatment patterns and HCRU in Spanish clinical practice. The most common patterns for relapsed/refractory pts are bortezomib and lenalidomide-based schemes. Data should be interpreted with caution given the small sample size; final analyses of this study with the complete sample population will be presented at EHA.

PB2098

PHARMACOECONOMIC MODELLING OF TARGET THERAPY CHRONIC MYELOGENOUS LEUKEMIA: EARLY AND LATE SWITCHING

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Background: Early molecular response (EMR) achievement (*BCR-ABL*≤10% by IS) at 3 months of tyrosine kinase inhibitors (TKI) treatment of chronic myelogenous leukemia (CML) is recognized as an important prognostic marker of subsequent therapy efficiency and survival. The time point of TKI switch from first to second generations is one of the main differences between different CML treatment guidelines. The attractive perspective of further improvement in CML management is treatment free remission phase (TFR),-stop the TKI therapy in patients with deep sustained molecular response under thorough molecular monitoring.
Aims: The pharmacoeconomic modelling of comparison between early (3 moths) and late (6 months) switching from Imatinib to second generation TKI (TKI2) depending on EMR achievement (*BCR-ABL*≤10% at 3 months of treatment) with subsequent TKI therapy cessation in patients with stable long-lasting (2 years) deep molecular response (MR4.0=*BCR-ABL*≤0,01% by IS).
Methods: We have used previously described Markov chain model for CML management[1]. We constructed two model variances for early (3 months) and late (6 months) time points of switches from Imatinib to TKI2 depending on EMR achievement with subsequent TFR phase for patients with MR4.0 2-years duration. The model size was 700 newly diagnosed CML patients yearly and time horizon was 5 years. The transition rates (TKI response rates, MR4.0 rates, successful TFR rates) have been chosen from clinical trials (ENESTnd, TIDEL-II, STIM, FILMC, DADI and own data). We have recalculated the total costs from Russian roubles to Euros for clearly representation (February 2016).
Results: Early *versus* late switching lead to more frequent successful transition to TFR (13,59% for early switch and 10,46% for late switch). The early switching takes 8.54% more expenses (figure 1), but provided 6099 additional quality-adjusted life years (QALY). Incremental cost-effectiveness ratio for early compared to late switching is 543.2€ per 1 additional QALY, that is reasonable to economically based implementation. Sensitivity analysis shows that in case of TKI second generic substitution the costs for early and late switching should be equal. It can yield extra cost-free efficiency.

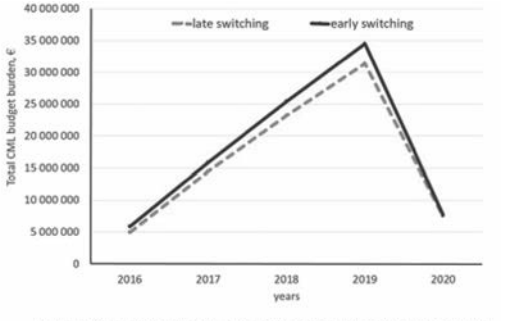

Figure 1. Chronic myelogenous leukemia budget burden for early and late switching strategy depend on early molecular response achievement.

Figure 1.

Summary/Conclusions: The pharmacoeconomic modelling can simulate budget burden for various modifications of diagnostic and therapeutic techniques in CML management with evaluation of its economical and clinical worth.

References

1. V.A.Shuvaev, A.S.Abdulkadyrova, I.S.Martynkevich, V.Y.Udaleva, M.S.Fominykh, I.I.Zotova, R.A.Golovchenko, A.A.Zhernyakova, L.S.Martynenko, E.V.Petrova, M.A.Kozlovskaya, M.P.Ivanova, N.Y.Cybakova, A.V.Schmidt, K.M.Abdulkadyrov. Bonus free life's in CML-pharmacoeconomic modeling first and second generation TKIs in first-line CML treatment with therapy cessation ELN Information letter October 2013.-p.14.

PB2099

Abstract withdrawn.

PB2100

THE APPLICATION OF GENOMIC MEDICINE IN HAEMATOLOGY: THE CHALLENGES AND ETHICAL IMPLICATIONS

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Background: Two scientists walked into a Cambridgeshire pub in 1953 and announced that they had discovered 'the secret of life'. Since the discovery of DNA by Watson and Crick, the field of human molecular biology and genetics has grown in leaps and bounds over the last few decades. Although it took almost ten years for the first ever human genome to be sequenced, today, with technology referred to as the 'next generation sequencing', millions of fragments of DNA from a sample are analysed on parallel sequencing platforms, facilitating high-throughput sequencing and allowing the entire genome to be sequenced in less than a day, at a considerably less cost. The effects of these advancements have been felt in clinical haematology, arguably more so than in the other specialties, given the application of cancer genomics in haematology. 'Targeted therapy' and 'personalised medicine' have become the buzz words in any specialty dealing with cancer. Are these new developments the pinnacle of modern medicine as we are often led to believe?
Aims: Although there is an abundance of studies on the scientific aspects of genomic medicine, there is surprisingly sparse amount of literature exploring the ethical implications and challenges of genomic medicine, even more so as it applies to haematology. This study aims to evaluate the place of genomic medicine in clinical haematology and provide a critical appraisal, focussing on the challenges it poses and ethical implications that arise from it.
Methods: MEDLINE database was searched using the keywords 'genomic', 'haematology' and 'ethics' to identify the relevant literature. The governing principles of modern medical ethics were applied to the topic in question.
Results: Several issues were identified from studies looking at ethical issues arising from genomic medicine. A common theme was responsibility placed on researchers to disclose potentially beneficial genetic information to the participants or patients. This is further complicated when the patient has made an advance decision explicitly expressing that s/he would not want to be contacted should further information become available. Although most would respect the patient's autonomy in this context, some might argue (controversially) that depending on the gravity of information being withheld, a paternalistic approach would be warranted. A similar conundrum may also arise when a clinician may be aware of genetic information of potential benefit to a patient which they may have acquired from the patient's relative. Although next generation sequencing has made genomic medicine much cheaper, it still remains a commodity which is not commonly used in everyday clinical practice. There is inter-regional and intraregional variation in the availability of this specialist service, raising the ethical issues of resource allocation, justice and equity. Of recent times, there have been many large population based genomic studies such as the 1000 genomes project and the recently launched 100 000 genomes project in the UK. Although some of these projects obtain genomic data from patient populations who donate their genetic material with altruistic motives, it can open up opportunities for pharmaceutical companies to benefit financially from these studies, creating a very grey area in modern ethics.
Summary/Conclusions: Although there has been tremendous benefits to patients from the advances in genomic medicine, particularly as it applies to cancer genomics and targeted therapy in haematology, it is not without challenges. Several ethically ambiguous issues arise, particularly in the conduct of research in this rapidly advancing field.

PB2101

PROSPECTIVE MULTICENTER PROGRAM OF REMOTE CONTROL IN ANTIVITAMIN K ANTICOAGULANTS

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Background: The use of oral anticoagulant therapy with VKA (OAT) has progressively increased in the cardiovascular diseases, such as atrial fibrillation, valvular diseases,and venous thromboembolic disorders. The OAT is currently used in 1.7-2% of the population. The grade of anticoagulation control is a vital aspect in the quality of clinical care of these patients. The fact that they remain above or below the optimal range exposes the patients to an increased risk of bleeding or stroke. The new technologies of information and communication allow us to optimize the control of these treatments significantly improving their quality of life and safety
Aims: 1. To evaluate the efficacy and safety of remote control (RC) of OAT in a group of 100 patients. 2. To analyze the degree of satisfaction and quality of life of patients included in the program.
Methods: Prospective multicenter study of the first 100 patients included in our program. The study has been carried out from June 2015 to nowadays. The institutional review board waived informed consent for this health care program that was compliant with the Spanish law in force. The participating centers are: Hospital Universitario Fundación Jiménez Díaz, Hospital Universitario Infanta Elena, Hospital Universitario Rey Juan Carlos and Hospital General de Villalba. Patients were included if meeting the following inclusion criteria: a history of use of OAT for at least three months, a valid chronic indication for the treatment and patients that were capable of handling computerized devices. The exclusion criteria were: patients under age of 16 years, patients with severe diseases or with less than 3 month of treatment with OAT and those who expressed their desire not to participate in the study. We created a reference unit of OAT composed of doctors and specialized nurses: the doctors made the prescription and gave the specific instructions to the nurses. Once they were taught, they are in charge of the active education of patients.
Results: In the first 100 patients included, there there isn't any complication with the treatment and the preliminar results of the quality life surveys are very good. The patients were evaluated in the OAT clinics, and were included when meeting the above mentioned inclusion criteria. Once they had signed the informed consent. After work, in a remote service,we generated a note that was accesible online to the patients in the web Patient Folder. The medical note consists of the prescription. When the control was needed, patient can introduce the result of the digital INR in the same Patient Portal. This web interaction prevents unnecessary movements of the patients and/or family members. There is no separation from the center.
Summary/Conclusions: Our RC program is a new technological system that assists patients in the control of OAT from their home.The program consists in adapting new technologies to the healthcare system to gain in quality of life, with fewer complications, greater safety and comfort for patients and reducing travelling to the hospital and costs. With this program the patients are an actives subjects in the management of their disease.

PB2102

A NEW MODEL OF ORAL HYGIEN FOR REDUCTION AND PREVENTION OF MUCOSITIS IN PATIENTS UNDERGOING MYELOABLATIVE STEM CELL TRANSPLANTATION

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Background: Mucositis is a very common complication of allogeneic stem cell transplantation (allo-SCT). The damage due to radio-chemotherapy is combined with the risk of superinfection. Protocols of oral hygiene play an important role to prevent the onset of oral infections and may improve disconfort due to mucositis.
Aims: We are we presenting data on mucositis prevention using professional oral hygiene and specific dental aids.
Methods: We prospectively enrolled patients who underwent allo-SCT for haematological malignancies. We joined to standard procedures (toothbrush, clorexidine mouthwashes) a set of specific tools, with a daily schedule of application. An oral hygiene session was performed in all patients before the date of recovery. The protocol provide, from the first day of hospitalization, the use of: manual toothbrush with bristles of 0,12 mm of diameter, super-soft; antibacterial toothpaste containing colostrum and antimicrobial enzymes; antibacterial mouthwash containing lisozyme, lactoferrin, lactoperoxidase and extracted colostrum, fluorine, xilitole and aloe vera. From the day 1 after stem cell infusion: manual toothbrush with bristles of 0,10 mm of diameter, ultra-soft; mouthwash containing maltodextrin, propylene glycol, hydroxyethylcellulose, sodium hyaluronate, sodium saccarhin and citric acid. Patients were clinically supervised every day, until engraftment or resolution of mouth lesions, through clinical examination, WHO grading of mucositis, VAS scale for the pain.
Results: We prospectively enrolled 8 patients that underwent to myeloablative conditioning regiment with TBF for haematological malignancies, comparing results with an historical cohort. Results are expressed in figure.

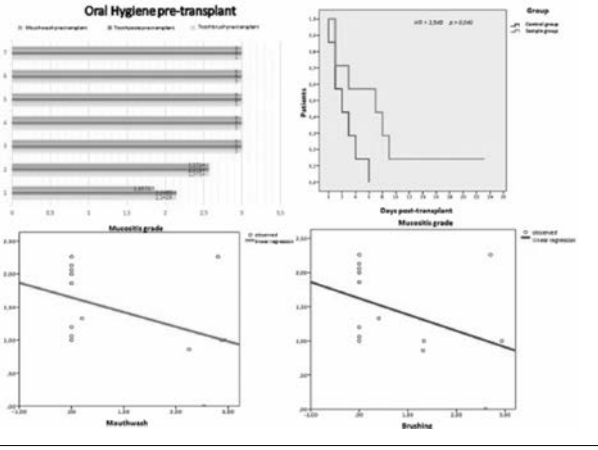

Figure 1.

Summary/Conclusions: Patients who followed a regular schedule of oral hygiene, before and after the transplant, had a lower pain reported in VAS scale, a significant lower level of mucosyte, and a later onset of mouth lesions, in comparison of patients who did not follow the procedure and in comparison of a control group, extracted by the clinical records of allo-SCTs of the previous

year. It has implication for the administration of analgesic drugs (opioids), notably lower in patients treated with protocol. This finding demonstrate the importance of oral hygiene in allo-SCT setting in preventing high-grade mucosytis, suggesting the institution of specific profession of dental hygienist for haematological patients.

PB2103

EVALUATION OF PRE-ANALYTIC AND POST-ANALYTIC PHASES OF THE COAGULATION LABORATORY IN HACETTEPE UNIVERSITY HOSPITALS

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Background: The pre-analytical and post-analytical phase in a test cycle contributes up to 93% of total laboratory errors. Our coagulation laboratory provides routine and stat tests for inpatients and outpatients in Hacettepe University Hospitals, including 1000 beds. Joint Commission International guidelines is followed in our laboratory; we document the unsuitable samples rejected by laboratory and we record critical value notifications, including the person who received the notification (physician, nurse or medical staff), the time and the date of communication. Hence, rejection of unsuitable samples and critical value notification are the quality indicators of our laboratory.
Aims: In this study, we aimed to evaluate the pre-analytical problems and critical values recorded on coagulation and hemostasis tests over one year period in 2015.
Methods: Venous blood samples for routine coagulation testing were considered unsuitable for analysis according to the following specimen rejection criteria of our laboratory; inappropriate clinical orders, inappropriate volume (inadequate blood to anticoagulant ratio), incorrect tube, clotting, delayed transport and visible hemolysis or fibrin following centrifugation. During day time and night shift, authorized laboratory secretary was responsible for reporting of critical values by telephone communication and reverse reading. The critical values in our laboratory were as follows; prothrombin time/international normalized ratio (PT/INR)>5, activated partial thromboplastin time (aPTT)>100 seconds, fibrinogen<100 mg/dL, factor levels<5% and anti-thrombin III<50%.
Results: Total coagulation test request was 155.945 in one year and *5.*090 tubes were rejected according to the rejection criteria of our laboratory. On overall, the more frequent pre-analytical problems could be referred as clotting (38.4%), following inappropriate volume (33.2%), inappropriate clinical orders (7.3%), misidentification (6.5%), hemolysis (6%), incorrect tube (3.8%), fibrin (3.3%) and delayed transport (1.5%), respectively. Among 155.945 tests performed in 2015, we reported 475 critical values, the ratio was 0.3% in total. The critical value notification ratio was 56% for INR, 23% for factor levels, 12.2% for fibrinogen, 5.5% for antithrombin III and 3% for aPTT. The critical value reporting rate was 97-99%, dropped call ratio was approximately 1-3% of all.
Summary/Conclusions: We detected an overall specimen rejection rate of 3.2% in coagulation laboratory. By documentation of rejected samples and periodic training of healthcare personnel, we expect to decrease sample rejection ratios below 1% and to improve total quality management of the laboratory. Our critical value reporting rate was 97-99%, and clinicians were notified of patients' life-threatening results within 15 minutes. We believe that rapid notification of abnormal test results has an impact on patient outcomes. This is the first report on the pre-analytical and post-analytical phases of coagulation laboratory in Turkey. To improve patient outcome, each laboratory has to establish its own specimen rejection criterias and reporting policy for critical values.

PB2104

DEVELOPMENT OF PATIENT-REPORTED OUTCOMES SYMPTOM MEASURE FOR PATIENTS WITH NON-TRANSFUSION-DEPENDENT THALASSEMIA (NTDT-PRO)

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Background: Currently, there is no disease-specific Patient-Reported Outcomes (PRO) measure available to assess key symptoms experienced by patients with non-transfusion-dependent thalassemia (NTDT).
Aims: To develop a symptom outcomes measure for patients with NTDT for use in clinical trials.
Methods: A qualitative study was conducted to develop the NTDT-PRO symptom measure consistent with regulatory requirements. The instrument was developed based on concept elicitation interviews, input from clinical experts, and refined through a process of cognitive interviews. Concept elicitation interviews were conducted among a total of 25 NTDT patients recruited from three countries (Lebanon, Greece, and Canada). Findings from these interviews were used to generate an item pool, inform response options, and determine appropriate recall period. Cognitive interviews were subsequently conducted among subjects in Greece and Lebanon (N=21) to further support saturation and examine the relevance, clarity and understanding of the items among the target population. All interviews were conducted in person in the local language with written informed consent obtained, audio-recorded, transcribed and translated back into English for evaluation.
Results: Based on findings from concept elicitation interviews, saturation of important underlying concepts was obtained and a total of nine symptoms were included in the NTDT-PRO Version 1. Instructions and item stems were derived from patient language elicited during the interviews. An 11-point numeric rating scale (0-10) was used for the response options, anchored on either end by the absence of the symptom and extreme symptom. A daily recall period ("during the past 24 hours") was selected based on the day-to-day variability in the symptom experience. Findings from the cognitive interviews indicate that subjects understood the instructions and that the daily recall period was appropriate. Subjects understood the items as intended, with a few minor exceptions. Four items were deleted as they were not considered core symptoms, and one item was added to address the symptom both with and without physical activity. The NTDT-PRO Version 2 includes six items that address three key symptoms of tiredness, weakness, and shortness of breath both during physical activity and at rest.
Summary/Conclusions: The NTDT-PRO Version 2 consists of six items to assess the key symptoms of NTDT, including tiredness, weakness, and shortness of breath using a 24-hour recall period, with plans to further evaluate the measures in an ongoing observational study and for eventual inclusion into a randomized phase 3 study.

PB2105

IMPACT OF ECONOMIC CRISIS ON THE MANAGEMENT OF HEMOPHILIA PATIENTS IN GREECE

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Background: Economic crisis has had a major impact on public health in Greece during the last years, negatively affecting both ability for health insurance coverage and availability of medical services. As all patients suffering from chronic illnesses, hemophilia patients have had to face difficulties resulting from the austerity measures.
Aims: Purpose of the present retrospective study was to evaluate the impact of economic crisis on management of hemophilia patients for the 6 year crisis period (January 2010 - December 2015) in a single pediatric institution.
Methods: The study included hemophilia A patients followed at the Pediatric Center for Bleeding Disorders of Aristotle University of Thessaloniki, one of the two pediatric hemophilia centers in Greece. Data were recorded 23 for patients with severe hemophilia (FVIII <1%) or moderate hemophilia (FVIII 1-5%) presenting with a severe bleeding phenotype. Patients with inhibitor presence were excluded from the study. A subgroup consisting of 12 patients on regular prophylaxis throughout the studied years was separately evaluated. Data assessed included annual factor consumption (IU/kg) and annual cost for the years studied. The prices for factor concentrates were obtained from the Greek National Organization for Medicines. Changes in patients' family working status and subsequent insurance coverage were also recorded.
Results: Mean age of patients was 11.3 year (range 2-19 years). The mean annual factor consumption did not significantly differ between the onset of crisis and the following years in both groups studied (i.e. total and regular prophylaxis patients). Similarly, the average annual cost per patient did not significantly change during the crisis years for either group. In specific, mean factor consumption for the whole group at starting and closing years was 334.167±227.536 U/KG and 318.235±105.92 U/KG, respectively, with respective mean annual costs 114,864±82,097 and 108,989±62,862 euros. For the regular prophylaxis group mean factor consumption for starting and closing years was 350.16± 268482 U/KG and 318.235±105.92 U/KG, respectively, with mean annual costs 114,187±97,772 and 134,61±54,159 euros. With regards to employment and insurance ability, one patient lost coverage due to parents' unemployment. However, Social Security provided cost-free factor administration, enabling patient to continue his treatment. The only change recorded between crisis onset and following years was a significant increase in number of hospital visits required in order to have access to factor administration, as a result of limited amount of factor administered by the hospital at each visit for home treatment.
Summary/Conclusions: Although Greece has seriously suffered during the last years because of the economic crisis, provision for health has managed to survive - at least for hemophilia patients. Albeit, without some cost on the patients' part.

PB2106

TO PROMOTE THE MEDICAL QUALITY OF HEMATOPOIETIC MALIGNANCIES BY INTERGRATION OF QUALITY CONTROL CIRCLE AND PHS WIN-WIN CONCEPT

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Background: Infection and bleeding contribute to about 2/3 and 1/3 of mortality

associated with hematopoietic malignancies in Nanfang Hospital, China. Patients, hospital staff, medical students and social groups were involved in this QCC.
Aims: To promote the medical quality, two rounds of Quality Control Circle (QCC) were carried out, with the aim to solve the above mentioned complications.
Methods: 1 The perianal infection is the most common infection for hematopoietic malignancies (up to 60%—100%, Ann Hematol. 2003; 82:S167). With severe immunodeficiency, the perianal infection rate was 17.2% in the bone marrow transplantation center (2013Apr.-Sep.). Then QCC1 was carried out following the 10 steps of Plan-Do-Check-Action. Bacterial colony culture experiments from perianal skin before and after sanitization were performed. Since warm aqueous solution of potassium permanganate are recommended for sanitization, such bacterial colony culture is also used to find a proper drug concentration, water temperature and soaking time *etc.* Eventually a tool kit was developed (CN patent No.201520820954.7) to ensure the standardization of procedures. 2 With the team of QCC1, we started QCC2 for decreasing mortality due to bleeding, the shortfall being the insufficient supply of platelets. Tackling of this condition is more complicated and demanding, which also exists in many developing countries. We integrated the functioning of PHS, which stands for patients (P); hospital staff (H); students and social workers (S); in accordance with the complementary advantages (Table 1) for co-operation. Four strategies were incorporated as: Establishing a professional team, systematic education, setting up a platelet donor bank and a new platelet harvest station.
Results: 1 The perianal infection rate gradually decreased from 17.2% to 5.25% and subsequently declined in the following year. Apparently each case of perianal infection could detrimentally prolong hospitalization by ≥2w and approximately 28,000 Yuan of expenditure for treatment, as well as witnessing >4,000/year such hospitalized cases in Nanfang Hospital and from amongst 40,000 new findings of leukemia in China, this QCC1 was awarded the first Prize of Chinese QCC in 2014. 2 Comparing 9 months(m) before QCC2 (2014Feb.-Oct.) and 9 m later (2014Nov.-2015July), we found that the number of platelet transfusions in the department of Hematology increased from 2815U to 3674U (130.5%) and the success rate of applications increased from 58.67% to 75.77%. Within 9 m, QCC2 helped to establish a professional team named as Blood and Bone marrow China (BBCn) which have conducted 19 public lectures, a proficient platelet donors Bank with 448 volunteers and donated 135U of platelet directly. Interestingly, a new platelet harvest station is under construction in Nanfang Hospital, which will harvest thousands of new blood platelets each year. Mortality due to bleeding will significantly decrease thereafter. Table 1. Analysis of patient, hospital stuff, student and society (PHS)

Table 1.

	Presence of	Absence of
Patient	Diseases Social relationships Option to select a hospital	Knowledge Psychological comfort Blood/Platelet Money
Hospital stuff	Knowledge Experience	Time Enthusiasm
Student &Society	Time Money Enthusiasm Blood/Platelet, etc.)	Information Training Trust

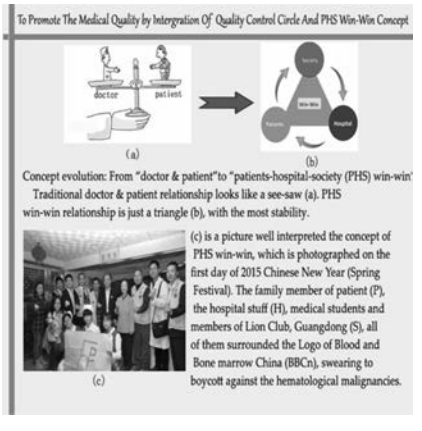

Figure 1.

Summary/Conclusions: QCC and PHS win-win concept could promote the medical quality of hematopoietic malignancies. Most beneficially, they will also alleviate the social conflicts between doctors and patients tremendously in China.

PB2107

PREGNANCY-ASSOCIATED ATYPICAL HEMOLYTIC UREMIC SYNDROME
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Background: Pregnancy-associated atypical hemolytic uremic syndrome (P-aHUS) is a rare catastrophic disorder defined by the occurrence of fibrin and/or platelet thrombi in the microvasculature, resulting in microangiopathic hemolytic anemia (MAHA) and thrombocytopenia without Shiga toxin and ADAMTS13 deficiency. An estimated incidence of P-aHUS is approximately 1 in 25,000 pregnancies during antepartum and puerperium. It is associated with a significant perinatal or maternal morbidity and mortality.
Aims: The evaluation of outcomes in P-aHUS. The establishment of adequate strategies for the timely diagnosis and treatment is very important to improve patient survival and pregnancy outcomes.
Methods: This is a retrospective analysis of aHUS during pregnancy treated in our center and other clinics via telemedicine between November 2010 and February 2016. Nine women mean age 30,4 years (range: 21-36) were diagnosed with aHUS. Most of them (8/9) presented postpartum, 1/9 developed aHUS in II trimester. All had MAHA, thrombocytopenia and severe organ dysfunction. 7/9 had at least one pregnancy before the onset of P-aHUS.
Results: Mean nadir hemoglobin was 60,3 g/L (range 46-69); mean nadir platelet count- 53,000/mm3 (range 16,000-100,000); mean peak serum creatinine- 454 mkmol/l (range 67-998), mean peak LDH- 2,953 IU/L (range 996–11,360). 6/9 required hemodialysis and mechanical ventilation. All underwent plasmaexchange or plasma infusion and all of them was appointed unfractionated or low molecular weight heparins. Signs of mild preeclampsia were observed in 8/9 patients with an average of 7 days (3-14) to debut P-aHUS, 4 of them were diagnosed with fetal death on 27-34 gestation weeks. Live births resulted in 5/9 of pregnancies on 28-38 weeks (median 32). Since the onset of the disease state of the patient rapidly deteriorated: acute renal failure was detected in 8/9 patients (6 cases performed hemodialysis), adult respiratory distress syndrome, requiring mechanical ventilation, was diagnosed in 6/9, 6/9 have pankreatit, neurological symptoms were accompanied by observations of 5/9, 2/9 observed in dilated cardiomyopathy and ischemic colitis in one case. The most common organ dysfunction was kidney failure (8/9). 8/9 patients received therapy with fresh frozen plasma. Treatment with eculizumab was started on 4 patients on 5-17 days from the time of P-aHUS debut (median, 7), but the full course was not held anyone: performed from 1 to 3 injections of 900 mg (median 1). All patients received prophylactic anticoagulation. Outcomes as a whole are extremely unfavorable: 5 of 9 patients died (3/5 causes of death-septic complications, thrombosis- 1, hemorrhage- 1), 2/9 - reached end-stage renal failure by 1 month, 2/9 gained the renal function impairment (CKD3-4) by last follow-up.
Summary/Conclusions: P-aHUS is a severe life-threatening disorder associated with a significant perinatal and maternal morbidity and mortality. We evaluated the outcomes of P-aHUS. Currently, they are extremely pessimistic now. Diagnostic and therapeutic approaches for this disease requires further study in order to optimize results. Clinical suspicion, early identification and effective approaches including target therapy can improve the pregnancy outcomes and prognosis in general.

PB2108

LAMIVUDINE FOR PREVENTION OF REACTIVATION IN OCCULT HEPATITIS B IN PATIENTS WITH NON-HODGKIN LYMPHOMA CD20+ UNDERGOING CHEMOTHERAPY: A COST-EFFECTIVE ANALYSIS
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Background: Occult HBV infections (OBI) are defined by the persistence of HBV in the liver without serum HBsAg and HBV-DNA. They can represent a life threatening event during immunosuppressive CHT. OBI occur in approximately 18% of HBcAb+ patients. Guidelines suggest surveillance for HBV markers in immunosuppressed patients, in particular in treatment with monoclonal antibodies. In our study, the prevalence of OBI reactivation in NHL, in 498 patients of our centre, was 10.42% in HBcAb+ HBsAb- patients. In this work, a cost-effectiveness analysis regarding the use of Lamivudine for the prevention of reactivation in OBI in patients with NHL undergoing chemotherapy with or without Rituximab was performed. In fact, considering guidelines and literature, universal prophylaxis should have been applied to all HBcAb + HBsAg - patients. A cost-benefit issue arises: is it more cost-effective to treat all the HBcAb + HBsAg - patietns with Lamivudine to prevent the OBI reactivation occurence in a small quote of them, or may it be more effective a "wait and see" protocol?
Aims: Our idea was to perform a cost-effectiveness analysis, comparing the costs of prophylaxis of an eventual HBV reactivation and the "monitoring" approach that was used in our patients based on international guidelines.
Methods: The cost of Lamivudine prophylaxis was calculated in a time interval of 12 months, which encompasses the time of a standard Rituximab-containing regimen and a minimum time of follow-up. It has been noticed that, very often, NHL patients need more than one treatment to obtain remission, and, sometimes, if they do not obtain a CR, undergo to long-term "maintenance" with Rit-

uximab. These patients (HBcAb +) are at high risk of HBV reactivations, due to long periods of immunosuppression.
Results: Nevertheless, even if our calculations underestimated the costs of prophylaxis, the "monitoring approach" resulted cost-effective. Moreover, even though in our series no serious events in terms of morbidity and/or mortality occurred, in other papers a monitoring approach did not guarantee patients survival. These detrimental results could be ascribed to the delayed start of lamivudine treatment if the monitoring is not adequately strict. Also, it has been reported that performing only the transaminase monitoring should not be acceptable to prevent severe reactivations.

Table 1.

	Unitary Cost	n. patients	Total per patient	Duration [days]	Total
Cost of prophylaxis					
Lamivudine	€ 3,18	48	€ 152,64	360	€ 54.950,40
HBV DNA monitoring	€ 130,00	48	€ 6.240,00	6	€ 37.440,00
HBsAg monitoring	€ 17,00	48	€ 816,00	6	€ 4.896,00
AST/ALT monitoring	€ 5,74	48	€ 275,52	12	€ 3.306,24
Total	**€ 155,92**	**48**	**€ 7.484,16**		**€ 100.592,64**
Cost of HBV Reactivation					
HBV DNA monitoring	€ 130,00	48	€ 6.240,00	6	€ 37.440,00
AST/ALT monitoring	€ 5,74	48	€ 275,52	12	€ 3.306,24
Cost of DRG 205 [v24 Grouper]	€ 3.769,10	5	-	-	€ 18.845,50
Total	**€ 3.904,84**	-	-	-	**€ 40.746,24**

Drg 205: Liver disease except malignancies, cirrhosis, alcoholic hepatitis with cirrhosis.

Summary/Conclusions: Our monitoring approach resulted efficacious probably because of the monthly ALT assay was strictly observed.

PB2109

AN HPLC AND 1H NMR STUDY OF THE CYTARABINE DEGRADATION IN CLINICAL CONDITIONS TO AVOID DRUG WASTE, DECREASE THERAPY COSTS AND IMPROVE PATIENT COMPLIANCE

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Background: Cytarabine, the 4-Amino-1-(β-D-arabinofuranosyl)-2(1H)-pyrimidinone, (ARA-C), is an antimetabolite cytidine analogue used worldwide as key drug in the management of leukaemia. As specified in the manufacturers' instructions, once the components-sterile water and ARA-C powder-are unpackaged and mixed, the solution begins to degrade after 6 hours at room temperature and 12 hours at 4°C.
Aims: To evaluate how to avoid wasting the drug in short-term low dose treatment regimens, the reconstituted samples, stored in the dark at 25° and 4° C, were analyzed every day of the test week by reversed phase *high-performance liquid chromatography* (RP-UHPLC) and high-field nuclear magnetic resonance spectroscopy (^{1}H NMR).
Methods: All the samples remained unchanged for the entire week, which corresponds to the time required to administer the entire commercial drug package during low-dose therapeutic regimens. The drug solution was stored in a glass container at 4 °C in an ordinary freezer and drawn with sterile plastic syringes; during this period, no bacterial or fungal contamination was observed. After one month, the samples presented evidence of a degradation product (0.8% of starting material), identified as 1-(β-D-arabinofuranosyl)-pyrimidine-2,4-(1H,3H)-dione (ARA-U).
Results: Our findings provide evidence of an optimal physico-chemical stability and microbiological sterility of ARA-C solution stored for one week in the dark, at 4°C. This encourages the use of the reconstituted drug for the time required for short-term multi-dose treatments, avoiding drug waste, patient stress and hospital crowding. Moreover, it seems possible to leave in the same container surplus of different ARA-C packages, improving the cost-effectiveness of the treatment without affecting its efficacy and safety. An additional advantage is the fact that patients are able to have the treatment administered at home.
Summary/Conclusions: Our results show that a solution of reconstituted ARA-C could be employed for a longer period that what suggested by the manufacturers. In fact, patients could receive a safe aliquot to be used at home for short-term treatments, thus optimizing the use of aliquot residues and avoiding vial manipulation and the production of special waste material.

PB2110

COST ANALYSIS OF THE END OF LIFE CARE IN HEMATOLOGICAL MALIGNANCY PATIENTS

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Background: Most hematological malignancies remain chemo-sensitiveness even in the end stage, unlike solid tumors. Sometimes it is difficult to determine the timing to switch to palliative care. Therefore, aggressive treatments often apply to patients in the end of life (EOL). On the other hand, aggressive EOL care causes deterioration of patients' quality of life, depression among family members, and increase of medical expenses.
Aims: We analyzed the contents of medical treatment and the costs to clarify the issues of aggressive EOL care for patients with hematological malignancies.
Methods: Hematological malignancy patients who died in the hemato-oncology unit of a general acute hospital from September 2010 to August 2015, and as the control group, all patients who discharged alive in the same period were studied. The duration of hospital stay, medical cost, contents of treatment, treatment policy, intervention by palliative care team, disease, and disease status were analyzed. T-test and univariate analysis of variance were used to test the factors associated with the cost using SPSS version 23.
Results: We analyzed 2984 patients who were discharged alive and 164 patients who died in our hospital. In patients who died, the mean age was 65.3 years old, 116 (70.7%) were men. Diseases were 54 (32.9%) with multiple myeloma, 38 with (56.1%) malignant lymphoma, 27 (16.5%) with leukemia, 41 (25.0%) with myelodysplastic syndrome. Twelve (7.3%) were in complete response, 6 (3.7%) in partial response, 19 (11.6%) in stable disease, 105 (60.4%) in progression of disease, and 22 (13.4%) were with newly diagnosed disease. Treatment policies were 95 (57.9%) in aggressive anti-tumor and/or support therapy, 69 (42.1%) in palliative care. In patients who died in the hospital, mean medical cost of last hospitalization was 60,200 euro, and the duration of stay was 63.4 days. Those were significantly higher than the mean cost (14,550 euro, p<0.001) and the duration of hospital stay (23.3 days, p<0.001) of patients who were discharged alive. Though the number of patients who died was only 5% of total number of inpatients, the medical expense accounted for 18.5% of total medical cost in the hematology department. Although, the treatment policy was shifted from aggressive therapy to palliative care in most patients (68% in the last 2 weeks, and 71% in the last week), the medical cost per week increased (p=0.020). The half of the cost in the last 2 weeks was the fee for blood transfusion and antibiotics. In the last 2 weeks, 11 days of blood sugar monitoring, 9 times of blood examination, and 5 times of roentgenological examination was performed per patient. In the last week, intravenous hyperalimentation was given in 50.6% of patients, vasopressors was used in 31.7%, hemodialysis was performed in 8.5%, and 6.9% was admitted to ICU. Analysis using univariate analysis of variance revealed that the significant factors which contributed to saving medical cost were palliative care policy on admission (p=0.020), older age (p<0.001), and patients who have care giver(s) (p=0.048). The intervention by palliative care team did not affect the cost.
Summary/Conclusions: In this study, we clarified that aggressive EOL care was given in the most hematological malignancy patients. Blood transfusion and antibiotics were continued until death. Furthermore, prospective study on the aggressiveness of EOL care and quality of life is needed.

PB2111

AMBULATORY MANAGEMENT OF ANEMIA: A RETROSPECTIVE VIEW FROM AN ITALIAN MULTIDISCIPLINARY TEAM

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Background: Anemia is one of the most prevalent clinic condition leading to a specialist medical consult. In 2014 our Internal Medicine unit started a Multidisciplinary Anemia Ambulatory (Internist, Immune-Hematologist, Hematologist) with the purpose to rapidly manage, diagnosis and treatment of anemic pts, giving a direct connection between general practitioners and hospital services.
Aims: Evaluate if Multidisciplinary Anemia Ambulatory and its diagnostic-therapeutic path, with the involvement of different Specialists, resulte in an improvement of the coordination and continuity of care, reducing sanitary cost in terms of hospitalization, drugs rationalization and quality of life for patients.
Methods: Retrospective analysis of 212 patients came to our attention for internist consult due to anemia from January 1st 2014 to January 2015.
Results: A total of 212 patients came to our attention for internist consult due to anemia: 165 female and 47 male, medium age 63,23 years (range 19-100). A precise classification of anemia was determined for 187 pts: 130 had iron deficiency anemia (IDA, 61,32%), 17 multifactorial anemia (inflammatory disorders, chronic kidney disease and combined deficiency, 8,02%), 16 combined deficiency anemia (iron and vitamins, 7,55%), 9 chronic kidney disease related anemia (4,25%), 7 anemia secondary to inflammatory chronic disorder (3,30%), 5 B12 deficiency (2,36%), 2 both folate and B12 deficiency (0,94%), 1 folate deficiency (0,47%). Twenty-five pts were not classified due to lack of data. Severity of anemia was defined according to WHO criteria: 53 pts (25%) presented mild anemia, 123 (58%) moderate anemia, 33 (15,6%) severe anemia.We considered comorbidities of internistic relevance, which could be worsened by anemia: cardiovascular (coronary heart disease, arrhythmias, heart failure), 30 pts; neurologic (ischemic and degenerative diseases), 19 pts; respiratory disease (COPD and asthma), 11 pts.Pts were treated according to clinical practice in relation to

type, severity and clinical manifestation of anemia.130 pts needed more than one access to ambulatory to correct anemia; data from the second access were: pt responders (normalization of Hb levels or improvement of at least 20 g/L): 78 pts; partial responders (improvement of Hb levels from 5 to 20 g/L): 34 pts; non responders: 18 pts. Fourteen pts needed at least 1 blood red cells transfusion, 12 with severe anemia and 2 with moderate anemia. A total of 93 pts needed deep diagnostic insight through specialist pathways, such as hematologic (4 pts), gastroenterologic (39 pts), gynecologic (37 pts), both gastroenterologic and gynecologic (13 pts). All pts were managed as outpatients, except for 8 pts which required hospitalization due to severity of clinical findings: 4 pts were hospitalized.Among IDA pts, 92 were treated with intravenous iron supplement: 32 with sodium ferric gluconate (SFG) (medium 16,68 vials, range 8-43) and 50 with ferric carboxymaltose (FC) (medium 1,04 vials).FC patients fully responded in 76% and 22% were partial responders.
Summary/Conclusions: These preliminary data shows that Multidisciplinary Anemia Ambulatory and its diagnostic-therapeutic path, with the involvement of different Specialists and Operative Unit, resulted in an improvement of the coordination and continuity of care, reducing sanitary cost in terms of hospitalization, drugs rationalization and quality of life for patients. More data will derive from the newborn Anemia Regional Register, which will lead to a better comprehension of the real size of anemia in our local epidemiology.

PB2112

DYNAMICS OF QUALITY OF LIFE IN ANEMIC PATIENTS WITH LYMPHO PROLIFERATIVE DISORDERS TREATED WITH RED BLOOD CELL TRANSFUSIONS AND ERYTHROPOIESIS-STIMULATING AGENTS
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Background: Anemia is a frequent complication of lymphoproliferative disorders (LPD) and antitumor therapy decreasing overall survival rate and Quality of Life (QoL). Anemia's pathogenesis is based on suppression by proinflammatory cytokines, decreasing erythroid precursor's sensitivity to serum erythropoietin and the myelosuppressive effects of chemotherapy. The main methods of anemia's correction are red blood cells transfusions (RBCsT) and using of erythropoiesis-stimulating agents (ESA). Both of them increase hemoglobin concentration and improve QoL. However RBCsT are used in patients with severe anemia and ESA are used to treat mild or middle anemia therefore alteration of QoL after correction may differ.
Aims: The aim of the work was to compare dynamics of QoL in LPD patients during anemia's correction using RBCsT and applying of ESA.
Methods: In this study anemic patients with lymphoproliferative disorders were included. The efficacy of anemia's correction by means RBCsT and applying of ESA was assessed in the base of clinical, laboratory methods and Quality of Life by using the questionnaire FACT-An.
Results: In the first group patients (n=54) with severe or middle anemia with initial Hb concentration of 70.0±1.6 g/l were included. To correct anemia they were prescribed RBCsT. After transfusions (Me=3 Units) the Hb level was increased up to 93.1±1.2 g/l. In the second group patients (n=77) with mild or middle anemia with initial Hb concentration of 88.4±1.4 g/l were included. All these patients were treated with erythropoiesis-stimulating agents. After ESA-therapy a positive response as increase of Hb concentration ≥20 g/l was in 52 (67.5%) out of 77 patients. Herewith the Hb concentration of patients with positive response increased up to 123.1±2.4 g/l. Analysis of dynamics of QoL patients after RBSsT revealed the significant alteration in "Physical well-being", "Emotional well-being", "Functional well-being" and "Anemia" scales. After EPO-therapy significant alteration were revealed in "Physical well-being" and "Anemia" scales. However in comparative analysis of QoL in both groups of patients the maximal improvement was revealed in the scale of "Physical well-being" (in the first group after RBCsT it was from 12.9±0.7 to 11.0±08 points; p<0.001, in the second one after ESA-therapy-from 11.6±0.7 to 9.6±0.7 points; p<0.02) and scale of "Anemia": after RBCsT it was from 41.1±2.0 to 34.2±2.1 points (p<0.001), after ESA-therapy-from 34.5±1.7 to 30.1±1.6 points (p<0.001).
Summary/Conclusions: RBCsT and ESA-therapy may significantly increase the Hb concentration and improve Quality of Life. However QoL of patients after ESA-therapy was better than after RBCsT because Hb concentration was achieved to normal level after applying erythropoiesis-stimulating agents. Nevertheless both methods for anemia correction are effective and ESA-therapy may be prescribed to prolong treatment of anemia after RBCsT.

PB2113

THE TREATMENT- ASSOCIATED SIDE EFFECTS IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA
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Background: Idiopathic thrombocytopenic purpura (ITP) is a disease characterized by the presence of antiplatelet antibodies which accelerate platelet destruction and prevent the release of platelets from megakaryocytes, resulting in various types of bleeding. The primary objective of treatment is to prevent bleeding by increasing the platelet count by reducing destruction of antibody-coated platelets (e.g. corticosteroids, immunoglobulins, splenectomy) or by stimulating platelet production (thrombopoietin receptor agonists, TPO-RAs) with the fewest possible side effects associated with treatment.
Aims: To evaluate the side effects associated with treatment and the quality of life in patients with ITP treated with corticosteroids *versus* TPO-RAs.
Methods: We studied 34 patients with ITP hospitalized in the Clinic of Hematology of Craiova (informed consent obtained) in between 2013 and 2015. Twenty-five patients received corticosteroids (prednisone or high dose dexamethasone) as first line therapy and nine patients with refractory or relapsed ITP were treated with eltrombopag (50 mg once daily as initial dose, followed by 25 mg once daily after platelet count ≥150.000/μL). We evaluated the side effects associated with treatment in both groups. We mention that patients treated with eltrombopag did have not hepatitis B/C or HIV infection, severe cardiovascular diseases or risk factors for thrombosis.
Results: Nineteen patients (76%) treated with corticosteroids presented one or more complications: infections (fungal, bacterial, viral) - 10 cases, arterial hypertension - 4 cases, duodenal ulcer hemorrhage - 1 case, dyspepsia - 2 cases, decompensation of diabetes mellitus - 2 cases, hyperglycemia - 3 cases, cataract - 1 case, insomnia - 2 cases, anxiety - 1 case, peripheral edema - 4 cases. Five patients (55%) treated with eltrombopag presented: headache - 1 case, transient increase of alanin aminotransferase - 3 cases, indirect hyperbilirubinemia - 1 case.
Summary/Conclusions: The treatment with eltrombopag was responsible for less side effects (and also less severe) in comparison with the treatment with corticosteroids, and contributed to the improvement of the quality of life in patients with ITP.

PB2114

MANAGEMENT OF MYELODYSPLASTIC SYNDROMES WITH ERYTHROPOIESIS STIMULATING AGENTS (ESAS): A REAL-LIFE EXPERIENCE
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Background: ESAs are the frontline treatment in low-risk anemic MDS patients and an employment of this therapy in the earlier stage of the disease can delay the need for RBC transfusion, hypothetically by slowing the disease course. It's matter of debate whether the clinical response is a result of proliferation and maturation of the dysplastic clone or stimulation of residual normal erythropoiesis by ESAs.
Aims: Macrocytosis is one of the cytological hallmarks of dyserithropoiesis in MDS: an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non trasfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian register, was performed.
Methods: 159 patients, treated with standard-dose ESAs, have been retrospectively analyzed (Table 1). Data analysis was performed, according to IWG 2006 criteria, at the baseline, after 3 and 6 months of continuous treatment, with a subanalysis of the patients according to WHO and R-IPSS risk stratification. ESAs were started at mean Hb concentration of 9.43 g/dl, mean serum EPO concentration: 42 mU/L, after a mean time from diagnosis of 6 months (r.1-118).
Results: Overall response rate (ORR) was 83.6% (133/159), no difference among WHO and IPSS subgroups was found :75.6% achieved response after 3 months of treatment, while other 8% after 6 months. 2 patients with SD (non responders IWG), in which treatment was continued, achieved response after 9 months. In the macrocytic-responders group 85.5% exhibits again macrocytosis after 3 months, while 13% become normocytic. In the normocytic-responders group 91.7% exhibits again normocytosis, while 4/52 (8%) become macrocytic: in these 4 patients after 3 months there was a contemporary worsening in neutropenia and thrombocytopenia, with transfusion-dependence, regarded as first signs of progression of disease. Non responders were 27/159 (16.9%): in the macrocytic-non responders group 89% exhibit again macrocytosis after 3 months, while 11% become normocytic; in the normocytic group 80% exhibits again macrocytosis, while 20% become normocytic (r.1-23).
Summary/Conclusions: These preliminary data can suggest that, in the majority of MDS patients responsive to ESAs, the increase of Hb concentration occurs mainly stimulating erythroid production in MDS clones; in the minority of patients probably it happens recruiting residual polyclonal erythropoiesis. It is interesting to note that stimulating effects of ESAs last even when the expression of dysplasia progresses.

PB2115

USE OF RADIATION THERAPY FOR THE TREATMENT OF SPLENOMEGALY IN NEOPLASTIC HEMATOLOGICAL DISORDERS
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Background: Splenic irradiation has been used as first treatment for several hematological neoplasms, including chronic leukemia or myeloid malignancies, but with the availability of new drugs its application was restricted.
Aims: In selected cases, not only with palliative intentions, irradiation can be useful treatment modality
Methods: Our study included 13 patients: 5 with chronic lymphocytic leukemia, 6 with high-grade B-cell lymphoma and 2 with diagnose of polycythaemia Vera. In 5 patients the treatment was with radical intention (all of them with high grade lymphoma) and the rest were palliatives as treatment of pain or normalization of red blood cell that allows more time between transfusions. The doses were generally low with range between 5 and 10 Gy in 0.5Gy daily fractions because doses higher than 10Gy did not provide benefits according to literature.
Results: We got 5 complete responses confirmed by PET but after 2 years 2 of them relapsed and were treated with radiotherapy again with the same scheme and obtain the same response to the present day. In terms of palliative intention, splenic irradiation provided a relief of pail from 6 to 12 months, and in 4 patients the disease progressed without new splenic symptoms. One patient received 3 courses of radiotherapy for painful splenomegaly with a gap of 12, 9 and 6 months without acute toxicity and died due to non-splenic leukemia progression.
Summary/Conclusions: In selected patients who are not responsive, not suitable for systemic treatment or palliative, splenic irradiation can be an efficient therapy with little toxicity and sustained response over time. f of pail from 6 to 12 months, and in 4 patients the disease progressed without new splenic symptoms. One patient received 3 courses of radiotherapy for painful splenomegaly with a gap of 12, 9 and 6 months without acute toxicity and died due to non-splenic leukemia progression.

Red blood cells and iron - Biology

PB2116

MOLECULAR DIAGNOSIS OF RBC ENZYMOPATHY BY MULTI-GENE TARGET SEQUENCING IN KOREA

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Background: RBC enzymopathy occurs due to the intrinsic deficiency or a defect in red blood cell (RBC) enzyme and defective function of RBC enzyme causes hemolysis. Practically, functional analysis of enzymes related to RBC enzymopathy are not available easily. Furthermore, differential diagnosis form paroxysmal nocturnal hemoglobinuria, hemoglobinopathy and congental dyseryhtropoiesis is required.
Aims: We aimed to investigate the frequnecy and patterns of gene mutation relevant to RBC enzyme in Korea.
Methods: We performed targeted sequencing in 15 patients suspected for RBC enzymopathy in whom RBC membranopathy was excluded after laboratory workup. Forty two genes were targeted including 20 genes relevant with enzymopathy and 17 genes relevant with RBC memebranopathy. For the differential diagnosis, Phosphatidylinositol Glycan Anchor Biosynthesis Class A (PIGA gene), 3 thalassemia genes (hemoglobin alpha1, hemoglobin alpha2, hemoglobin beta, and hemoglobin beta), and congenital dyserythropoietic anemia II gene (Sec23 homolog B) were included in target sequencing, additionally. Targeted sequencing of 42 selected genes was performed using IlluminaHiSeq 2500. Putative mutations were analyzed in comparison to normal reference control population.
Results: The mean sequencing depth for the 60 samples was 984X (range 534–1171X). PKLR mutation in 10 patients, ALDOB mutation in 1 patient and G6PD mutation in 1 patients. Of note, gene mutation related to PNH and thalassemia were detected in 2 patients; PIGA mutation in 1 patient and thalassemia alpha point mutation in 1 patient. Among 15 patients, no mutation was detected in 1 patient. Collectively, target sequencing revealed gene mutations related to RBC enzymopathy in 80% and gene mutations other than enzymopathy in 13.3% among 15 patients suspected for enzymopathy in Korea.

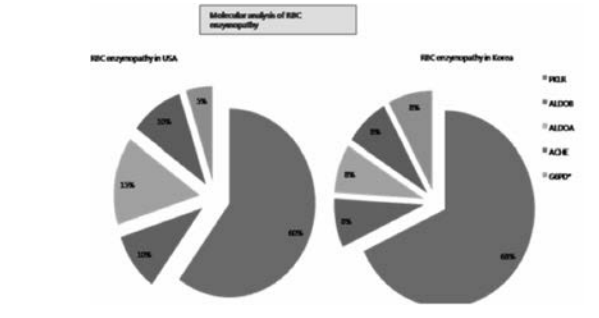

Figure 1.

Summary/Conclusions: Distribution of RBC enzymopathy mutations was similar between USA and Korea. Considering that 13.3% of patients showed gene mutaions unrelevant to RBC enzymopathy, molecular analysis of RBC enzymopathy is necessary for clinical diagnosis.

PB2117

A NOVEL IN-VITRO REVERSAL OF SICKLED ERYTHROCYTES BY COCOS NUCIFERA WATER AND HIGH K+ -ISOTONIC SOLUTIONS

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Background: Red cell sickling and adhesion are favoured by cellular dehydration, which increases the rate of hemoglobin polymerization and cell sickling. Potassium chloride co-transport and calcium-activated potassium channel (Gardos channel) mediate erythrocyte dehydration in sickle cell disease and β-thalassemia, but their role in vaso-modulation is less defined. Blocking the potasium leakage has been suggested as a means of inhibiting dehydration and enhancing normal shape of the sickle cells. We investigated the in-vitro effect of various concentration of K^+ ion in physiological solutions (PSS) as well as in cocos nucifera water which is a natural drink known for its natural high potassium content and health benefits.
Aims: This study has therefore been designed to highlight the possibility of reversing the Na^+/K^+-membrane counter-movement in the extra cellular medium and observe the impact on sickled erythrocyte especially in the dehydrated

state using a natural medium with very high potassium but isotonic solution - Cocos nucifera water
Methods: Twenty blood samples from sickle cell anaemia subjects were collected and studied with different high potassium isotonic media. Firstly with Cocos nucifera water (with K^+ of 240mM); erythrocytes sickling test was done, then repeated after incubation with the fluid for 30 mins. Another part was treated with Sodium Metabisulphite ($Na_2S_5O_7$) solution to induce maximum sickling as controls, then later incubated with the fluid. Subsequently, we subjected the samples to different high concentrations of K^+ in Physiological Salt Solution (PSS) (40mM, 80mM, respectively) and their percentage sickling activities were enumerated using a thin blood smear stained with Leishman dye before and after the treatment. Later experiments to study the effects of the Cocos nucifera water on Osmotic fragility of red cells from different haemoglobin protein electrophoresis before and after incubation with coconut water.
Results: The result showwed statistically significant counts of discoid shaped cells in the blood samples incubated with 80mM and Cocos nucifera water ($P<0.05$, respectively) over the native and the sickled- induced samples while the 40mM PSS did not show significant discoid cells over the sickled cells ($P>0.05$). In 80mM K^+PSS, values of (18% against 78%; before and after treatment); Cocos nucifera (22% against 85%; before and after treatment) were obtained. In addition, all the samples treated with cocos nucifera water have significantly reduced osmotic fragilities ($P<0.05$, respectively) irrespective of their haemoglobin protein status.
Summary/Conclusions: High extracellular potassium in an isotonic medium could reverse the membrane permeability to K^+ exflux from the sickled erythrocyte by possibly overwhelming the Na^+/K^+ counter movement across the membrane thereby promoting rehydration through K-Cl cotransporter and ultimately reverses the sickling. The inhibition of Gardos channel is suggested as a possible mechanism in the interim, also cocos nucifera water has shown in this study, the tendency to reduce osmotic pressure lysis of sickle cells and possibly prolong its life span.

PB2118

ERYTHROBLASTS IN PERIPHERAL BLOOD AS AN OCCASIONAL FINDING IN MULTIPLE SCLEROSIS PATIENTS TREATED WITH NATALIZUMAB
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Background: Natalizumab (NTZ) is a recombinant humanized IgG4 monoclonal antibody directed against the α4 subunit of the α4 β1 (VLA-4) integrin of the lymphocytes. VLA-4 plays a critical role in erythropoiesis and is involved in mobilization of hematopoietic progenitors to peripheral blood. NTZ is used in autoimmune diseases such as multiple sclerosis and Crohn disease, and has been reported to induce CD34+ hematopoietic progenitor cells mobilization and erythroblasthaemia in multiple sclerosis patients.
Aims: To identify and report the presence of erythroblasts in peripheral blood in our cohort of NTZ-treated patients.
Methods: Peripheral blood smears (PBS) of patients treated with NTZ from September 2014 to February 2016 were retrospectively reviewed. PBS were prepared and dyed with May-Grünwald-Giemsa stain and examined by light microscopy by an experienced hematologist. The cohort included 53 patients treated on a monthly basis at different time points.
Results: A total of 82 PBS were reviewed in 53 patients. Fifteen of them (28.3%) showed 1 or more erythroblasts in peripheral blood. Mean time on treatment when PBS was reviewed was 20.5 months (1-74). Mean time on treatment when erythroblastaemia was documented was 14 months (1-71). Once erythroblastemia was described, mean follow up was 10.67 months. No hematologic malignancy was reported in these patients.
Summary/Conclusions: The pressence of erythroblasts in peripheral blood is usually a sign of alarm requiring further investigation since it is generally associated with severe underlying disorders. As previously data support, NTZ is a cause of erythroblastemia and should be taken in consideration in differential diagnosis in cases with erythroblasts in peripheral blood, at any time while on therapy. Knowledge of this frequent side effect is crucial for the correct interpretation of PBS in NTZ-treated patients and to avoid unnecessary diagnostic procedures. Investigation of the mechanisms underlying this effect is needed in order to estimate its repercussion and potential benefits.

PB2119

ROLE OF GENETIC DIAGNOSIS FOR PATIENTS WITH SUSPICION OF THALASSEMIA INTERMEDIATE/MINOR
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Background: Armenia is situated in the link of Mediterranean and Indo-Mediterranean belts of thalassemia. However, due to the scarcity of information restricted only for very rare reported cases it was traditionally underestimated the need of provision of genetic analysis for thalassemia associated with variable degree of anemia and hematological findings. Our previous results from population screening study suggested rather low carrier frequency however with a higher rate for alpha than for beta thalassemia.
Aims: To provide sound data about the mutation spectrum of alpha and beta globin genes in Armenia and to assess the role of genetic testing for patients with ssuspected clinical phenotype, we carried out the first genetic analysis in a cohort of Armenian patients.
Methods: All 48 sporadic and 11 familial cases of Armenian ethnicity with suspicion of mainly thalassemia intermedia or thalassemia minor had genetic testing for alpha and beta thalassemia genes who have been defined by specific hematological features, mainly by hypochromia, microcytosis, and marked variations in size and shape of the red blood cells and/or suffering from minimal anemia. Screening of 22 beta globin gene and 21 alpha globin gene mutations common for the Mediterranean and Indo-Mediterranean regions was performed using multiplex PCRs of DNA purified from blood samples of affected cases and family members. Further biotinylated amplification products were reverse-hybridized to allele-specific oligonucleotides immobilized on membrane teststrips.
Results: 30 patients were diagnosed as simple heterozygotes for one of the following b^0 or b^+ globin gene mutations (-30 [T>A], cd8 [-AA], cd15 [TGG>TGA], cd44 [-C], IVS2.1 [G>A]) responsible for thalassemia minor or intermedia with specific hematological features. In 2 cases 3.7 alpha single gene and 20.5 alpha double gene deletion were detected, while a compound heterozygous genotype of alpha genes consisting of 20.5 alpha double gene deletion and a1 cd59 [GGC>GAC] (Hb Adana) mutation was detected in one patient. More interestingly, two patients were detected with simple heterozygous beta globin gene mutations (cd8 [-AA] or IVS1.110 [G>A]) with the co-inherited triplicated alpha globin gene rearrangement which indeed developed a clinical phenotype of intermediate beta-thalassemia. All 5 families were detected with alpha or beta globin gene mutations. Particularly, sibs in four families were detected with single b^0-mutations or 20.5 alpha double gene deletion. A proband of another family had compound heterozygous genotype for beta globin mutations (cd8 [-AA]/IVS2.848 [C>A]) and parents were carriers of the beta globin mutations along with anti-3.7 alpha globin gene triplication. 13 patients had no alpha globin neither beta globin gene mutations detected.
Summary/Conclusions: This is the first report of genetic screening of suspected thalassemia cases in Armenia. Despite of former pessimistic view on thalassemia in the country compared to the neighboring countries, 80% detection rate of mutations among symptomatic cases emphasizes the importance of development of genetic screening among patients with suspected phenotype with minor or intermediate thalassemia. Meanwhile, the genetic diagnosis has become possible only after having more differentiated clinical and hematological analysis. Furthermore, the screening data does not reject possibility of detection of other mutations, particularly in patients with no mutation through sequencing analysis which raises the importance of genetic testing. In contrast to the population screening data, beta thalassemia was found more frequent than alpha thalassemia. Further larger screening study is required to better understand and assess the role of mutations identified in heterozygous beta-thalassemia cases and the clinical-molecular relationships, as well as mechanisms leading to clinical and hematological severity in simple heterozygotes.

PB2120

IDENTIFICATION OF CARRIERS OF GENETIC ALTERATIONS IN THE HBB GLOBIN CLUSTER: HEMATIMETRIC AND BIOCHEMICAL DATA IN 462 HETEROZYGOTE INDIVIDUALS
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Background: The β globin cluster (HBB cluster) contains several genes differentially expressed over development. Most common alterations in this cluster are point mutations in the β globin gene (β^o, β^+ or β^{++}-mutations depending on the severity) that produce β thalassemia. Additionally, large deletions of one or more genes can occur in the HBB cluster, leading to *β like* thalassemias (εγδβ, δβ or β thalassemia) or Hereditary Persistence of Fetal Hemoglobin (HPFH). Homozygotes or compound heterozygotes for HBB cluster alterations present variable transfusional requirements, depending on the combination of inherited alterations. In many centers, identification of carriers of these alterations is commonly done at hematimetric and biochemical level. Thus, effective identification of carriers and discrimination between different types of HBB alterations at this level is necessary for an appropriate genetic counseling.
Aims: To describe the hematimetric and biochemical profile of individuals bearing heterozygous alterations in the HBB cluster in order to facilitate their identification.
Methods: Large deletions and β gene point mutations were identified by Multiplex Ligation dependent Probe Amplification (MLPA) and sequencing, respectively. Most common alterations in the HBA globin cluster were assessed with the Alpha-Globin StripAssay. Hematimetric data were obtained with a Coulter LH 750 analyzer. HbF and HbA2 levels were obtained by ion exchange HPLC.

In line with literature, individuals were categorized according to the type of deletion (εγδβº, δβº, HPFH or βº) or point mutation (βº, β^+ or β^{++}) in the HBB cluster. Coinheritance of alterations in the HBA globin cluster was a reason for exclusion. 462 carriers of alterations in the HBB cluster were finally included in the study.
Results: Means and standard deviations for hematimetric data variables, HbF and HbA2 levels for different HBB alterations are summarized in Table 1.

Table 1.

Table 1. Hematimetric and biochemical profile of individuals bearing heterozygous alterations in the HBB cluster.

Genetic alteration	n	Age	IIb (gr/dl)	MCV (fl)	MCH (pg)	RDW (%)	IIbA2 (%)	IIbF (%)
Deletions	197							
βº	6		12.2±1,5	66,8±6,6	20.5±2,1	18.8±2,8	**4,8±0,9**	7,7±5,8
(δβ)º	168		12.1±1,2	66.9±4.3	20.9±1,5	**20,1±2,5**	2,8±0,3	**5,9±4,2**
HPFH	9		12,9±1,3	**84,1±6,1**	**27,3±2,5**	16,2±0,8	2,3±0,3	**27,4±7,6**
(εγδβ)º	5	neonates	**7,6±1,8**	68.7±7,6	22.3±2,1	25.1±5,1	1,6±1,2	21,8±8.2
	9	>6 months	10,9±1	59±2,1	18,6±0,6	16,6±1,2	**2,9±0,3**	**1,7±1,6**
Point mutations	265							
βº	170		11,4±1,3	64,1±4,5	20,2±1,4	17,3±1,5	**5,2±0,5**	1,9±1,9
β^+	64		11.8±1,4	65.6±4,3	21,4±1,4	16.5±1,5	**4,8±0,4**	1,4±1,1
β^{++}	31		13,1±1,2	71,8±5	23,2±1,3	15,6±1,4	**4±0,6**	1±1,6

Mean±SD
Key features of each group are presented in bold

Summary/Conclusions: Within deletions, only βº deletions have elevated HbA2 levels (≥3,5%). The main differences between δβ thalassemia and HPFH were HbF levels, MCV and RDW. εγδβ thalassemia cases had variable features in relation to age; severe microcytic anemia was observed in neonates (4 out of 5 required transfusions) and marked microcytosis with mild anemia in *adults*. Many εγδβ thalassemias are caused by de novo deletions, so this condition should be suspected when severe microcytic anemia occurs in neonates, even if there is no history of familiar microcytosis. Roughly, 10% of δβ thalassemias have normal HbF (≤2%) and mimic an α thalassemia phenotype (microcytic and hypocromic red cells, normal HbA2 and HbF levels). When an alpha thalassemia trait is present, elevated RDW (≥16) makes us to suspect that the patient is actually a carrier of a δβº deletion. When a point mutation is present, phenotype aggravates accordingly to β globin production impairment. Generally, these individuals can be easily identified because of elevated HBA2 levels. In conclusion, phenotypes associated with alterations in the HBB cluster are frequently distinctive, although phenotypic overlap can be seen in a subset of cases and molecular analysis may be required.

PB2121

ERYTHROCYTES OF INDIVIDUALS WITH HEMOGLOBIN C TRAIT ARE DENSER AND SMALLER THAN ERYTHROCYTES OF OF INDIVIDUALS WITH HEMOGLOBIN S TRAIT

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Background: Since HbS and HbC are by far the two most prevalent structural hemoglobinopathies in our geographical area, it seems reasonable to develop efficient and rapid tools to discriminate between them in daily practice. Carriers are asymptomatic, and clinicians need to accurately identify them and provide adequate genetic counselling to them in order to prevent the occurrence of homozygous or compound heterozygous offspring.
Aims: The aim of our study was to identify red blood cell (RBC) laboratory parameters of the complete blood count (CBC) that discriminate between HbAS and HbAC individuals.
Methods: The CBC of 355 HbAS and 104 HbAC subjects were run on an Advia 2120 analyser (Siemens Medical Solutions Diagnostics, Tarrytown, New York, USA). All of these Hb variants were detected by HPLC in the HA-8160 analyzer (Menarini Diagnostics, Florence, Italy) and further characterised according to their alkaline and acid electrophoretic pattern using cellulose acetate electrophoresis. Classic hematological parameters and RBC populations of the complete blood count (CBC) were assessed in all subjects. Independent sample t-test was used to compare laboratory parameters between HbAS and HbAC, and receiver operating characteristic (ROC) curves were plotted.
Results: The following parameters were significantly higher in HbAC than in HbAS: RBCs ($5.09x10^{12}$/L *vs* $4.91x10^{12}$/L, p=0.004), Hb (13.97 g/dL *vs* 13.47 g/dL, p=0.017), MCHC (35.35 g/dL *vs* 33.29 g/dL, p<0.001), RDW (15.16% *vs* 14.51%, p=0.002), HDW (2.68% *vs* 2.48%, p=0.001),%MICRO (7.35% *vs* 4.58%, p=0.006),%HYPER (3.04% *vs* 0.82%, p<0.001) and M/H (22.37 *vs* 4.59, p<0.001). The following parameters were significantly higher in HbAS than in HbAC: MCV (82.92 fL *vs* 79.15 fL, p<0.001),%MACRO (0.45% *vs* 0.27%, p=0.033) and%HYPO (4.51% *vs* 1.27%, p<0.001). When only HbAS and HbAC without iron deficiency and α-TT were evaluated, differences remained significant only in MCV, MCHC,%MICRO,%HYPER and M/H (Table 1).%HYPER (AUC=0.842), M/H (AUC=0.839) and MCHC (AUC=0.833) were the most efficient parameters to discriminate between HbAS and HbAC. A cut-off point of 1.15% for%HYPER provided a sensitivity of 79.9% and a specificity of 74.4%. A cut-off point of 34.55 g/dL for MCHC provided a sensitivity of 80.6% and a specificity of 72.1%. A cut-off point of 7.3 for M/H provided a sensitivity of 81.6% and a specificity of 76.7%.
Summary/Conclusions: RBCs of HbAC subjects are denser than HbAS erythrocytes, possibly because the K+:Cl− cotransporter may be more active in HbAC subjects, since lysine (HbC) is more positively charged than valine (HbS), and thus its binding to band 3 may be even further increased. If a patient with a variant Hb not yet identified shows hyperchromia (% HYPER >1.15% and/or MCHC >34.55 g/dL and/or M/H >7.3), the diagnosis is more likely to be HbAC.

PB2122

MUTATIONS FOUND, IN THE PROMOTER REGION OF THE B-GLOBIN GENE, IN ONLY ONE HOSPITAL IN MADRID

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Background: The β^+-thalassemia is characterized by reduced production of beta chains, which can be produced by mutations in the promoter area (either the CACCC or TATA box). Depending on the extent of reduction of beta globin chain, the β^+-thalassemia mutations may be classified into mild and silent. Silent β-thalassemia is characterized in the heterozygous state by normal MCV and MCH, normal or more frequently borderline HbA_2, and normal HbF. Globin chain synthesis ratio is only slightly imbalanced, and sometimes even normal. This phenotype may escape detection by the usual methods, so DNA study being necessary for proper identification. Compound heterozygosity of these mutations with a severe mutation always produces very mild thalassemia intermedia. Frequently the diagnosis is made in adulthood or even in the elderly. Mild β-thalassemia mutations are characterized by a residual high β-chain production, show moderate thalassemia-like hematological features and imbalance chain synthesis. This group of molecular defects includes transcriptional mutants in the proximal CACCC box and in the TATA box. Homozygotes or compound hetrozygotes for these mild mutations usually have thalassemia intermedia. So far a total of 37 alterations have been described in the promoter region, some of which are ethnic specific, -87 C>T are common in Mediterranean areas, while -29 A>G and -88 C>T are frequently found in blacks.
Aims: In this report we present a compilation of the mutations found, in the promoter region of the β-globin gene, in only one Hospital in Madrid.
Methods: The hematological parameters were determined with Coulter LH750 Analyzer. HbA_2 and HbF levels, as well as the different hemoglobins, were measured and analyzed by ion-exchange HPLC (VARIANT™ II). Hemoglobins were studied by capillary zone electrophoresis (CE) (Sebia). Genetic analysis of the β and Gγ globin genes was carried out by automatic sequencing and, in the case of α genes, by multiplex PCR.
Results:

Table 1.

	Hb (g/dL)	VCM (fL)	HCM (pg)	HbA2-HbF (%)	OTHER MUTATION	SEX/AGE	ETHNIC
-27 (A>T) β+ [I]	13.9	80.4	26.6	4.2-0.5		M/39	C
-28 (A>G) β+							
II	6.4	71.0	21.3	1.9-98.0	CD71/72 (+A) β^0	F/2	Ch
III	6.8	72.5	21.5	2.0-97.5	CD71/72 (+A) β^0	F/2	Ch
IV	8.9	73.5	25.7	2.7-27.9	CD41/42 (-TTCT) β^0	M/5	Ch
V	13.9	75.8	24.3	4.9-0.9		F/29	Ch
VI	13.0	77.3	25.1	4.4-1.1		F/23	Ch
-29 (A>G) β+							
VII	12.6	74.2	25.3	5.1-5.5	$-\alpha^{3.7}$/αα	M/31	B
VIII	12.8	72.3	22.6	5.3-3.8	$-\alpha^{3.7}$/αα	F/28	B
IX	10.1	73.5	23.3	6.9-3.3	$-\alpha^{3.7}$/αα	M/20	B
X	11.7	73.3	23.5	6.5-4.1	$-\alpha^{3.7}$/αα		B
XI	12.3	75.8	24.6	5.0-3.9	$-\alpha^{3.7}$/αα	F/21	B
XII	14.2	78.3	26.1	7.0-4.0	$-\alpha^{3.7}$/αα + [HbS]	M/42	B
XIII	6.8	83.2	26.0	5.7-10.3	$-\alpha^{3.7}$/αα + [HbS]	M/33	B
XIV	12.1	68.9	21.3	4.2-6.3		F/22	C
XV	15.2	72.7	23.7	3.8-5.0		M/25	B
XVI	10.9	62.5	18.2	4.3-0.4		M/17	B
XVII	12.8	67.1	19.8	4.0-0.8	IVS-2-nt843 (T>G) β^+	F/44	B
XVIII	14.1	74.3	23.5	4.6-1.7	IVS-1-nt5 (C>G) β^{++}	M/53	C
XIX	9.5	88.5	26.7	3.0-17.0		M/23	C
XX	12.5	78.0	25.2	5.0-3.5		F/24	B
XXI	6.9	62.0	27.0	6.0-9.5	-29 (A>G)	M/26	B
XXII	6.8	61.2	28.9	8.0-30.0	-29 (A>G)	M/25	B
XXIII	12.8	73.4	25.7	5.2-3.4		F/20	B
-56 (G>C) β+ [XXIV]	13.0	72	23.5	5.3-4.1		M/23	B
-86 (C>A) β+							
XXV	15.2	67.3	22.3	3.6-6.0		M/77	C
XXVI	11.8	71.9	22.1	3.5-1.6		F/30	C
XXVII	15.3	73.2	24.9	3.2-2.0		M/32	C
-88 (C>T) β+							
XXVIII	11.8	76.9	23.6	5.3-5.7		F/25	B
XXIX	13.1	71.8	23.3	5.9-5.6		F/39	C
XXX	13.3	72.3	22.9	5.3-3.4		F/50	C
XXXI	13.2	73.6	23.2	5.6-2.9		F/20	C
XXXII	11.0	66.1	22.1	5.2-15.8	[HbS]	F/4	B
XXXIII	12.4	73.7	23.7	4.1-4.1	[HbS]	M/27	B
XXXIV	12.4	72.7	24.2	6.0-10.0	[HbS]	F/27	B
-101 (C>T) β+ [XXXIV]	11.3	72.5	23.9	3.8-1.5		M/2	B

Summary/Conclusions: All these mutations show the importance of the promoter region in the β globin genes. Except for the mutation located at the distal CACCC (-101 C>T) that presents a β-thalassemia silent, the rest behaves as a β-thalassemia mild, generally high levels of HbA_2 HbF and being the cause most likely the partial removal of competitiveness to limit transcription factors, facilitating their access to the promoters of both genes as the δ γ genes. Thus,

as seen, any alteration in the CACCC proximal box and the TATA box, cause a moderate decline in the synthesis of the β globin chain, which has been tested both cases of thalassemia intermedia that they made their debut in the second decade of life with a moderate clinical as when associated with a HbS where the disease is mild sickle cell anemia as there is a residual activity of 20% HbA. The identification of such mutations is important for a good genetic counseling that will provide information to individuals and couples at risk (both carriers) in relation to the mode of inheritance, the genetic risk of having affected children and history nature of the disease, including treatment and thus make an informed decision about their reproductive options.

PB2123

PERFORMANCE EVALUATION OF RETICULOCYTE COUNT ADOPTING NEW CALIBRATOR XN CAL ON THE SYSMEX XN SERIES

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Background: Reticulocyte counts have been known as an accurate index of erythropoietic activity. Currently, automated hematology analyzer could measure the reticulocyte count with increased precision and accuracy. The newly introduced Sysmex XN (Sysmex Corporation, Kobe, Japan) has been introduced calibration of reticulocyte count that was not applied by the previous hematology analyzer XE series.
Aims: to clarify the effect of calibration, we evaluated the performance of reticulocyte counting with the Sysmex XN and XE series.
Methods: The XN-9000 was calibrated with new calibrator XN CAL and total 40 blood samples were analyzed to evaluate accuracy of reticulocyte count. The results from the XN-9000 and previous hematology analyzer (XE-2100) were compared with those obtained by manual reticulocyte count as reference method. The precision profiles of the XN-9000 were also evaluated with quality control materials according to Clinical & Laboratory Standards Institute (CLSI) document EP05-A3.
Results: There were perfectly matched results between the XN-9000 and reference method, however the XE-2100 consistently gave significantly lower values compared with reference method. In precision evaluation, the XN-9000 showed impressively improved precision profiles than those of the XE-2100.
Summary/Conclusions: Our study has shown that correlation between the two instruments are good, but the bias of reticulocyte count significantly exists in the XE-2100, thus the effort of calibrating reticulocyte is critical to minimize instrument-to-instrument difference. By calibrating reticulocyte count, the XN series could provide more accurate results of reticulocyte count, as well as improved precision.

Red blood cells and iron - Clinical

PB2124

EVALUATION OF A MORE EFFECTIVE TREATMENT REGIMEN AND DOSE OF RECOMBINANT HUMAN ERYTHROPOIETIN FOR MEDICATION OF ANEMIA IN PREGNANCY

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Background: There is evidence that pathogenesis of anemia in pregnancy (AP) is multifactorial. It has not relation to ineffective erythropoiesis, caused by iron or folate deficiency only. Blunted erythropoiesis is one of main AP causes. According to our own data inadequately low production of erythropoietin (EPO) for the degree of the anemia found at more 50% of anemic pregnants. That is why recombinant human erythropoietin (rHuEPO) combined with iron is effective method in the therapy of anemic pregnant women, who had been ineffectively treated with iron alone. Because EPO does not cross the placenta, this protects the fetus from its use during pregnancy. Most recent studies show overall the effect of rHuEpo is of clinical benefit in treating of anemia in pregnancy. But there is no standard dose for this medication during pregnancy.
Aims: To carry out the comparative study to identify more effective treatment regimen and dose of rHuEPO for medication of AP.
Methods: Three groups of anemic pregnant women were enrolled. All groups were stratified according to age, gestational aged and initial mean Hb levels at anemic pregnant women. All of them met the following criteria for inclusion in the study: gestational age above 20 weeks, Hb concentration <9.5 g/dl, inefficiency of iron therapy alone for at least 4 weeks, and absence of pregnancy complications, or severe systemic diseases. The treatment protocol comprised a combined therapy with rHu-EPO (epoetin-α) subcutaneously and 200 mg iron sulphate orally daily. We used next regimens of rHuEPO dosing: Group 1 (n=18)-75 IU/kg three times per week (225 IU/kg per week); Group 2 (n=21)-100 IU/kg three times per week (300 IU/kg per week) and Group 3 (n=16)-120 IU/kg two times per week (240 IU/kg per week). We evaluated Hb levels weekly beginning before start of rHuEPO therapy during three weeks at all groups of anemic pregnants. The advance of target Hb level (10.5 g/dl for I trimester and 11.0 g/dl for II trimester) in 2 week of rHuEPO therapy we considered as complete response to the medication. Informed consent was obtained from all patients for being included in the study.
Results: Response to the rHuEPO therapy depended on the regimen of dosing (Table 1). The mean Hb levels, before and in 3 weeks after medication, in group 1 were 8.45±0.28 g/dl and 9.87±0.21 g/dl, in group 2-8.17±0.53 g/dl and 10.52±0.28 g/dl, in group 3-8.16±0.46 g/dl and 9.87±0.28 g/dl respectively (Fig.). We did not observe any serious adverse effects during the therapy with rHu-EPO.

Table 1.

Groups of anemic pregnant women	Regimen of dosing, IU/kg/dose	Weekly dose, IU/kg	The number of treated anemic pregnants with complete response to the medication (effectiveness of rHuEPO therapy) n (%)
1 (n=18)	75 three times per week	225	9 (50%)
2 (n=21)	100 three times per week	300	16 (76.5%)
3 (n=16)	120 two times per week	240	7 (43.8%)

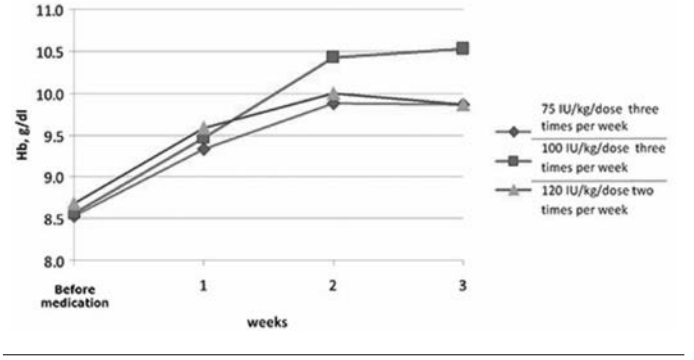

Figure 1. Comparison of hemoglobin trends in groups of anemic pregnant women who treated with different doses of rHuEPO.

Summary/Conclusions: Effectiveness of rHuEPO therapy is dose-dependent. The use of rHu-EPO at dose 100 IU/kg subcutaneously three times per week is more effective treatment regimen for medication of AP.

PB2125

ADENOTONSILLECTOMY IN SICKLE CELL DISEASE: IS TRANSFUSION NEEDED?

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Background: Adenotonsillar hypertrophy in patients with sickle cell disease (SCD) can predispose to several complications including obstructive sleep apnea, vasocclusive crisis (VOC), pulmonary hypertension, and acute chest syndrome. Traditionally, all children with sickle cell disease (SCD) indicated for adenoidectomy and/or tonsillectomy have been exposed to exchange transfusion preoperatively aiming at reduction of both surgical and SCD-related complications.
Aims: To address the need for transfusion in patients with SCD undergoing adenotonsillectomy and to report its risks and benefits.
Methods: All patients with SCD admitted in SQUH for adenotonsillectomy from July 2006 till January 2015 were included in the study. Between 2006 and 2012, patients' files were reviewed for retrospective data recording. After 2012, the study has been prospective.
Results: The current cohort included 70 patients with SCD. They were categorized into three groups. (No transfusion, simple transfusion and exchange transfusion group). Almost 33% of patients with SCD didn't need any preoperative transfusion before surgery (23 out of 70 patients). There was no statistically significant difference between the 3 studied groups as regards the development of surgical or SCD related complication. Development of postoperative vaso-occlusive crisis and acute chest syndrome was comparable in the three groups. As an advantage, there was a markedly significant less blood used in patients given simple top-up transfusion as compared to exchange transfusion group. All results are shown in table 1.

Table 1.

Table (1): Pre- and Post-operative data of the three studied groups:

	Non-transfusion (group 1) n=23	Simple transfusion (group 2) n=33	Exchange transfusion (group 3) n=14	*P* value
Hb (g/dl) Mean± SD Range	8.4 ± 0.36 7.3-11.2	8.1 ± 0.21 6.8 – 9.8	9.7± 1.2 7.5 -12	<0.001 (1 vs 2& 2 vs3)
Hemoglobin S (%)	76.2± 19.2	73.1 ± 21.4	77.8 ± 14.3.3	>0.05
Preoperative transfused PRBCs - Total (ml) - Mean (±SD) - ml/kg	NA NA	6085 (184.4± 105.8) 9.1	12500 (892.9± 446.3) 17.7	0.0001 0.000
Postop. Surgical Complications: -No -Primary Hge -Secondary Hge	21 1 1	28 3 1	14 0 0	0.076
Postoperative Non-surgical Complications: - No -VOC - ACS	20 2 1	30 1 2	12 1 1	0.64

PRBCs: Packed red blood cells, Hb: Hemoglobin, Hge: Hemorrhage, Postop.: Postoperative, VOC: vaso-occlusive crisis, ACS: Acute chest syndrome

Summary/Conclusions: We conclude that it is safe to do adenotonsillectomy in patients with SCD with hemoglobin above 8 g/dl without any transfusion. Exchange transfusion before surgery is not indicated and it does not improve surgical or SCD-related outcome.

PB2126

CARDIAC AND HEPATIC IRON ASSESSMENT BY MR IMAGING IN PATIENTS WITH BETA THALASSEMIA: SINGLE CENTER EXPERIENCE

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Background: Cardiac and hepatic magnetic resonance imaging for iron load becomes more of an issue in patients with thalassemia. The planning of chelation therapy needs more guidance in terms of signs or markers.
Aims: The aim of the study is to assess the relationship between cardiac and hepatic magnetic resonance imaging (MRI) values with serum ferritin level, splenectomy status, and chelation therapy in patients with thalassemia major and intermedia.
Methods: A total of 117 patients (58 male, 59 female) with thalassemia major (n=90) and thalassemia intermedia (n=24) who were followed up in Istanbul Medical Faculty Thalassemia Center were enrolled in the study. Their cardiac and hepatic magnetic resonance imaging was evaluated by specialist radiologist and T2* with R2* values were calculated. The chelation therapy status and splenectomy status of patients was recorded. Patients were divided into three groups according to Cardiac T2* findings: high risk group (T2* MRI <10 ms), medium risk group (T2* MRI 10–20 ms) and low-risk group (T2* MRI >20 ms). The statistical analysis between MRI findings and chelation therapy was performed by SPSS 21 version.
Results: The mean age was 24,77±9,87 years. The mean length of disease was 21,36±10,11 years. Splenectomy was performed in 40 patients. Fifty-four patients used DFX (deferasirox), 13 patients used DFP (deferiprone), 6 patients received DFO (deferoxamine), 2 patients received DFO plus DFX, and one patient was administered DFX plus DFP. The mean level of ferritin was 2093 ±1,97 ng/ml (median 1252 ng/ml). The correlations of ferritin with MR parameters were executed and results were found as following: a weak negative correlation with Cardiac T2* values (p=0,00, r=-0,35), a strong positive correlation with Hepatic R2* and liver iron concentration (p=0,00, r=0,61; p=0,00, r=0,77 respectively). There was also weak positive correlation between ferritin and ALT and AST (p=0,00, r=0,39; p=0,00, r=0,36 respectively). The gender effect on findings was found Hepatic R2* (296,7 *vs* 495,8 Hz) and liver iron concentration (9,6 *vs* 15,8 mg/g) were significantly lower in females (p=0,00). When analyzed according to chelation therapy, there was no significant relationship apart from Hepatic T2* values which were significantly higher in patients receiving DFX (6,7 *vs* 3,9 ms; p=0,04). The ferritin (2291 *vs* 1262 ng/ml) and liver iron concentration (13,8 *vs* 8,7 mg/g) were significantly increased in patients with thalassemia major compared to intermedia group. The ferritin level was significantly decreased from medium risk group to low-risk group (4038 *vs* 1758 ng/ml; p=0,00). The liver iron concentration was significantly decreased from high risk group to low-risk group (21 *vs* 19 and 11 mg/g; p=0,03 and p=0,02 respectively).
Summary/Conclusions: Cardiac T2* and Hepatic R2* were better correlated with ferritin. When combined with negative correlation of ferritin with Cardiac T2* values, it may be supposed that the choice of chelation therapy may be done according to Cardiac T2* and Hepatic R2* results.

PB2127

A INSIDIOUS LINE BETWEEN THALASSEMIA INTERMEDIA AND LEFT VENTRICULAR NON-COMPACTION DISEASE: THE ROLE OF CARDIAC MAGNETIC RESONANCE

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Background: Differentiation of left ventricle non-compaction (LVNC) from hypertrabeculated LV due to a negative heart remodeling in thalassemia intermedia (TI) can depend on the selected cardiac magnetic resonance (CMR) criterion.
Aims: We verified whether the diastolic non-compacted to compacted myocardium (NC/C) ratio criterion could actually discriminate the abnormal trabeculations observed in β-TI from LVNC patients and we compared this diagnostic approach with the CMR criterion suggested by Grotoff M *et al.* (Eur Rad 2012), which has been reported to be highly sensitive and specific for the LVNC diagnosis.

Table 1.

Parameter	LVNC (N=20)	β-TI (N=18)	P value
Age (years)	48.19 ± 18.78	42.18 ± 7.20	0.209
Male sex, N (%)	16 (80%)	5 (27.8%)	0.003
LV EDVI (ml/m2)	107.50 ± 29.35	103.12 ± 27.46	0.582
LV ESVI (ml/m2)	54.45 ± 33.71	39.71 ± 12.69	0.222
LV SVI (ml/m2)	52.72 ± 16.81	65.88 ± 16.50	0.040
LV EF (%)	50.30 ± 17.78	61.29 ± 4.87	0.034
LV Mass Index(ml/m2)	90.06 ± 38.77	67.19 ± 12.72	0.093
RV EDVI (ml/m2)	83.15 ± 27.12	92.24 ± 35.27	0.382
RV SVI (ml/m2)	36.45 ± 23.41	36.53 ± 18.46	0.927
RV SVI (ml/m2)	48.00 ± 14.83	53.47 ± 19.79	0.399
RV EF (%)	59.90 ± 14.95	60.65 ± 9.82	0.891
Myocardial fibrosis, N(%)	9 (45%)	3 (16.7%)	0.086

Methods: CMR images were analyzed in 180 patients with β-TI consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network and 20 patients with proved diagnosis LVNC studied at FTGM MRI Lab in Pisa from 2002 to 2014. The CMR diagnostic criteria applied in β-TI patients were: a modified CMR Petersen's criterion proposed by Piga et al (Am J Haem 2012) based on a more restrictive ratio of diastolic NC/C >2.5 at a segmental level and the Grothoff's criteria (percentage of trabeculated left ventricular myocardial mass (LV-MM) ≥25% of global LV mass and a total LV-MMI NC ≥15 g/m2). In

the 20 patients with LVNC the final diagnosis was performed based on Grotoff's criteria and on the clinical/functional criteria for LVNC to further increase the pre-test probability of the disease.
Results: In β-TI patients at least 1 positive NC/C segment was found in 18 patients (10%). Compared with LVNC patients, in 18 β-TI patients the non-compaction areas were less frequent (3.70±2.22 *vs* 1.62±1.16; P=0.007). The LV-MM NC percentage and LV-MMI NC g/m2 were significantly higher in LVNC than in β-TI patients 27.21±2.45% *vs* 10.88±3.96%, P<0.001; 20.35±5.60% vs.7.30±4.77%, P<0.001). None of the β-TI patients fulfilled the Grothoff's criteria. The table shows the comparison of CMR parameters. LVNC patients had significant lower LV stroke volume index and LV ejection fraction and they had an higher frequency of myocardial fibrosis detected by the LGE technique, although the statistical significance was not reached.
Summary/Conclusions: Differentiation of LVNC from hypertrabeculated LV in β-TI patients due to a negative heart remodeling depends on the selected CMR criterion. Based on our data in all β-TI patients with a NC/C ratio >2.5 we suggest to use Grothoff's criteria to improve the specificity of the diagnosis of LVNC.

PB2128

GENOTYPE/PHENOTYPE PATTERN IN EGYPTIAN PATIENTS WITH SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) refers to all different genotypes that cause this clinical syndrome, yet secondary effectors genes are likely to modulate its phenotype.
Aims: This study aimed to evaluate genotype variation and allele segregation among SCD patients and its relation to the different clinical presentations.
Methods: This cross-sectional study included 38 SCD patients. Genotyping for beta thalassemia gene(BT) mutations was performed using polymerase chain reaction (PCR) and reverse hybridization. Assessment of genotype relation to phenotype was based on: Onset of the disease, transfusion history, baseline MCV, HbF%, frequency of pain episodes, and hospitalization, percentage of patients who suffered from acute chest syndrome, stroke and avascular necrosis, sickling score, echocardiographic, carotid doppler findings.
Results: The most common BT gene mutation among SCD patients was Codon 6[A>T] HbS/β^0 IVS1-1[G>A] (18%), then Codon 6[A>T] HbS/β+ IVS1-110[G>A] (8%). 55% of the patients were diagnosed by baseline Hb electrophoresis as Sβ+. Only 52% of sickle cell disease patients by genotype had MVC normal and 72% had HbA2 <3.6%. Eighty eight% of $S\beta^{0}$patients by genotype had low MCV, while half of Sβ +patients had HbA2 >5%. Although, we found a higher baseline Hb S% and a lower Hb F% among homozygous sickle cell patients compared with the 2 other groups, yet the results did not reach statistical significance Homozygous SCD patients had a significantly earlier age of onset, a higher baseline MCV and a lower HbF% compared with $S\beta^0$ and $S\beta^+$ patients, but a comparable baseline markers of hemolysis. Eighty percent of our studied Sickle cell patients had acute painful episodes, 18% suffered from at least one attack of acute chest syndrome, 5.3% had avascular necrosis of femur 15.8% had single episode of stroke while 6% had cardiac morbidity, 13% had impaired left ventricular contractility, (8%) had cardiomyopathy, none had pulmonary hypertension, and all had normal transcranial Doppler. We did not find significant difference between all 3 groups as regard the frequency of painful Crises, the need for hospitalization, the percentage of patients with avascular necrosis, cerebral stroke, acute Chest Syndrome and the sickling score. Causes for transfusion varied among different groups, the most common cause in homozygous sickle cell disease and sickle β^0 patients was painful crises and stroke (36%&20%), while in sickle β^+ thalassemia patients, the only cause was anemia(100%).This variation was not statistically significant, and all 3 groups had comparable mean pretransfusion Hb level, number of transfusion, transfusion requirement. Moreover, we did not find significant difference between the three groups as regard number of patients on Hydroxyurea treatment (76% among homozygous sickle cell patients, 67% among $S\beta^0$ and 75% among $S\beta^+$patients.
Summary/Conclusions: the presence and the nature of associated β-thalassaemia mutations influences the clinical presentation of sickle cell disease.

PB2129

DETECTING THE PREVALENCE AND AWARENESS OF CARRIER RATE OF THALASSEMIA IN A COHORT OF UNIVERSITY STUDENT POPULATION IN SRI LANKA

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Background: The thalassemias are a group of autosomal recessive disorders caused by a reduction or absent production of one or more of the globin chains that make up the haemoglobin tetramers. Two main types, α and β thalassemias are seen according to the type of globin chain involved, and there are other types although not common such as δβ γδβ thalassemias which are identified. Thalassemias pose an increasing health problem for the Indian subcontinent and many Asian countries. Worldwide 56,000 conceptions have a thalassemia major of which approximately 30,000 are affected by β-thalassemia major and 3500 succumb to hydrops fetalis syndrome. Most of these patients are born in developing and low income countries where they create an enormous health burden. There is a significant increment of thalassemia carrier rate in Sri Lanka, probably an unestimated increment in alpha thalassemia. The increment of the number of patients with thalassemia form 2,094 in the year 2005 to 4,924 by the year 2010 in Sri Lanka is a major concern where further attention is necessary in investigating in to the failure of the general understanding and counselling the of thalassemia carriers. An analytical cross sectional study was done on 384 undergraduates in University of Sri Jayewardenepura, Sri Lanka during year 2015. Objective of this study was to determine the prevalence and awareness of thalassemia trait among the students of University of Sri Jayewardenepura.
Aims: Aim is to screen the university student population who are the next generation to be married and a potential group of the population where effective screening and genetic counseling for thalassemia can be done. This university represents students from all parts of the country and hence a cross section of the country is represented.
Methods: Venous blood was obtained from the students from the four faculties and pre tested self-administered questionnaire was used to gather information regarding awareness about thalassemia trait among the participants. Samples were analyzed by the fully automated hematology analyzer Sysmex SX 500i. The initial diagnosis of thalassemia carrier was done by using red cell indices of the analyzer report combined with the blood picture reported by the consultant haematologist. Final confirmation was done by quantifying the HbA2 levels by HPLC. (High Performance Liquid Chromatography)
Results: Of the 384 blood samples tested, 53 (13.8%) had hypochromic microcytic red cell indices (MCV <80 fL, MCH <27 pg) with normal iron studies. Fifteen students (3.9%) were possible alpha thalassemia carriers where they showed thalassemia indices with normal iron profile and HPLC (A2 levels <3.5%) with the blood picture showing typical changes of thalassemia carrier. Eleven students (2.9%) were carriers of beta thalassemia. Out of 384, twenty seven students (7%) showed a haemoglobin level between 7-11.5g/dl with no obvious thalassemia indices or blood pictures finding of thalassemia and they had low serum ferritin levels. Out of 384 questionnaires, only two hundred and twenty two (77.6%) were aware of thalassemia major and out of them, only hundred and sixty five (88.7%) knew that pre- marital screening could prevent thalassemia. This knowledge was mainly obtained by news items and television.
Summary/Conclusions: The awareness of thalassemia in the cohort of students tested was not satisfactory and it highlights the importance of introducing methods to improve their basic knowledge of the disease. This also detected the Beta thalassemia carrier rate to be 2.9% which are compatible with the results of the studies done previously on thalassemia in Sri Lanka, however there seems to be a trend in higher levels of suspected alpha thalassemia rate which was found to be 3.9% which needs attention. These need to be confirmed by genetic studies which are financially difficult in the current set up. An incidental finding of anaemia of 27 students was important in a young university student population who are the future workforce of the country. This study highlights the importance of continuing the screening campaigns specially the student screening programmes, pre-marital counseling and new born screening service that are of paramount importance in reducing the births of thalassemia major.

PB2130

REFRACTORY AUTOIMMUNE HAEMOLYTIC ANEMIA IN ELDERLY EFFECTIVELY TREATED WITH MYCOPHENOLATE MOFETIL

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Background: Autoimmune haemolytic anemia (AIH) is a rare disorder that sometimes may be lifethreatening. The treatment is not evidence based and corticosteroids, azathioprine, cyclophosphamide, rituximab, and mycophenolate mofetil have been used effectively although there is not enough evidence for the effectiveness and safety of the later one.
Aims: We present here 7 patients with AIH that were treated effectively with mycophenolate mofetil.
Methods: Seven patients, 6 males and 1 female aged 75-82 years were refered to our department diagnosed with autoimmune haemolytic anaemia 12 to 60 months ago. Three patients had received corticosteroids, azathioprine cyclophosphamide and rituximab without gaining a PR or better and without obtaining stable condition maintaining Hb levels about 9g/dl. Two patients obtained PR with costicosteroids and rituximab but relapsed within 6 months and could not get rid of the drugs for more than 4 months. The remaining 2 were on contineous corticosteroid treatment and occasionally rituximab for more than 4 years maintaining hemoglobin levels between 8 and 10 g/dl. All

the patients started mycophenolate mofetin for 3 months and corticosteroid tapering thereafter.
Results: Within 6 months 6 patients reached normal levels of haemoglobin and reduced DAT titer to equal or less than half. They continued the drug for 26-36 months when DAT was negative and remained in remmision for 15-22 months without any drug. The 7th patient continues taking mycophenolate for 5 years with haematological remission (Hb=14g/dl) but DAT (+). All the patients tolerated the drug very well without major adverse events. Lymphocytopenia grade 2 and hypoglobulinaemia grade was a common condition in all patients without infections. The 7th patient was prophylactically indroduced to antiviral therapy as well as to cotrimoxazol/azathioprine.
Summary/Conclusions: Mycophenolate mofetil is an effective and well-tolerated drug in elderly patients with haemolytic anemia refractory to conventional therapies.

PB2131

DIFFERENTIAL IRON STATUS AND TRAFFICKING IN BLOOD AND PLACENTA OF ANEMIC AND NON-ANEMIC PRIMIGRAVIDA SUPPLEMENTED WITH DAILY AND WEEKLY IRON FOLIC ACID TABLETS

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Background: Iron deficiency (ID) globally impact billions of people; mostly pregnant women. Anemia during pregnancy is mainly associated with ID; and prophylaxis includes daily supplementation of iron folic acid (IFA) tablets. During pregnancy, increased foetal iron demand leads to fluctuations in body iron reserves; thus supplemental iron is recommended to meet the iron demand. The effectiveness of weekly IFA supplementation in comparison to daily IFA intake during pregnancy is still shadowy, as the available data from well-defined clinical trials is inconsistent. The amount of quantifiable iron circulating in blood and stored in placenta, in both the supplementation schemes and their correlation with pregnancy outcomes is pre-requisite to reach any firm conclusion. Owing to this, the present study was conducted to providing better understanding of hemodynamics and molecular mechanism in human placental iron transfer.
Aims: To investigate the iron dynamics in anemic and healthy pregnant women supplemented with daily and weekly IFA supplementation and its relation with clinical outcome.
Methods: The subjects include non-anemic primigravida (NAP) (Hemoglobin (Hb) >11 g/ dl, N= 60) and anemic primigravida (AP) (8.0 <Hb <11 g/ dl, N=60) were followed across pregnancy at three time-points *i.e.* baseline (13-16 weeks of gestation), after three months (25-28 weeks) and 6 weeks postpartum. The subjects (NAP and AP) were randomly allocated to daily dose comprised 100 mg iron and 500 µg folic acid tablets and weekly dose contained two IFA tablets/ week till six weeks postpartum. Corresponding changes in hematological markers, iron status indicators viz. ferritin, iron and soluble transferrin receptor (sTfR) in blood and placental ferritin expression was studied. Birth outcome, gestational length, infant weight and placental weight were recorded. The statistical significance level ($p < 0.05$) between the groups were assessed by applying unpaired t-test using SPSS (version 20.0).
Results: Weekly IFA supplementation for three months has significantly altered the Hb, hematocrit and red blood cell (RBC) count ($p<0.05$) and increased the level of serum sTfR ($p<0.002$) at postpartum in AP. After three months of supplementation, serum iron and sTfR were significantly different ($p<0.01$) between the two supplemental groups in NAP; whereas, serum ferritin ($p<0.001$) was significantly high at postpartum in daily IFA group of NAP. Placental ferritin expression was increased in NAP supplemented with daily IFA in comparison to weekly IFA. The placentas obtained from AP supplemented with weekly IFA showed significantly low ferritin ($p<0.05$) expression when compared with daily IFA supplementation. However, birth outcome, infant weight, gestational length and placental weight were comparable in both the supplementation groups.
Summary/Conclusions: The placental ferritin expression and its serum level showed similar trend among anemic and non-anemic primigravidas supplemented with daily and weekly IFA tablets. Although, significantly lower placental ferritin was observed in AP supplemented with weekly IFA tablets; but there was not any difference in birth outcome, infant weight or gestational length between two supplementation groups. The modulation in iron transporter expression may be involved in anemic placentas to flux iron for developing fetus, thereby maintaining the healthy pregnancy. The results of this study revealed that blood iron status/ hematological markers may be helpful to assess the optimum amount of supplemental iron required during pregnancy.

PB2132

THE USE OF NEW ANTIVIRAL DRUGS FOR THE TREATMENT OF HEPATITIS C VIRUS (HCV) INFECTION IN PATIENTS WITH THALASSAEMIA: PRELIMINARY DATA ON SAFETY AND EFFICACY

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Background: Several adult patients with thalassaemia major (TM) and talassaemia intermedia (TI) following the failure of combination therapy with pegylated interferon (PEG-IFN) and ribavirin are still affected by Hepatitis C virus (HCV) infection and need to be treated with new antiviral drug. To date, no data are available on interaction between chelation treatment and new antiviral drugs.
Aims: Here we report our preliminary data on their efficacy and safety during different chelation treatments on the first four weeks of antiviral therapy.
Methods: Since December 2015, 17 patients with TM, 2 with TI, 2 with sickle cell/thalassaemia were considered for treatment. Twelve received Ledipasvir-Sofosbuvir, 7 Ledipasvir-Sofosbuvir-ribavirin, 1 sofosbuvir-ribavirin, one daclatasvir-sofosbuvir. All patients but three, were and continued to be under chelation treatment: five were under deferasirox (DFX), 3 under deferiprone (DFP), 2 under desferioxamine (DFO), 6 under alternate DFO/DFP, 1 under DFO and DFX combined treatment. During the first four weeks of therapy all patients had weekly monitoring of serum ferritin, liver enzymes, creatinin level, complete blood count and biweekly monitoring HCV RNA copies.
Results: All treatments were very well tolerated; patients receiving ribavirin still not presented significant increase in blood transfusion requirements. Following one week of treatment liver enzyme decreased significantly from baseline: mean±SD alanine transaminase (ALT) and aspartate transaminase (AST) decreased from 103±127 to 35±U/L ($p=0.03$) and from 83±85 to 33±27 ($p=0.02$) U/L, respectively; mean±SD ferritin level and HCV RNA copies decreased from 1556±1436 to 1334±1355 ng/ml ($p=0.68$) and from 1,650000±1472378 to 0 copies/ml ($p=0.000014$). A further decline was observed at fourth weeks in mean (± SD) ALT, AST and ferritin level which dropped to 28±29 U/L ($p=0.02$), 30±20 U/L($p=0.01$) and 1058±1011 ng/ml ($p=0.26$), respectively. No increase in glomerural filtration rate (GFR) and impairment in urinary albumin/creatinine ratio were observed among patients treated with DFX with respect to basal level. No event of neutropenia and agranulocytosis neither reduction in neutrophil count with respect to basal level occurred among patients treated with DFP
Summary/Conclusions: All treatments were effective in promptly drop viral copying. The observed reduction in ferritin level is likely linked to reduced hepatic lysis and shouldn't be used to modify chelation therapy. Our opening data show that chelation treatment is feasible and safe. The termination of treatments and further studies on larger population are needed to confirm these preliminary observations.

PB2133

CMR FOR MYOCARDIAL IRON OVERLOAD ASSESSMENT: CALIBRATION CURVE FROM THE MIOT PROJECT

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Background: The measurement of myocardial iron by T2* cardiovascular magnetic resonance (CMR) has been established as fundamental to the best practice management of thalassemia. However, iron calibration data in humans is limited and CMR calibration varies according to instrumentation and technique.
Aims: The aim of this study was to calibrate the T2*-CMR technique for non-invasive cardiac iron assessment, by considering a segmental approach.

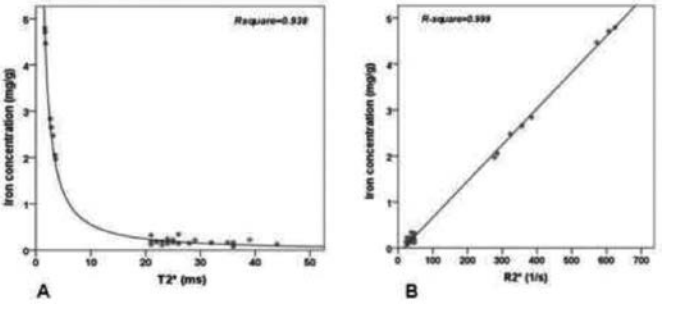

Figure 1.

Methods: Four human hearts were studied from transfusion-dependent

patients after their death within the MIOT network (Myocardial Iron Overload in Thalassemia). A multislice multiecho T2* approach was adopted. After CMR, used as guidance, the heart was cut in three short-axis slice and each slice was cut into different equiangular segments, the same ones in which the T2* was assessed. Tissue iron concentration in the segments was measured with inductively coupled plasma atomic emission spectroscopy.
Results: T2* and iron concentration were overall assessed in 36 myocardial segments: 6 in the first heart (year 2004), 6 in the second one (year 2004), 8 in the third one (year 2005), and 16 in the fourth one (year 2010), Figure 1A shows the segmental iron concentration (in milligrams per gram dry weight) plotted *versus* the correspondent segmental T2* value (in milliseconds). As expected, the relationship was not linear. In Figure 1B the R2* values (R2*=1000/T2*, in s^{-1}) were considered. Regression analysis yielded a linear calibration of the following form: $[Fe]_{R2^*}$=0.0079xR2*-0.1262 (R-square=0.999).
Summary/Conclusions: As in the only previously proposed calibration curve by Carpenter et al (Circulation 2011), we did not collected hearts with an intermediate iron burden. We found an excellent linear agreement between R2* and cardiac iron with a model similar to the calibration curve in the gerbil showed by Wood J et al (Circulation 2005). The results further validate the current clinical practice of monitoring cardiac iron *in vivo* by CMR.

PB2134

REDUCED INSULIN NEED IN PATIENTS WITH TYPE 2 DIABETES MELLITUS (T2DM)WITH IRON DEFICIENCY ANEMIA THREATED WITH SUCROSOMIAL IRON VS INTRAVENOUS SODIUM FERRIGLUCONATE. MULTICENTRIC PROSPECTIVE STUDY
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Background: About one out of four diabetic patient shows iron deficiency anemia. I.v. sodium ferrigluconate is frequently used as iron deficiency anemia therapy. The sucrosomial iron is a new compound used in the therapy, made of sucrose ester containing liposomal iron. Sodium ferrigluconate increases the amount of free radicals. The increase of free radicals increases inflammation. Inflammation reduces insulin sensitivity in diabetic patients.
Aims: To assess whether the use of sucrosomial iron involves less use of insulin in patients with T2DM with iron deficiency anemia
Methods: This study is a multicentric randomized study. We considered 40 T2DM patients with iron deficiency anemia, with TIBC saturation <10% and hemoglobin <10 g/dl, without documented infections, tumors or autoimmune diseases. All patients received diabetic diet. They received lispro insulin TID + glargine insulin once daily. In group A 20 patients, M: F=12/8, median age 75 years (R65-82), median blood glucose 230 mg/dl (R170-350), median CRP at onset 10 mm/lh (R2-22), were treated with sodium ferrigluconate 62.5 mg in 250 cc NS ic iv in 4 hours/day for 12 days. In group A, 10 patients had anemia by gastrointestinal hemorrhage, 5 by atrophic gastritis, 5 by insufficient intake. In group B, 20 patients, M: F=11/9, median age 78 years (R67-83), median blood glucose 220 mg/dl (R180-380), median CRP at onset 12 mm/lh (R2-20), were treated with sucrosomial iron 1CP 30 mg orallyx2/day for 30 days. In group B, 12 patients had anemia by gastrointestinal hemorrhage, 4 by atrophic gastritis, 4 by insufficient intake. Differences between the two groups were not statistically significant. Statistical analysis was done with Fisher exact test and with Chi Square test.
Results: In group A at day 6 of iron support the median values of CRP were 38 mm/lh (R4-127), with 5-documented infections (urinary 3, lung 1, skin 1); only 8 patients achieved blood glucose values <or=140 mg/dl with a median total lispro insulin dose of 42 U(R25-60) and glargine insulin dose of 22U (R10-28). In group B at day 6 of iron support the median values of CRP were 12 mm/lh (R2-12), with 2-documented infections (urinary 2); 15 patients achieved blood glucose values <or=140 mg/dl with a median total lispro insulin dose of 20 U(R12-23) and glargine insulin dose of 20U (R10-22).
Summary/Conclusions: In diabetic patients with iron deficiency anemia supported with sucrosomial iron the median lispro insulin need appears to be lower than that of the patients supported with i.v. sodium ferrigluconate. This study needs confirmation on a larger cohort of patients.

PB2135

HYPERFERRITINEMIA: CLINICAL COURSE AND THERAPEUTIC STRATEGY
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Background: Hyperferritinemia is a common laboratory finding, which requires assessment of iron status and investigation for underlying disorders, such as hereditary hemochromatosis, inflammatory and malignant conditions. True iron overload is found in approximately 10% of patients presenting with hyperferritinemia (Adams & Barton. Journal of Hepatology 2011;453-8). Although research of the last decades has given insight into new molecules and mutations and their role in iron homeostasis, the clinical consequences of all these mutations are not straighforward, and there are still cases of hyperferritinemia with no underlying cause identified.
Aims: Objective of the study is to assess the clinical course of patients with hyperferritinemia and the therapeutic manoeuvres performed.
Methods: Data was collected retrospectively for 17 patients with hyperferritinemia, followed up for a mean of 5.75 years. They were negative for classic HFE mutations (C282Y homozygosity, C282Y/H63D compound heterozygosity) and had no underlying malignancy, inflammation or alcohol abuse. Hepatic iron was assessed with MRI T2* or liver biopsy. The decision to propose therapeutic phlebotomy was based on presence of iron overload, eligibility for blood donation and patient preferences.
Results: Seven patients were heterozygous for ferroportin (FPN) R178Q mutation. At diagnosis their mean serum ferritin level (SF) was 2,017ng/ml (range 800-5077) and trasferrin saturation (TS) was normal. Three patients with moderate iron overload were offered regular phlebotomies once per month; iron overload was diminished, while SF did not change significantly. In the maintainance phase, phlebotomies were performed once in 3 months, and interrupted during pregnancy and puerperium. Patients without iron overload were encouraged to become blood donors. Seven patients, albeit negative for tested HFE and ferroportin mutations, had mild to moderate hepatic iron accumulation, with mean SF at 880ng/ml (range 435-1351); three of them had elevated TS, that is over 45%, and two had fatty liver on imaging studies. Venesections were performed to all of them, once in 1-2months initially and then every 3-6 months. Iron accumulation and SF were reduced even down to normal ranges; venesections were well tolerated at the aforementioned frequency. A patient homozygous for HFE-H63D mutation, with high SF and high TS, had no iron overload; SF was reduced without any intervention during the follow-up. A teenage girl with ferroportin polymorphism had isolated hyperferritinemia without iron overload nor high TS, which remained so after 8 years. A patient with hyperferritinemia, high TS and catarract, was tested positive for the light ferritin 5'-UTR iron response element (L-FT IRE) mutation, which has been identified in the so-called 'hereditary hyperferritinemia-catarract syndrome'. No iron overload was detected at diagnosis and after 5 years of follow-up. These changes of SF and TS from baseline values are depicted in the figure (blue lines: FPN heterozygotes, orange lines[wt]:no mutation identified).

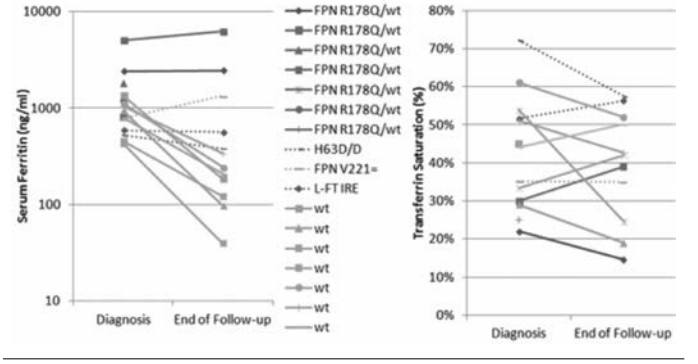

Figure 1.

Summary/Conclusions: In all of the described cases, a benign course of the patients with hyperferritinemia is evident. Even in the cases with moderate hepatic iron overload, a less stringent phlebotomies program (once per 1-2 months, compared to higher frequency of weekly venesections, recommended for classic and juvenile hereditary hemochromatosis types by European Association for the Study of the Liver, Journal of Hepatology 2010;53:3–22) seems to provide satisfactory response, removing excess iron from tissues and being very well tolerated. This proposal could be incorporated into prospective trials, in order for clear evidence-based guidelines to be developed in the future.

PB2136

EFFECT OF L-TYPE CALCIUM CHANNEL BLOCKER (AMLODIPINE) ON MYOCARDIAL IRON DEPOSITION IN PATIENTS WITH THALASSEMIA MAJOR: A RANDOMIZED CONTROL TRIAL
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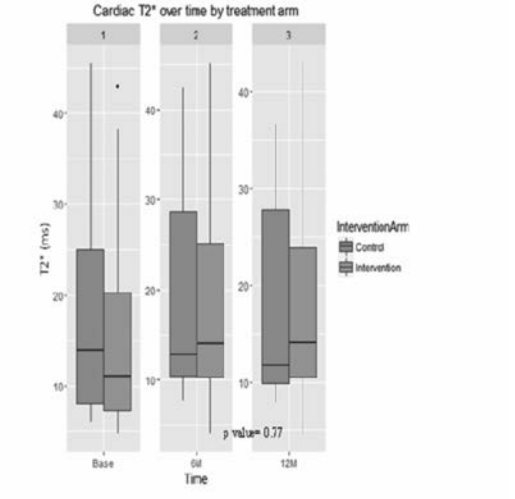
Background: Sideroblastic cardiomyopathy is a feared complication in Thalassemia Major (TM). Recent evidence suggests a role for L-type-Ca^{2+}-chan-

nels (LTCC), in addition to standard chelation therapy, in mediating myocardial iron-uptake.
Aims: Our primary objective was to determine the efficacy of Amlodipine in reducing myocardial iron-load when compared to controls. Changes in echocardiographic derived LV function, liver T2* and iron-content were also sought.
Methods: Participants managed for over a year with a transfusion need of ≥180 ml/kg/year, having received ≥10 blood transfusions in life-time and with serum ferritin ≥1000 ng/dL were invited. All patients with known hypersensitivity to amlodipine, history of developing tetany with calcium channel blockers or systolic blood pressures ≤ 2 SD for age at baseline and pre-existing cardiac conditions were excluded. Twenty patients in a 1:1 allocation ratio were randomized into the intervention and control arms. Conventional echocardiographic measures, global longitudinal strain (GLS), T2* CMR and liver T2* were obtained at baseline, 6 and 12 months. Continuous and categorical variables were reported as mean (SD and CI)/median (IQR) and frequency/percent respectively. Mann-Whitney U test and Fisher exact test were used. Change in outcomes with respect to time and treatment were tested using repeated measure ANOVA. P-value of <0.05 was considered significant.
Results: 10 participants in intervention arm (median age 18 IQR 15.8-19.3 years) and 9 controls (median age 16 IQR 13-18 years) were analyzed. The two groups were statistically similar at baseline. Adjusting for age, years on transfusion and chelation compliance, a statistically non-significant trend of improved cardiac T2* was seen from baseline (15.8ms, CI 6.9-24.6) to 6 months (T2*= 17.3ms, CI 8.8-25.9) in the intervention arm (p=0.77) when compared with controls. This change was sustained at 12 months (figure 1). MIC, conventional echocardiographic parameters and GLS, liver T2* and LIC did not change significantly throughout the study between the two arms.



Change in Cardiac T2* values from baseline at 6 months and 12 months by study group.

Figure 1.

Summary/Conclusions: Calcium channel blockers such as amlodipine show promise but larger clinical trials are needed to establish their role.

PB2137

IRON STATUS IN BETA THALASSAEMIA TRAIT
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Background: Iron overload remains the cause of morbidity and mortality in patients with beta thalassaemia major. Repeated transfusions and ineffective erythropoiesis lead to excessive iron stores in the body. However, not much is known about the status of iron in carriers of beta thalassaemia mutation. In Pakistan nearly 1/3rd population has iron deficiency and the policymakers have suggested global flour fortification with iron. This may have implications for the nearly 7% BTT population. Without knowing whether the BTT individuals are prone to develop iron overload or deficiency, this may be potentially harmful to such individuals.
Aims: The aim of this study was to determine whether carriers of beta thalassaemia trait could develop iron deficiency or iron overload.
Methods: Beta thalassaemia trait was diagnosed with HPLC (HbA2>3%) and serum Ferritin levels were measured using sandwitch ELISA method. A total of 101 individuals (50 males and 51 females) with BTT were assessed for serum ferritin levels. None of these subjects were on oral or injectable iron therapy or has any recent inflammatory event.
Results: Overall, 93% carriers of beta thalassaemia trait were anaemic whereas 7% had Hb within the normal range. Serum ferritin levels of 18.8% were low (mean serum ferritin while 8.3±4.25 ng/ml) while 75.2% individuals were iron replete. Interestingly, 6% individuals had serum ferritin levels higher (mean 911±788 ng/ml) than the reference range. This iron overload was more common in adults (4%) and males (4%). The results imply that iron deficiency a common occuring in beta thalassaemia trait individuals in Pakistan. However, a smaller proportion of carriers may also develop iron overload.
Summary/Conclusions: This pilot study was carried out to determine the status of iron balance in beta thalassaemia trait individuals. Both iron deficiency and overload were observed. These findings are significant and provide a rationale for more robust epidemiological studies.

PB2138

ACQUIRED IRON-REFRACTORY IRON DEFICIENCY ANEMIA (ACQUIRED IRIDA): A TUMOR RELATED DISORDER
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Background: Mutations in the TMPRSS6 (matriptase-2) gene are associated with severe iron-refractory iron deficiency anemia (IRIDA) resulting from an overexpression of hepcidin. Microcytic anemia is the hallmark of this disorder. A similar form of acquired microcytic anemia has been observed in several situations, including benign tumors (1) or malignant tumors (1), treatment with m-TOR inhibitors (3).
Aims: To evaluate the prevalence of this acquired form of microcytic anemia.
Methods: From the group of patients with anemia studied in our red cell pathology unit for a year those with microcytic anemia and biochemical data similar to IRIDA were clinically evaluated.
Results: A total of 3911 anemia patients were studied for a year, including 882 (22.5%) with microcytic anemia. Nine of these 882 patients (1% of microcytic anemia, and 0.2% of total) showed a pattern similar to IRIDA. Main analytical data of these 9 patients are included the table below.
The microcytic anemia showed mixed inflammatory (increased SR and SF) and iron deficiency (low SI and high sTfR) signs. In all cases the diagnosis of the microcytic anemia was at the onset of the disease, and in 8 out of the 9 was related to neoplasia, including: renal carcinoma (2 cases), carcinomatosis peritoneal due to bladder neoplasia, disseminated mesotelioma, bladder carcinoma and lung carcinoma, renal lymphoma, disseminated neoplasia of probable bladder origin, rectal adenocarcinoma with a perirenal mass. Six of them died. The remaining case was an HIV patient with pneumocystis jirovecii pneumonia, after pneumonia treatment anemia disappeared.

Table 1.

Pt	Hb	MCV	HCM	PLT	Leu	SR	FE	TIBC	SI	SF	sTfR
51/M	93	69	20	590	10	90	3	44	6	849	7
71/M	119	76	24	552	9	80	2	27	8	927	5.4
69/M	98	75	24	454	15	84	1	25	4	1827	5.5
35/M	97	74	23	546	9	90	5	39	13	835	5.8
69/F	73	74	23	157	5	90	5	38	14	2097	4
55/F	87	77	24	473	5	87	7	42	17	635	4.4
78/M	116	74	25	117	13	92	15	51	29	1127	3.6
58/F	70	72	22	452	14	87	2	29	2	294	7.6
85/M	95	71	23	393	7	64	2	30	8	571	5.1

Pt: Patient age in years; M: male, F: female; PLT: platelet; Leu: leucocytes; SR: sedimentation rate in mm Hg; FE: serum iron in µmol/l; TIBC: total iron binding capacity µmol/l; SI: saturation index in%; SF: serum ferritin; sTfR: soluble transferrin receptor in mg/l (normal values: 1.5-4.5)

Summary/Conclusions: This microcytic anemia was rare, but it was related with malignant tumor with very poor prognosis, mainly involving kidney. Despite its rarity, this diagnosis should be considered owing to the poor prognosis. The term acquired IRIDA is proposed for this type of acquired microcytic anemia.

References
1. Weinstein DA, Roy CN, Fleming MD, Loda MF, Wolfsdorf JI, Andrews NC. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. Blood 2002;100:3776-81;
2. Kamai T, Tomosugi N, Abe H, Arai K, Yoshida KI. Increased serum hepcidin-25 level and increased tumor expression of hepcidin mRNA are associated with metastasis of renal cell carcinoma. BMC Cancer 2009, 9:270;
3. Kaplan B, Qazi Y, Wellen JR. Strategies for the management of adverse events associated with mTOR inhibitors. Transplant Rev (Orlando) 2014;28:126-33.

PB2139

GENOTYPE IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL DISEASE: RELATION TO VASCULAR COMPLICATIONS AND SUBCLINICAL ATHEROSCLEROSIS
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Background: Sickle cell disease (SCD) has a wide spectrum of complications.

Many factors contribute to the variability in SCD including genetic determinants.
Aims: To assess the relation between different genotypes among 38 children and adolescents with SCD and vascular markers, iron overload as well as vascular complications and subclinical atherosclerosis.
Methods: SCD patients (21 males and 17 females), in steady state were studied. All patients were subjected to full history and thorough clinical examination with special emphasis on history of sickling crisis, cardiopulmonary disease, acute chest syndrome, stroke, bone manifestations, nephropathy, spleen status, transfusion history and hydroxyurea/chelation therapy. Laboratory investigations included hematological profile, liver and kidney functions, markers of hemolysis (lactate dehydrogenase [LDH] and indirect bilirubin) and serum ferritin. Soluble CD163 (sCD163) was measured by enzyme linked immunosorbent assay while analysis of platelet microparticles (PMPs) was done by flow cytometry. Pediatric SCD severity index was assessed based on both clinical and laboratory variables. DNA genotyping was identified using on polymerase chain reaction and reverse hybridization. Echocardiography and assessment of carotid intima media thickness were performed.
Results: Based upon DNA genotyping, patients were categorized as either homozygous sickle cell disease (HbSS) or heterozygous sickle cell disease (Hb S/β); 24 (63.2%) patients had sickle cell anemia (SCA), 10 (26.3%) patients had sickle β^o thalassemia and 4 (10.5%) patients had sickle β^+ thalassemia. Comparison between patients with SCA (n=24) and those with sickle β-thalassemia (n=14) revealed no significant difference as regards age, sex, anthropometric measures, Tanner stage, family history or residency (p>0.05). A trend towards higher incidence of consanguineous marriage among families of patients with sickle β-thalassemia was found (p=0.082). Patients with SCA had significantly higher incidence of pulmonary hypertension, acute chest syndrome, frequent sickling crisis, avascular bone necrosis and nehropathy while those with sickle β-thalassemia had higher incidence of splenectomy, viral hepatitits and heart disease (p<0.05). The degree of hemolysis and iron overload was increased in sickle β-thalassemia patients compared with SCA group as shown by elevated transfusion index, LDH and serum ferritin (p<0.05). Patients with SCA displayed an evident state of inflammation, vascular injury and subclinical atherosclerosis reflected by high white blood cells (p=0.018), monocyte count (p=0.017), HbS, sCD163 levels (p<0.001), PMPs (p<0.001) and CIMT (p=0.029). Upon comparing genotypes among patients with mild/moderate *versus* severe disease, SCA genotype represented the higher incidence in severity (80.8%) followed by sickle β^o thalassemia (19.2%) while all patients with sickle β^+ thalassemia (n=4) were in the mild/moderate group. Genotype, in addition to HbS and parameters of vascular dysfunction (sCD163, PMPs and CIMT) were independently related to disease severity in logistic regression analysis. The cutoff values the studied vascular markers in relation to disease severity were determined.
Summary/Conclusions: Our findings could be of pathophysiological importance, because they provide evidence that genotyping may not only have a role in predicting disease severity in young SCD patients but it is potentially involved in determining the type of vasculopathy developed in those patients, allowing better individualized treatment.

PB2140

MARKED HYPERFERRITINEMIA (SERUM FERRITIN>10000MG/L). A POOR PROGNOSIS FACTOR

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Background: Iron overload is a poor prognosis factor in patients with myelodysplasic syndromes or patients undergoing hematopoietic stem cell transplantation (HSCT) when considering a serum ferritin (Ft) of 1000 µg/l as a threshold. Marked hyperferritinemia (MHFT) is a hallmark of hemophagocytic syndromes and is a diagnostic criterion of the hemophagocytic lymphohistiocytosis.
Aims: The characteristics of patients with MHFT (Ft>10000µg/l) were studied.
Methods: Cases with hyperferritinemia (>1000 µg/l) and the characteristics of the patients with MHFT (Ft>10000 µg/l) were studied for a year. Serum Ft was evaluated using an Architect c.i. 16200 (Abbott Diagnostics).
Results: A total of 1056 samples presented Ft ≥1000 µg/l, 40 of which showed a MHFT(3.8%) in 25 patients. Of these, 12 died (48%) between 2 and 67 days after the determination, including: 4 non hematological neoplasias with metastasis; 3 with infection after HSCT; 2 with hemophagocytic syndrome associated with chronic myelomonocytic leukemia and diffuse large B-cell lymphoma (DLBCL); 1 respiratory infection during T-cell lymphoma treatment; 1 acute myeloid leukemia in pancytopenia postchemotherapy and 1 chronic lymphatic leukemia that evolved into a Hodgkin's lymphoma. Of the 13 who survived, 7 presented infection while undergoing HSCT (viral infection caused by CMV in three cases, type A influenza virus in 2 and 1 viral encephalitis; 1 fungal infection (aspergillus) and 2 bacterial sepsis (pseudomonas). The six remaining cases showed 2 hepatopathy (1 hepatic cirrhosis with MRSA sepsis and 1 on the onset of autoimmune hepatitis); 2 presented MHFT during chemotherapy (1 acute myeloid leukemia and 1 for DLBCL plus aspergillus infection); 1 was a myelodysplastic undergoing HSCT with severe previous transfusional hemosiderosis; and, finally, 1 with pancytopenia in a kidney transplanted patient.
Summary/Conclusions: MHFT is a poor prognosis factor and half of the patients died after a few days. In those who survived, MHFT appeared during infection, especially viral infections, in patients undergoing HSCT or receiving chemotherapy. In this regard, MHFT is a useful sign of viral infection. Further studies are warranted to confirm the clinical value of MHFT.

PB2141

LIPOSOMIAL IRON IS SAFE AND COST-EFFECTIVE IN HCV PATIENTS WITH TYPE II DIABETES AND ANEMIA DUE TO ESOPHAGEAL OR GASTRIC BLEEDING

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Background: HCV patients frequently show anemia due to esophageal or gastric bleeding and type 2 diabetes. Iron support reduces degree of anemia. Iron support might cause hepatic function worsening or hepatocellular cancer onset. Non transferrin bound iron may sustain inflammation and increase insulin resistance.
Aims: Aim of this study is to verify if sucrosomial oral iron support *vs* ferric gluconate iv iron support *vs* transfusional support is safe and effective in patients with HCV treatment with iron deficiency anemia.
Methods: 35 patients with HCV related anemia for esophageal varices and gastric bleeding with a median Hb level of 8 g/dl (R8-9.5 g/dl), were treated 15 with sucrosomial oral iron 30 mg 1 tablets t.i.d for 3 months (group A), 10 with i.v. ferric gluconate 62.5 mg/day for 15 days (group B) and 10 with 1 blood transfusion/day (group C) until Hb increase level of 1 g/dl was reached. Median Hb and glucose level in group A were 8 g/dl and 140 mg/dl respectively, in group B 9.5 g/dl and 130 mg/dl, in group C were 7 g/dl and 160 mg/dl. All patients received an abdomen echography to detect hepatocellular carcinoma (HCC) at 1, 3, 6 months.
Results: Patients in group A gained 1 g/dl Hb after 1 month (R 3-6 weeks), with a median blood glucose level of 130 mg/dl (R120-230) and a median cost of 30€/month (R 20-80), patients in group B gained 1 g/dl in 7 days (R 6-13 days), with a median blood glucose level of 310 mg/dl (R190-430) and a median cost of 1240€/month (R 830-2800), patients in group C gained 1 g/dl in 1 day (R 2-4 days), with a median blood glucose level of 210 mg/dl (R160-330) and a median cost of 400€/month (R 350-950). Only 1 patient in group B and 1 patient in group C developed HCC at 6 months. Worsening of liver function blood test was observed only in group C.
Summary/Conclusions: Liposomial iron is safe and cost-effective in HCV patients with type II diabetes and anemia due to esophageal or gastric bleeding.

PB2142

THROMBOCYTOPENIA AFTER IRON INFUSION: A CASE SERIES OF FOUR PATIENTS AND REVIEW OF LITERATURE

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Background: Among all the etiologies of anemia worldwide, iron deficiency remains the leading cause and might be present in 1 to 2% of adults. A thrombocytosis between 500 to $700x10^9$/L or more rarely a thrombocytopenia under $100x10^9$/L have also been described. The management of iron deficiency involves the research and treatment of a bleeding; an iron uptake quantification with a dietary survey followed by an oral or intravenous iron supplementation. However, published cases of secondary thrombocytopenia after an iron injection remain troublesome and underline our partial understanding of its mechanism of action.
Aims: The objective of our study was to describe the clinical and biological characteristics of thrombocytopenia occurring after iron infusion in patients.
Methods: Two strategies have been applied to collect cases. On one side, we have searched through the French Pharmacovigilance Database (FPVD); and on the other side, we have performed a systematic review. We used "thrombocytopenia" as high level term (MedDRA 11.0) and drug exposition was defined by the presence in the report of intravenous iron coded "suspect" according to the WHO criteria, whatever the level of causality assessment.
Results: This study found 9 patients (1M, 8F) having thrombocytopenia after intravenous iron products, four from the FPVD and five from the literature. The median age at diagnosis was 37 years old (range 16-90). The spectrum of iron supplementation included bleeding (n=6), malabsorption (n=1), a peritoneal dialysis (n=1), and an inflammatory anemia (n=1). The hemoglobin level was 5.7±2.3 g/L (range 3.1-9.6) with a median MCV 63 fL and a median platelet

counts of 172±133x10^9/L (range 102-434). Two patients had thrombocytosis before admission. Before intravenous iron products, four patients have received oral iron drugs and units of packed red blood cells for two patients. Treatment of intravenous iron consisted of iron sucrose (n=8) and ferric carboxymaltose (n=1). One patient was treated with intramuscular injection of iron sucrose. The median time to the onset of the thrombocytopenia was 3±2.4 days (range 2-8). The average decrease in platelets count compared to the baseline was 85%. The available bone marrow aspirations showed a megakaryocytic hypoplasia (n=4) or no abnormality of the megakaryocytes (n=2). Two patients have experienced hemorrhagic events. One has epistaxis and the other one purpuric ecchymosis. Only one patient received packed of platelets. We aimed to identify some subsets of patients according to their baseline characteristics. However, there was no difference between patients with early (<3 days) or late (>3 days) thrombocytopenia.
Summary/Conclusions: As we reported only nine cases in this study, we hypothesize that thrombocytopenia secondary to an iron infusion remains an uncommon adverse drug reaction. Our statement is also supported by the absence of similar data from the European Medicines Agency and the Food and Drug Administration. The appearance of thrombocytopenia does not seem specific of the intravenous form because some cases of thrombocytopenia have been reported with oral iron products. Withdraw of IV iron remained the first option of treatment and platelet transfusions was added if necessary. The main entity in the differential diagnosis of iron-induced thrombocytopenia is thrombocytopenia associated with IDA. In conclusion, clinicians should be aware of this possibility, as in one hand iron is the treatment of thrombocytopenia and on the other hand, iron might be the trigger of the thrombocytopenia.

PB2143

AUTOIMMUNE HEMOLYTIC ANEMIA IN CHILDHOOD. LONG TERM EXPERIENCE FROM A SINGLE CENTER
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Background: Autoimmune hemolytic anemia (AIHA) is an immune mediated cytopenia rarely encountered in the first years of life. Childhood AIHA is characterized by diagnostic complexity in many cases and prognostic uncertainty in most, while therapeutic approach remains not well determined.
Aims: Aim of study was to report on the clinical presentation, management and long term outcome of cases with AIHA followed at a single pediatric hematology department during a 12year period.
Methods: The study was retrospective, covering the period between January 2004 and December 2015. Hospital files were used to record data including age at diagnosis, personal and family medical history, laboratory investigation, treatment, time of follow up, complications and relapses.
Results: During the given time period, 16 patients were diagnosed with AIHA. Mean age at diagnosis was 7.8 years (4 months-13 years) and mean time of follow up 5.08±4.18 years (4 months-12 years). A family history of autoimmune disorder was reported in 2/16 patients (12.5%). A known history of congenital hemolytic anemia was reported in 3/16 patients (18.7%) and of autoimmune thyroiditis in 1/16 patients (6.25%, while a history of recent infection was recorded in 11/16 patients (68.7%). In 12/16 cases (75%) AIHA was considered primary and in 4/16 (25%) found to be secondary to other conditions - EBV infection in 2 cases, systemic lupus erythematosus in one case and autoimmune lymphoproliferative syndrome in one case. Mean Hb at diagnosis was 6.29 ±2.22 gr/dl and median reticulocyte count 11.2% (0.8-32%) - 2 patients initially presenting with reticulocytopenia. In all but 2 cases (93.7%) direct Coombs test was IgG-IgG/C3d positive, in one case IgA positive and in one case initially negative. As to immunology studies, 8 patients presented with transient ANA, 1 patient with findings consistent with SEL (presence of ANA with low C3 and C4) and 2 patients with positive ATA (one already diagnosed with autoimmune thyroiditis). As to management, in 7/16 cases (43.75%) at least one urgent transfusion was required, while in 5/16 cases (31.26%) more than two. All patients received IVIG 1-6g/kg and solu-medrol pulse therapy at least once depending on clinical course, while 4/16 patients (25%) were put on monthly administration for 12 months. Oral prednisolone was administered in 11/16 patients (68.5%) for 3 to 118 months and cyclosporine in 7/16 patients (43.75%) for 3 to 24 months. In total, 6/16 patients (37.5%) required multiple pharmaceutical agents, *i.e.* IVIG, corticosteroids, cyclosporine and rituximab. As to complications, 4 patients presented with gall bladder lithiasis and 1 with growth retardation and adrenal insufficiency due to chronic corticosteroid use. With regards to clinical course, 3/16 patients (18.7%) presented with an acute course lasting for less than 3 months and 13/16 patients (81.3%) presented with a chronic course. Of these 13 patients, 8 presented with 1 or more (up to 6) relapses during follow-up.
Summary/Conclusions: AIHA in children may present with chronic course and unsatisfactory control of hemolysis, requiring prolonged immunosuppressive therapy. In addition, complications related both to disease and treatment may develop.

PB2144

Abstract withdrawn.

PB2145

SHOULD WE ALWAYS USE THE MCV TO DEFINE ANAEMIA?
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Background: How reliable is it to base testing serum ferritin on the presence of a microcytic anaemia? Traditional use of the term 'hypochromic microcytic anaemia' has fallen from favour. Most publications now concentrate on the 'microcytic anaemias'.
Aims: The aim of this study was to ascertain in a large regional teaching Hospital whether MCH or MCV is better at predicting for a low serum ferritin (SF) indicative of iron deficiency.
Methods: This study was based upon data extracted from our Hospital's laboratory information and results management system. Our large combined laboratory uses Sysmex XE2100 analysers. The majority of UK haematology labs use Sysmex analysers. We collected a year's worth of full blood count (FBC) data and paired the results with serum ferritin values that were just below the lower limit of normal range. The request for SF had to be made within 4 weeks of the FBC.
Results: Of the 501 patient samples that could be paired, a greater proportion of serum ferritin levels between 10 and 12 mcg/l inclusive, showed hypochromia in comparison to microcytosis (72% *vs* 31% respectively). The median results were: Haemoglobin 117g/l, MCV 83.6fl, MCH 26.6pg (men and women combined). Over a four day period by analysis of 100 consecutive samples microcytosis was observed to be the greater trigger for a serum ferritin request compared to hypochromia (39% *vs* 28% respectively). Over the same 4 day period, of approximately 7000 samples, hypochromia was seen in 6% and microcytosis in 3%.
Summary/Conclusions: In conclusion, although in the literature and in practice microcytosis is the predominant trigger for serum ferritin testing, it is hypochromia that has the better predictive capacity in our laboratory for the presence of iron deficiency. Clinicians need to look at the MCH and consider iron deficiency when the value is low, even in the presence of a normal MCV.

PB2146

MRI PROSPECTIVE SURVEY ON CARDIAC IRON AND FUNCTION AND ON HEPATIC IRON IN NON TRANSFUSION-DEPENDENT THALASSEMIA INTERMEDIA PATIENTS TREATED WITH DESFERRIOXAMINE OR NON CHELATED
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Background: Few studies have evaluated the efficacy of iron chelation therapy in thalassemia intermedia (TI) patients.
Aims: Our study aimed to prospectively assess by quantitative Magnetic Resonance imaging (MRI) the efficacy of Desferrioxamine (DFO) and its advantages with respect to the absence of chelation therapy in non transfusion-dependent (NTD) TI patients.
Methods: Among the 185 TI patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network and with a MRI follow-up (FU) study at 18±3 months, we selected 65 NTD patients. Cardiac iron overload was assessed by the multislice multiecho T2* technique. LV function parameters were quantified by cine SSFP sequences. Liver T2* values were converted into liver iron concentration (LIC) values.
Results: We considered 18 patients who have not received any chelation therapy (50% males; mean age: 37.83±14.29 years) and 33 patients who had received DFO alone between the two MRI scans (51.5% males; mean age: 38.85±7.83 years). The two groups were comparable for age, sex and baseline MRI data. No patient treated with DFO had cardiac iron. At baseline only one non-chelated patient showed a pathological global heart T2* value (<20 ms) and he recovered at the FU. The percentage of patients who maintained a normal global heart T2* value was 100% in both groups. A significant increase in the right ventricular ejection fraction was detected in DFO patients (-3.48±7.22%; P=0.024). The changes in cardiac T2* values and in the global systolic biventricular function were not significantly different between the two groups (Table

1). In patients with hepatic iron at baseline (MRI LIC ≥3 mg/g/dw), the reduction in the MRI LIC values was significant only in the DFO group (-2.20±4.84 mg/g/dw; P=0.050). The decrease in MRI LIC values was comparable between the groups (P=0.155).

Table 1.

	DFO (N=33)	None (N=18)	P
Mean Diff Global Heart T2* (ms)	0.53 ± 5.70	-0.21 ± 3.82	0.767
Mean Diff N seg. with T2* < 20 ms	-0.45 ± 1.92	0.17 ± 0.79	0.239
Mean Diff Mid septum T2* (ms)	0.69 ± 9.79	-2.33 ± 5.82	0.256
Mean Diff LV EF (%)	0.71 ± 5.58	0.77 ± 5.51	0.977
Mean Diff LV EDVI (ml/m²)	-2.18 ± 15.29	-2.88 ± 14.93	0.891
Mean Diff RV EF (%)	3.48 ± 7.22	0.92 ± 4.79	0.244
Mean Diff RV EDVI (ml/m²)	-0.28 ± 17.73	0.56 ±14.43	0.881
Mean Diff MRI LIC (mg/g dw)	-1.36 ± 4.04	1.07 ± 5.38	0.110
Mean Diff Ferritin (ng/ml)	1.03 ± 151.15	26.27 ± 192.80	0.674

Summary/Conclusions: In this small population of sporadically or non transfused TI patients, DFO therapy showed no advantage in terms of cardiac iron but its administration allowed and improvement in right ventricular function and hepatic iron overload.

PB2147

CHELATION THERAPY IN NON-TRANSFUSION-DEPENDENT IRON LOADING ANEMIA. EXPERIENCE IN A TERTIARY HOSPITAL

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Background: Iron loading anemia (ILA) encompasses a group of inherited and acquired anemias characterized by ineffective erythropoiesis, low hepcidin levels, excessive intestinal iron absorption and secondary iron overload. This iron accumulation occurs more slowly compared to transfusion-dependent patients, and complications do not arise until later in life. It remains crucial monitoring and appropriately treat their iron burden, for this reason it is essential to manage it appropriately with iron chelation therapy, especially since phlebotomy is not an option in these patients.
Aims: To describe the clinical management of chelation therapy in patients with non-transfusion-dependent iron loading anemia.
Methods: This retrospective study was conducted at Cruces University Hospital in Barakaldo-Spain, from February 2009 to February 2016. Clinical data and results of Hemoglobin, serum ferritin and transferrin saturation, Liver Iron Concentration (LIC) and Cardiac T2* measured by MRI was obtained from patient's medical records. All patients were treated with Deferasirox 500 mg/day as compassionate use since none was transfused. Safety profile was studied in terms of gastrointestinal side effects, rash and change in serum creatinine and AST-ALT values.
Results: Seven patients were included (5 male, 2 female). Medium age was 57 years (min 26, max 74). 1 patient was diagnosed with non-severe Aplastic anemia/Paroxysmal nocturnal hemoglobinuria (AA/PNH), 3 with Glucose-6-phosphate dehydrogenase (G6PD) deficiency, 2 Congenital Dyseritropoietic Anemia (CDA) and 1 Refractory anemia with ring sideroblasts (RARS). None was splenectomized. Medium serum ferritin before treatment was 1109,5 ng/mL (min 644, max 1561) and after at least 12 months post-treatment was 538 ng/mL (min 86, max 969). In 5 patients LIC was evaluated after treatment, in 4 of them a reduction was observed. At baseline 4 patients were evaluated with T2* and anyone showed cardiac iron overload. No adverse events were observed in any patient.

Table 1.

			Pre-treatment						Post-treatment					
Age	Sex	Diagnostic	Serum Ferritin (ng/mL)	Transferrin saturation (%)	AST (U/L)	ALT (U/L)	Liver Iron Concentration (mg/g)	T2* (ms)	Serum Ferritin (ng/mL)	Transferrin saturation (%)	AST (U/L)	ALT (U/L)	T2* (ms)	Liver Iron Concentration (mg/g)
60	M	AA/PNH	1190	61,6	19	14	14		86	32,4	22	19		1,2
45	M	G6PD Deficiency	644	70	21	23	6,4	33	576	55	20	28		6,4
26	M	G6PD Deficiency	939	81	27	27	7	28	493	31	30	29		4,1
40	F	CDA	798	96,9	22	25	5,6		537	51	30	36		
74	M	RARS	1342	85,2	20	17	7,6		294	42	18	16		
38	M	G6PD Deficiency	1561	78,7	32	38	7,6	39	969	53,6	22	19	23	5,5
60	F	CDA	737	98	30	42	13,5	35	352	81	17	19	37,9	8,8

Summary/Conclusions: There are currently no standard clinical practice guidelines for the treatment of iron overload in Iron Loading Anemia. In our experience, using Deferasirox in this kind of patients is a useful and safely strategy to manage Iron overload. Prospective studies are needed to evaluate efficacy and safety of chelation therapy in this setting.

PB2148

EFFECT OF SICKLE CELL DISEASE AND SICKLE CELL TRAIT ON PARAMETERS OF BONE METABOLISM

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Background: Sickle cell disease (SCD) has sequelae for bone health including infarcts, low BMD and osteomyelitis. Sickle cell trait (SCT) is clinically silent but common in individuals of African descent (AD), many of whom are unaware of their status. Although SCD poses increased risk for low BMD and vitamin D, there are no data on the relationship between SCT status and BMD, vitamin D level, and serum markers of bone metabolism. We hypothesized that pre-menopausal AD women with SCT have a significantly lower BMD and higher serum bone turnover markers than race- and age-matched control AD women and are intermediate between healthy controls and subjects with SCD.
Aims: To compare BMD, vitamin D level, and serum bone turnover markers in pre-menopausal AD women with and without SCT and SCD.
Methods: To assess the effect of sickle cell disorders on bone homeostasis in pre-menopausal AD women, we recruited AD subjects with SCT and SCD following informed consent and compared serum and radiographic bone parameters to control ADs. We compared vitamin D level, hemoglobin (g/dL), body mass index (BMI), daily calcium intake, BMD of lumbar spine (L-spine), femoral neck (FN) and total hip (TH), as well as serum bone turnover biomarkers between control, SCT and SCD premenopausal AD women. Serum bone turnover markers included CTX, P1NP, osteocalcin, sclersostin, vitamin D binding protein, IGF-1 and iPTH. Subjects were 35-45 years old with regular menstrual periods, not taking oral contraceptives or medications that influence bone metabolism, and without known metabolic bone disorders. Statistical analysis included a one-way ANOVA test and two-group t-tests for comparing group means using SPSS statistical software.
Results: A preliminary data analysis was performed on 21 subjects including 9 controls, 5 with SCT, and 7 with SCD. The mean age of the cohort was 40.8±4.8 years, and mean BMI was 29.8±7.6 kg/m^2,.BMI was lower in the SCD group compared with SCT (24.5 *vs* 34.1 kg/m^2, p=0.01). Mean hemoglobin levels were lower in SCD (8.7±2.8 g/dL) *versus* control (12.7±0.9 g/dL) and SCT (12.3±2.8 g/dL) subjects (p=0.001). Daily calcium intake did not differ between groups. Mean vitamin D level for the entire cohort was 21±6 ng/dl and there was no difference between groups. BMD T-scores were normal for all groups at all anatomic sites; however, compared to controls, mean L-spine and FN BMD was significantly lower in the SCD group (1.4±0.2 g/cm^2,vs. 1.1±0.2 g/cm^2, p=0.04; 1.1±0.2 g/cm^2 *vs* 0.9±0.1 g/cm^2, p= 0.03, respectively). FN BMD in the SCD group was lower than SCT (p= 0.046). Overall, BMI correlated with mean TH BMD (r=0.666, p=0.001); and serum IGF-1 correlated with mean FN (r= 0.633, p=0.003) and mean TH BMD (r= 0.513, p=0.021). Mean serum sclerostin levels were significantly decreased in both SCT and SCD subjects (Control 2975±786, SCT 2141±402, SCD 1613±598, p=0.002). The groups did not demonstrate a difference in serum CTX, P1NP, osteocalcin, vitamin D binding protein or iPTH.
Summary/Conclusions: In this preliminary analysis we assessed parameters of bone metabolism in SCT and SCD compared with controls. In the overall cohort, IGF-1, a reflection of bone formation, positively correlated with FN and TH BMD. Sclerostin, an inhibitor of Wnt-signaling and bone formation, was decreased among SCT and SCD subjects, although the explanation is unclear. Further study in an expanded population is needed to verify and further elucidate the pathophysiologic basis of these findings.

PB2149

B-THALASSEMIA INTERMEDIA IN CHILDREN AND ADOLESCENTS: B CHAIN GENOTYPE IN RELATION TO DISEASE PHENOTYPE AND PULMONARY HYPERTENSION

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Background: Prediction of β-thalassemia intermedia (BTI) phenotype from the genotype remains problematic.
Aims: Our aim is to study the different β-chain mutations in a group of Egyptian patients with BTI, its impact on clinical presentation, laboratory parameters and complications laying stress on pulmonary hypertension.
Methods: A cross-sectional study including 37 patients with BTI was performed in Pediatric Hematology Clinic, Children's Hospital, Ain Shams University. Revision of hospital records for age of diagnosis, blood transfusion, frequency, transfusion index over the last year and recording of complications as pubertal

delay, gall stones, leg ulcers together with splenectomy status and hydroxyurea treatment was performed. Physical examination for weight and height standard deviation scores, liver and spleen size then calculation of clinical score. Laboratory investigations included complete blood picture, initial hemoglobin electrophoresis, total, direct bilirubin, LDH, s.ferritin and β-Chain mutation analysis. 35 patients underwent echocardiography with recording of tricuspid regurgitant jet velocity and estimation of right ventricular systolic pressure.
Results: The studied patients had mean age of 10.3±4.55 years (range 4-18), female/male 20 /1.7, mean age at diagnosis 4.14±1.77 years (range 2-8), 10 (27.0%) had been diagnosed less than the age of 3 years, 19 (51.4%) between 3-6 years, 8 (21.6%) from 6-8 years, with a mean hemoglobin 6.38±1.26 gm/dl (4-10). Positive consanguinity was detected in 14 (37.8%) patients and positive family history in 20 (54.1%). 18 (48.6%) became transfusion dependent (TD), 9 (22.5%) patients had never been transfused, 8 (20.0%) receive one or two yearly transfusion, 23 (57.5%) receive more than two transfusions per year. Transfusion index ranged from 38-186 cc/kg/year in the TD group. Their mean height SDS -0.81±1.17; 75.7% of them had no obvious thalassemic facies, and5.4% had definite facies. Mild Pubertal delay was present in 16.22% and advanced delay in 5.41%. Splenectomy was performed in 5 (13.5%) patients; 64.86% were on hydroxyurea therapy. As regards β-chain genotype results, 14 (37.84%) have compound heterozygous with IVS1.6/codon6 and IVS1.6/IVS1.1and IVS1.1/codon2.7 most frequent genotypes, 10(27.03%) had heterozygous with IVS1.1 mutation most common, and 13 (35.14%) have homozygous mutations with IVS1.6 the commonest mutation with no significant difference between the three groups as regards age at diagnosis, hemoglobin at diagnosis. Mean pretransfusion hemoglobin was higher in heterozygous 8.90 ±1.29 gm/dl compared to compound heterozygous 7.29±0.61 gm/dl and homozygous 7.92±0.49 gm/dl. As Regards pulmonary hypertension (PHTN), Patients with PHTN had higher total serum bilirubin 2.15±0.71mg/dl, higher LDH 515.21±190.86IU/L, serum ferritin 1757.00 ng/ml (1082-2239), reticulocyte count 3.13±1.05% compared to those with normal pulmonary pressure 1.55±0.56 mg/ml, P=.009, 333.55±96.00 IU/L, P=0.001, 576.00 (296-1716) ng/ml, P=0.042; 2.45±0.55%, P=0.019 respectively; while they had lower initial hemoglobin level 6.41±1.43 gm/dl P=0.008. Compound heterozygotes formed 57.1%, heterozygotes 14.3% and homozygote mutations 28.6% of patients with PHTN compared to 30%, 30% and 40% in patients without PHTN, P=0.267.
Summary/Conclusions: Conclusion: Compound heterozygous are the most frequent β-chain genotype in patients with β-TI and it presents with more severe hemolysis and more frequent pulmonary hypertension.

Stem cell transplantation - Clinical

PB2150

OUTCOME OF HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR NON-HODGKIN LYMPHOMA

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Background: Patients with non-Hodgkin lymphoma (NHL) who have relapsed or refractory disease, may not achieve long-term disease-free (DFS) even after autologous hematopoietic stem cell transplantation (auto-HSCT). The long-term survival or curability is still challenging. Given this situation, allogeneic (allo)-HSCT for the treatment of aggressive-relapsed or refractory NHL is appealing for the prospect of providing both a tumour-free graft and the graft-versus-lymphoma (GVL) effect. However, there have been only limited matched sibling dornor for allo-HSCT. For those patients who do not have suitable dornors, the related partially HLA-matched dornors may be a good choice. Related haploidentical (haplo)-HSCT may offer the opportunity to achieve long-term survival and cure for those select patients.
Aims: To explore the efficacy and safety of haploidentical hematopoietic stem cell transplantation(HSCT) for refractory-relapsed or highly aggressive non-hodgkin lymphoma(NHL) patients.
Methods: 26 refractory-relapsed or highly aggressive NHL patients who received haploidentical HSCT from Jan 2004 to Mar 2015 were analyzed retrospectively. 17 patients were treated with the conditioning regimen consisting of modified busulfan/cyclophosphamide(Bu/Cy) plus anti-human thymocyte globulin(ATG). 9 patients were given the regimen comprised of total body irradiation(TBI)/Cy plus ATG. All patients were pretreated with cyclosporin A(CsA), methotrexate(MTX), mycophenolate mofetil(MMF) and ATG to prevent graft-versus-host disease(GVHD).
Results: The patients included 4 cases of diffuse large B-cell lymphoma, 1 case of follicular lymphoma, 5 cases of B-lymphoblastic lymphoma/leukemia, 9 cases of T- lymphoblastic lymphoma/leukemia, 1 case of anaplastic large cell lymphoma(ALK-), 5 cases of peripheral T-cell lymphoma(NOS), and 1 case of NK/T-cell lymphoma. 19 patients were refractory or relapsed. All patients achieved full donor chimerism. With a median follow-up of 13.5(4-136) months, 20 cases(76.92%) survived, 15(57.69%) survived without lymphoma, and 7(26.92%) relapsed. 6 patients died, 4 because of recurrence/progress, 2 because of the complication of HSCT. 8 patients developed acute GVHD grades II-IV, which risk at 100 days was 30.80%. The risk of chronic GVHD at 2 years was 25.10%. The estimated 2-year recurrence rate was 42.20%. The estimated 1-year overall survival(OS) and disease-free survival(DFS) rate was 84.60% and 61.10%, respectively. The 2-year OS and DFS rate was 71.60% and 48.90%, respectively. Univariate analysis showed that disease status (complete remission/ not complete remission) before haploidentical HSCT may be a factor affecting OS and DFS.
Summary/Conclusions: Haploidentical HSCT is effective for relapsed-refractory or highly aggressive NHL. It may prolong DFS in part of those patients and even cure them.

PB2151

HIGH-SENSITIVE MONITORING OF CHIMERISM IN BLOOD AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDHOOD LEUKAEMIA

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Background: We hereby report from a study aiming to evaluate the ability of microchimerism to predict relapse in a retrospective Danish cohort. Final results are pending. Relapse is the primary treatment failure after haematopoietic stem cell transplantation (HCT) for acute leukaemia in childhood, with a cumulative risk of 20-35%. Early detection of increasing minimal residual disease (MRD) or recipient chimerism is increasingly important to monitor imminent post HCT relapse. Chimerism analysis by polymerase chain reaction (PCR) of short tandem repeats is the gold standard and has a sensitivity of 1-5%. However, chimerism analysis based on real time, quantitative PCR (RQ-PCR) analysis (microchimerism) is more sensitive, with a reported detection level of 0.1-0.01%. We aimed to evaluate the ability of microchimerism to predict relapse in a retrospective Danish paediatric cohort.
Aims: It is the purpose of this study to evaluate the ability of microchimerism to predict early relapse following allogeneic SCT in children with leukaemia, with clinical and molecular relapse as primary outcome parameters. Microchimerism will be compared to results from detection of MRD in bone marrow and to standard STR-PCR based chimerism analysis.
Methods: Children transplanted for acute lymphoid (ALL) or acute myeloid

leukaemia (AML), between 2008 and 2014 at the Department for Children and Adolescents, University Hospital of Copenhagen, were included. Microchimerism was analysed on peripheral blood DNA using a commercially available kit (GenDx), based on allele-specific RQ- PCR of insertions/deletion polymorphisms. Results were compared with bone marrow MRD analysis and with standard, peripheral blood (PB) chimerism in DNA from unseparated whole blood, and PB cells positive for CD66b, CD4 and CD8. Transplant related data was collected from the European society for Blood and Marrow Transplantation (EBMT) database. We defined complete chimerism (CC) as no detectable recipient-cell chimerism at any time point, stable mixed chimerism (SMC) as a single increase below upper sensitivity limit, and increasing mixed chimerism (IMC) as two or more increases or a single increase above upper sensitivity limit.
Results: We included 59 children (ALL n=34, AML n=25), seven of these were re-transplanted due to relapse or rejection. Two transplants were excluded from further analysis due to lack of available DNA. Median age at HCT was 9 (1-8) years, and median follow up time was 23 (1-72) months. In total, 686 samples of frozen DNA were available for analysis, with a median (range) of 11 (2-22) samples per transplant. HCT was performed in 1st complete remission (CR) in 30 (47%), 2nd CR in 23 (36%), 3rd CR in 6 (%) and five (8%) performed without remission. Transplant characteristics and outcome data are displayed in Table 1. Seven children (21%) with ALL and 9 (36%) with AML relapsed, a median of 7 (1-52) months from HCT. One child re-transplanted in second remission subsequently relapsed again. In total, seventeen (29%) children died a median of 266 (60-1391) days from latest HCT, 10 (59%) of relapsed disease and 7 (41%) from transplant-related causes. For standard chimerism, 12/16 with IMC relapsed, *versus* 2/16 and 3/25 with SMC and CC, respectively. Cumulative incidence between the groups SMC, CC and IMC was statistically different for relapse (P<0.0001), and remained so after grouping CC and SMC.

Table 1.

Table 1. Summary of patient and transplant characteristics.

	Transplants		
	Total	ALL	AML
Number of patients/ Number of transplants, n	59/64	34/35	25/29
Age, years (median, range)	9 (1-18)	8 (1-18)	10 (2-18)
Gender, n male (%)	42 (65.6)	23 (65.7)	19(65.5)
Disease state at SCT, n (%)			
CR1	30 (46.9)	19 (54.3)	11(37.9)
CR2	23(35.9)	12 (34.3)	11 (37.9)
CR3	6 (9.4)	4 (11.4)	2 (6.9)
Not in remission at HCT	5 (7.8)	0 (0)	5 (17.2)
Conditioning, n (%)			
TBI + Etoposide or CY.	23 (35.9)	22 (62.9)	1 (3.4)
Busulfan based regimen.			
Bu + Cy + Mel	22 (34.4)	3 (8.6)	19 (65.5)
Other Busulfan based regimen	9 (14.1)	8 (22.9)	1 (3.4)
Other myeloablative, chemotherapy based regimen	10 (15.6)	2 (5.7)	8 (27.6)
Donor type, n (%)			
MFD	13 (20.3)	7 (20.0)	6 (20.7)
MMFD (Haplotransplantation)	8 (12.5)	1 (2.9)	7 (24.1)
MUD	43 (64.1)	27 (77.1)	14 (48.3)
MMUD*	2 (3.1)	0 (0)	2(6.9)
Stem cell source, n (%)			
BM	48 (75.0)	29 (82.9)	19 (65.5)
PBSC	11 (17.2)	3(8.6)	8 (27.6)
UCB	5 (7.8)	3(8.6)	2 (6.9)
Follow-up			
Months of follow up, median (range)*	23 (1-72)	24 (2-72)	12 (1-71)
Chimerism analysis samples			
Peripheral blood, total n	686	392	294
Per patient, median (range)	11 (2-22)	11(2-22)	10 (2-22)
Outcome			
Relapsed disease, n (%)	16 (25.0)	7 (20.0)	9 (31.0)
Time to relapse, months, median (range)	7 (1-52)		
Deceased , n (% of relapsed)	9 (56.3)	4(57.1)	5 (55.6)
Re-HCT, n (% of relapsed)	6 (37.5)	2(28.6)	4 (44.4)
- *Relapse after 2. HCT, n (% of re-HCT)*	1	0	1
- *TRM, n (% of re-HCT)*	1	0	1
New malignancy, n (%)	1	0	1 (3.4)
Acute GVHD			
No, n (%)	38 (40.9)	14 (40.0)	16 (55.2)
Grade 1, n (%)	12 (18.8)	6 (17.1)	6 (20.7)
Grade 2-3, n(%)	21 (32.8)	15 (42.9)	6(20.7)
Grade 4, n (%)	1 (1.6)	0 (0)	1 (3.4)
DLI administered	5 (7.8)	2(5.7)	3 (10.3)
Deceased, n (%)	17 (26.6)	7(20.0)	10 (34.5)
Relapse n (% of deceased)	10 (58.8)	4(57.1)	6 (60.0)
TRM% of deceased)	7 (41.2)	3 (42.8)	4 (40.0)
Graft rejection	1	0 (0)	1 (3.4)

Abbreviations: AML=Acute Myeloid Leukaemia, ALL= Acute Lymphoid Leukaemia, TBI = Total Body Irradiation, CY= Cyclophosphamide, BU= Busulfan, MEL= Melphalan, MFD= Matched family donor, MMFD= unrecorded family donor, MUD=Matched, unrelated donor, MMUD= Mismatched, unrelated donor,
All percentages are of transplants unless otherwise stated.
*8/10 match for bone marrow or peripheral blood transplantation, 5/6 or 6/6 match for UCB transplantation.
*Follow-up of patients alive and in complete remission.

Summary/Conclusions: Final results of microchimerism analysis are currently pending and will be presented at the EHA conference.

PB2152

MULTIPLEX REAL TIME PCR SYSTEM FOR CHIMERISM MONITORING AFTER ALLOGENEIC HEMATOPOIETIC STEM CELLS TRANSPLANTATION
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Background: Modern chimerism techniques require high sensitivity and capacity to predict relapse after allogeneic hematopoietic stem cells transplantation (allo-HSCT) several months prior its clinical presentation. These techniques have to be simple in use, time saving and inexpensive permitting repetitive measure in the course of treatment. Here we propose multiplex real time polymerase chain reaction technique targeted to unique polymorphic sites in recipient/donor alleles conforming these requirements.
Aims: To develop high sensitive and versatile test-system based on multiplex real time PCR technique for donor/recipient chimerism analysis after allo-HSCT.
Methods: We have used the panel of primers and molecular probes, proposed by M. Alizadeh et al., targeted to biallelic polymorphisms sites (insertions/deletions) in the human genome. All probes were modified by one of four fluorescent dyes: FAM, R&G, ROX and Cy5, and appropriate quenchers: BQH1 or BQH2. Genomic DNAs of donors and patients were isolated from the bone marrow or peripheral blood. Informative markers for each donor/recipient pair were selected by initial screening. For special cases (sibling transplantation) with no informative markers found among Alizadeh set we extend analyzed panel with additional primers and Taqman probes for other genome SNPs (for example rs1801131, rs1801133, rs587776796). Chimerism was detected also with STR-PCR using COrDIS Plus multiplex kit for amplification of 19 polymorphic STR-markers and amelogenin loci ("GORDIZ"Ltd., Moscow, Russia).
Results: We have combined twenty pairs of primers and twelve Taqman fluorescent probes into seven parallel real time PCR multiplex reactions. Each of seven simultaneous PCR was analyzed by four channel, detecting FAM, R&G, ROX and Cy5 fluorescence. Alleles present only in donor or recipient DNA were considered informative and used for chimerism monitoring. Sensitivity of the method was estimated in control experiments with serial donor/recipient DNA sample dilutions. The same sample dilutions were analyzed with classic method, based on PCR of short tandem repeats (STR-PCR) followed by fragment analysis of the PCR-products. Multiplex method proposed detects as low as 0.03% recipient DNA admixture to donor DNA, while the standard STR-PCR method accuracy is limited to 1%.
Summary/Conclusions: We have developed fast multiplex Taqman real time PCR technique for evaluating polymorphic alleles informative for donor/recipient chimerism analysis after allo-HSCT. Method is convenient, simple, time saving, more sensitive and could replace classic STR-PCR approach.

PB2153

TREOSULFAN-BASED CONDITIONING AND UNMANIPULATED PERIPHERAL BLOOD HAPLOIDENTICAL TRANSPLANTATION (HAPLOSCT) FOR PRIMARY REFRACTORY AND RELAPSED AML: RESULTS IN 63 PATIENTS
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Background: Allogeneic stem cell transplantation is the only potentially curative therapy for primary refractory (PR-AML) and relapsed acute myeloid leukemia (R-AML) patients (pts). HaploSCT could be an option for eligible pts whose clinical status warrants expedient treatment and who could benefit from a more potent alloreactivity.
Aims: Retrospective analysis of 63 consecutive pts with PR-AML and R-AML who received a first unmanipulated haploSCT at our center
Methods: We retrospectively analyzed 63 pts undergoing a first unmanipulated haploSCT from January 2006 to December 2015. PR-AML was defined as the failure to achieve hematological remission following induction treatment.
Results: Median age was 56 years (y) (20-77); 40% of pts were classified as PR-AML after a median of 2 induction courses (1-5), while 60% of pts had R-AML (51% 1st and 9% 2nd relapses). Median blast cell count in bone marrow was 33% (55-93%); median HCT-comorbidity index by Sorror *et al.* (HCT-CI) was 2 (0-8). Overall, 51% of pts received a reduced intensity conditioning (RIC) regimen based on Treosulfan (14 g/m2/d from -6 to -4), Fludarabine (30 mg/m2/d from -6 to -2) and anti-thymocyte globulin (ATG); 49% underwent a myeloablative conditioning (MAC) based on the same Treosulfan-Fludarabine schedule with the addiction of TBI 4Gy and ATG (23%) or Melphalan 70 mg/m2/d from -2 to -1 (26%). Source of stem cells were T-cell repleted G-CSF-mobilized peripheral blood stem cells from haploidentical familiar donors. All pts received post-grafting immunosuppression with mycophenolate mofetil and sirolimus; moreover, pts undergoing the Treo-Flu-Mel conditioning received post transplant Cyclophosphamide (PTCy) as backbone for GvHD prophylaxis. The complete remission (CR) rate was 78% after a median of 30 days (d) (21-41). Neutrophil engraftment occurred in 92% of pts with a median of 16 d (9-51). The 100-d cumulative incidence (CI) of grade ≥2 acute GvHD (aGvHD) was 30±10% and of grade≥3 aGvHD 19±8%; the 2y CI of chronic GvHD was 14±8%. With a median follow-up for survivors of 24 months (4-95), the 2y probabilities of leukemia-free survival (LFS) and overall survival (OS) were 13±7% and 14±7%, respectively. For pts who achieved CR, the 2-y LFS and OS were 17±8% and 18±8%, respectively; CI of relapse at 1y and 2 y were 45±13% and 53±14%, respectively, with a median time to relapse of 160 d (47-727); CI of non-relapse mortality (NRM) at 1y and 2 y was 30±12%, with a median time of 63 d (42-375). In univariate analysis, the only factors influencing 2y-LFS were the intensity of conditioning regimen (29±14% for MAC and 5±4% for RIC recipients, p 0.05) and HCT-CI (35±13% if ≤2 and 4±3% if >2, p 0.01). In a Cox-multivariate model including pts age, blasts percentage, cytogenetic risk, HCT-CI, intensity of conditioning regimen, GvHD prophylaxis backbone (ATG *vs* PTCy), disease status (PR- *vs* R-AML) and interval from diagnosis to haploSCT, the only predictive factor for better LFS was an HCT-CI ≤2 with an hazard ratio of 0.46 (95% confidence interval: 0.2-0.8, p 0.01).
Summary/Conclusions: A consistent proportion of PR-AML and R-AML pts can achieve CR after an unmanipulated haploSCT. Median time to relapse of 160 days opens the window to early post transplant treatment aimed at a remission prolongation and enhancement of immune control.

PB2154

BIOSIMILARS OF FILGRASTIM HAVE AN EQUIVALENT EFFICACY WHEN USED AFTER PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Background: The biosimilars of G-CSF are sometimes the object of discussions about their efficiency and their harmlessness compared to historical products. Their use and efficiency for mobilization of the peripheral stem cells (PBSCH) or the regeneration post-reinjection of these CSP were little studied.
Aims: We wished to compare the efficacy of two products, biosimilars of filgrastim, Ratiograstim® and Zarzio®, compared to the original products in post-autograft stimulation.
Methods: Between February, 2008 and November, 2014, we identified all the patients who underwent autologous stem cell transplantation (ABSCT), in our Sector of Intensive Care, for of a lymphoma or of a multiple myeloma. The patients with lymphoma had a conditioning regimen by standard BEAM chemotherapy meanwhile the patients with myeloma had a high dose Melphalan (HDM) conditioning regimen. The reinjection of the PBSC was made 24 hours after the end of conditioning chemotherapy. All of the patients were stimulated by daily injection of G-SCF from J5 post-transplant until reach 1 giga/L of PNN. We collected the information concerning the times of hospitalization, the results of blood counts, transfusion needs, clinical data and infectious complications. The results) are expressed in median.
Results: We supported 187 patients (114 men and 73 women) for autologous stem cell transplantation (lymphoma/myeloma=99/88). We treated 51 consecutive patients Neupogen®-Amgen Inc (26 lymphoma and 25 myeloma, respectively), 74 patients Ratiograstim®-Ratiopharm AG (44 lymphoma and 30 myeloma) and 62 patients Zarzio® Sandoz AG (29 lymphoma and 33 myeloma). Within the patients with lymphoma, length of hospital stay was 22 days (16-64 days). The median use of filgrastim was for a period of 7.5 days (4-13 days). Cytopenias were deep in CSP post-reinjection with at least 10 days to less than 1 giga/L leukocytes and 13 days to less than 50 giga/L of plates. There was no difference for the results ragardinf the different types of filgrastim. The percent of patients transfused in red cells was 85%, and platelets 99%, without differences between the products. Three patients had bone pain (all under Ratiograstim®). Ten patients transferred into intensive care unit (ICU) because of infectious complications (5 under Zarzio® and 4 under Ratiograstim®). Four patients died within 100 days, two of them were under Neupogen®. Within the patients with myeloma, length of hospital stay was 16 days (13-42 days) and the administration of filgrastim was for period of 7.5 days (1-16 days). Cytopenias lasts for 6 days, under 1 giga/L leukocytes (the minimum of 6 days under Zarzio®, p=0.03), 0 days under 9 g/dL of hemoglobin (non-significant) and 6 days under 50 giga/L platelets s (more than 7 days within Neupogen®, p=0.04). The percent of patients transfused in red cells was 26% and in platelet 91%, without differences between the products. Eighteen patients had bone pain (9 under Ratiograstim® and 6 under Neupogen®). Four patients transferred into ICU (2 in Neupogen®). No patients died within 100 days post-autograft.
Summary/Conclusions: We compared the efficacy and safety in post-autologous of three G-CSF products, two biosimilars and one originator product. This retrospective analysis shows that for most of the parameters studied, the tree products have similar efficacy and side effects. By analyzing these results we concluded that biosimilars as well as princeps products may be use for stimulation after peripheral blood stem cell transplantation.

PB2155

DIAGNOSTIC VALUE OF SERUM STREM-1, PROCALCITONIN AND CRP IN PATIENTS WITH FEBRILE NEUTROPENIA

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Background: The triggering receptor expressed on myeloid cells (TREM)-1 is a transmembrane glycoprotein predominantly expressed on neutrophils and monocytes/macrophages and an important regulator of inflammatory responses. Under inflammatory conditions, TREM-1 is released in a soluble form (sTREM-1) which is of diagnostic value in patients with pneumonia or sepsis in the absence of neutropenia. Severe sepsis in patients with autologous stem cell transplantation is the most abundant cause of death in the first 30 days after transplantation. Early diagnosis of sepsis and adjustment of medical treatment might improve outcome of these patients. Up to date, the potential and diagnostic value of sTREM-1 to predict sepsis and outcome in neutropenic patients undergoing stem cell transplantation has not been explored.
Aims: The aim of our work is to elucidate the diagnostic value of soluble TREM-1 and the correlation with procalcitonin and CRP in patients with febrile neutropenia.
Methods: Blood serum was obtained from 12 male and 5 female inpatients (n=17; median age 62 -IQR 58 to 63y) with a hematological malignancy (8 multiple myeloma, 9 lymphoma) undergoing high dose chemotherapy, followed by autologous stem cell transplantation and presenting for the first time with fever (axillary temp. >38.3°) and neutropenia (PMN <500/µl). sTREM-1 was measured with our self made sTREM-1 ELISA. Procalcitonin and CRP were measured by immunoassays under routine laboratory conditions.All human studies were performed in accordance with the declaration of Helsinki and were approved by the Landesaerztekammer Rhineland-Palatine Ethics Committee according to the institutional guidelines.
Results: At the first episode of febrile neutropenia, the patients had increased levels of CRP of 91 (IQR 42.5 to 161.5) mg/l and PCT of 0.29 (IQR 0.16 to 1.5) ng/ml. Despite severe neutropenia (leukocyte count median 30 (IQR 20 to 60) /nl, median sTREM-1 levels were increased with 33.7 (IQR 14.7 to 98.5) pg/ml. In 12 of 17 patients a bacterial blood infection was documented by positive blood cultures. Patients undergoing a bacterial sepsis showed significantly upregulated levels of sTREM-1 in serum (p=0.001). Additionally elevated sTREM-1 levels significantly correlated with elevated PCT level (r^2=0.30; P=0.02). No significant correlation was found between sTREM-1 and CRP (r^2=0.04; p= 0.42) or CRP and PCT (r^2=0.18; p=0.08).The response to initial empiric antibiotic treatment as well as the subsequent development of serious complications were independent of sTREM-1 levels during first episode of fever. All patients survived 30 days after autologous stem cell transplantation.
Summary/Conclusions: We detect elevated levels of sTREM-1 in patients with febrile neutropenia after high dose chemotherapy and autologous stem cell transplantation in the absence of measurable amount of neutrophils and monocytes. Increased level of sTREM-1 significantly correlates with PCT and sepsis. Our results suggest that sTREM-1 might be a useful marker for the early detection of sepsis also in neutropenic patients. However, it is currently unclear whether the malignant hematological disease itself, chemotherapy or autologous stem cell transplantation have an influence on the serum level of sTREM-1. Therefore further prospective studies are on the way to evaluate of sTREM-1 as a clinically relevant or alternative sepsis disease marker in neutropenic patients compared to other well-known markers like PCT or IL-6.

PB2156

OUTCOMES OF HIGH GRADE GASTROINTESTINAL GVHD POST-HSCT IN CHILDREN

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Background: The high grade acute graft *versus* host disease (aGVHD) of typically involved organs (skin, gastrointestinal system (GIS) and liver) have specific features of therapeutic challenges, among which high grade GIS aGVHD has a distinctive and remarkable place in this context.
Aims: Our study intend to bring out the risk factors and clinical course of high grade gastrointestinal system (GIS) GVHD in children.
Methods: This is a retrospective analysis of 28 pediatric patients presented with a clinical diagnosis of stage 3 and 4 acute GVHD of the GIS who were selected from allogeneic hematopoetic stem cell transplantation (HSCT) performed. The demographics, the regimen used for conditioning and GVHD prophylaxis, clinical characteristics of GVHD including follow-up, laboratory parameters during GVHD, treatment modalities used for GVHD, response assessment in every week, complications of GVHD, and survival data were recorded.
Results: Patient and transplant characteristics were summarized in Table 1.

Table 1.

Characteristic	Value	
Diagnosis	Acute Leukemia 7 (25%) MDS 7 (25%) Fanconi anemia 5 (18%) Immunodeficiency 4 (14%) Hemoglobinopathy 2 (7%) Other 3 (11%)	
Donor and HLA match	Sibling n:2 Parent n:7 (four full-match, three haploidentical) Unrelated n:19 (six 10/10 and thirteen 9/10)	
Conditioning Regimen	Myeloablative n:23 Reduced Intensity regimen n:5	
Cell Source	Bone Marrow n:19 (68%) Peripheral Blood n:8 (29%) BM+PB n:1 (3%)	
GVHD prophylaxis	CsA and MTX n:17 CsA and MP n:5 Other n:6	
Median (range) onset day of GVHD	14 (5-217)	
Median (range) onset day of GIS GVHD First day of stage 3	21 (5-224) 32 (7-230)	
Treatment modalities		
Photopheresis	20	
Mesenchymal stem cells	18	
ATG	6	
MTX	6	
Sirolimus	6	
Other (rituximab, infliximab..)	3	
Survival		OAS
Dead	13 (46%)	2,4 months (1,4-6,4)
Alive	15 (54%)	17,3 months (3,0-56,3)

Summary/Conclusions: Overall survival at 3 months after the onset of stage 3 or 4 gut GVHD was 54%. Better outcome than the adult data might be related with more usage of bone marrow as a stem cell source or different character-

istics of pediatric gastrointestinal system. In general, GVHD started 14 days post-HSCT, extended to GIS GVHD in a week and progressed to high grade GIS GVHD in 10 days. The initial day of GVHD, days to extending to GIS GVHD and progressing to high grade did not influence the mortality. Melphalan and ATG usage in conditioning regimen and the drugs used in GVHD prophylaxis were not associated with survival. Low albumin level at any time of the severe GIS GVHD was associated with high mortality possibly due to more inflammatory GIS state of lost patients. Because ATG usage in the treatment of GIS GVHD significantly increased the mortality in our study, it should not be used in a routine manner. Better but not significant outcome with non-immunosuppressants seems a better treatment approach. Today best approach to GIS GVHD is to prevent and recognize it early. Treatment without immunosuppressive therapy like Photopheresis and mesenchymal stem cells seems a better approach which deserves further research with more patients.

PB2157

EFFECTIVENESS AND TOXICITY OF SECOND AUTOLOGOUS STEM CELL TRANSPLANTATION AS SALVAGE THERAPY FOR RELAPSED OR PROGRESSIVE MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is still an incurable disease despite the introduction of novel therapy. Relapse or progression (R/P) following autologous stem cell transplantation is common. Therapeutic options may include novel agents often followed by second ASCT.
Aims: We aimed to evaluate the effectiveness and toxicity of salvage ASCT in R/P multiple myeloma.
Methods: We retrospectively analyzed the outcome of patients who underwent second autologous stem cell transplantation as salvage therapy for R/P MM, between February 2007 and February 2014.
Results: Thirty four patients underwent salvage ASCT. Conditioning regimen was melphalan 200 mg/m^2(n=32) or 140mg/m^2 (n=2). The median age was 54 years (36- 60 years) at first ASCT and 57 years (37- 63 years) at second ASCT. Twenty patients (59%) were men. Median duration of response after first ASCT was 28 months (4-61 months), 6 patients (17%) relapsed within the first year of ASCT. Twenty nine (85%) patients received a salvage therapy: Thalidomide-Dexamethasone (n=17), Bortezomib based (n=10), Lenalidomide-Dexamethasone (n=1) and Melphalan-Prednisone (n=1). Median interval between first and salvage ASCT was 37.6 months (9-72 months). Twenty one patients (72%) were at least in partial response before second ASCT. Non relapse mortality (NRM) was 9%. Response was assessable at 3 months post-ASCT in 30 patients: 25 patients (83%) achieved at least partial response, 4 had progressed disease and 1 stable disease. After salvage ASCT, 9 patients received thalidomide maintenance therapy. The median progression-free survival (PFS) after second ASCT was 12 months (0.7-35 months) and median overall survival (OS) was 21 months (0.7-65.4 months).
Summary/Conclusions: Salvage ASCT is an effective therapeutic option for relapsed or progressive multiple myeloma although associated with a high NRM.

PB2158

THE EFFECT OF THE DHAP REGIMEN ON STEM CELL MOBILIZATION AND TRANSPLANT OUTCOMES OF PATIENTS WITH NON-HODGKIN LYMPHOMA WHO ARE CANDIDATES FOR UP-FRONT AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: Data on dexamethasone, cytarabine, and cisplatin (DHAP) as a mobilization regimen, compared to high-dose cyclophosphamide (HDC), for up-front autologous stem cell transplantation (ASCT) in non-Hodgkin lymphoma (NHL) are limited.
Aims: The aims of this study were to compare the efficacy of peripheral blood stem cell (PBSC) mobilization of DHAP regimen with HDC in combination with G-CSF in patients with aggressive NHL who were candidates for up-front ASCT, and to investigate the effect of DHAP mobilization on overall survival (OS).
Methods: We conducted a multicenter, retrospective cohort study on patients who were diagnosed with aggressive NHL and treated with CHOP or rituximab-CHOP and subsequent PBSC mobilization using HDC (4.0g/m^2) or DHAP (cisplatin 100mg/m^2 D1, cytarabine 4.0g/m^2 D2, dexamethasone 40mg D1-4) plus G-CSF regimens for up-front ASCT in 3 Korean institutions between 2004 and 2014. Successful mobilization was defined as collected CD34+ cell count ≥5.0x10^6/kg, but mobilization failure was defined as collected CD34+ cells <2.0x10^6/kg.
Results: Ninety-six patients (57 male, 39 female) with a median age of 48 years (range, 18-66) were included. Thirty-one patients (32.3%) received DHAP regimen and 65 (67.7%) received HDC. Diffuse large B-cell lymphoma (DLBCL, 54.2%) was the most common histologic type, and remaining included peripheral T-cell lymphoma (PTCL), not otherwise specified (28.1%), anaplastic lymphoma kinase-negative anaplastic large cell lymphoma (12.5%) and angioimmunoblastic T-cell lymphoma (5.2%). The number of total CD34+ cells collected per patient was significantly higher in the DHAP group than the HDC group (median, 16.1 *vs.* 6.1 × 10^6/kg, *P*=0.001). More patients in the DHAP group achieved successful mobilization compared to the HDC group (87.1% *vs.* 61.5%; *P*=0.011), whereas the rate of mobilization failure was higher in the HDC group (33.8% *vs.* 3.2%; *P*=0.032). In multivariate analysis, no bone marrow involvement (odds ratio [OR], 4.60 [95% CI, 1.20-17.61]), no prior radiotherapy (OR, 11.78 [1.68-82.39]), WBC counts at first apheresis day >1,785/uL (OR, 9.25 [2.89-29.58]), and the DHAP regimen (OR, 4.12 [95% CI, 1.12-15.17]) were independent predictors for successful mobilization. Febrile neutropenia developed 3 patients (9.7%) in DHAP group, which was less frequent than HDC group (N=21 [32.3%], *P*=0.043). Of the 96 patients, 2 patients did not proceed to up-front ASCT (1 failed mobilization, 1 patient's refusal). Following the reinfusion of PBSC, median time to neutrophil/platelet engrafts did not significantly different according to mobilization regimen. With a median follow-up of 57.4 months (range, 9.1-143.4), the 5-year OS rates were not significantly different between the DHAP and the HDC groups (74.0% *vs.* 72.2%; *P*=0.936). Because of the heterogeneous histologic types in the study, additional subgroup analyses were performed separately based on the histologic types, but the OS in patients with either DLBCL or PTCL was not different according to the mobilizing regimens (*P*>0.05).
Summary/Conclusions: Our study showed that the DHAP regimen was associated with higher efficacy for PBSC mobilization and less frequent episodes of febrile neutropenia compared to the HDC regimen. Although there was no clinically meaningful OS improvement in the DHAP group, the DHAP regimen reduced significant rates of mobilization failure. Therefore, DHAP plus G-CSF can be effective in patients with NHL undergoing up-front ASCT.

PB2159

IMPACT OF THE METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) C677T GENE POLYMORPHISM ON CLINICAL OUTCOMES OF HLA-MATCHED SIBLING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is widely used to treat various hematological malignant and bengin diseases. The occurrence of complications following HSCT-as graft *versus* host disease (GVHD), hepatic veno-occlusive disease (VOD), oral mucositis (OM), drug induced hepatic and renal adverse events- is highly variable and dependent on a multitude of host, donor, and treatment factors. Identifying important genetic variables will allow for better prediction of HSCT-related outcomes, that could help to develop targeted interventions. A common genetic polymorphism, C677T, has been described for Methylenetetrahydrofolate reductase (MTHFR);results in amino acid changes, at codons 222. The homozygous 677TT genotype has been shown to have 30% of the MTHFR wild-type enzyme activity in vitro, and the heterozygous (CT) genotype has approximately 60% of wild-type enzyme activity (Liew and Gupta, 2015 *Eur J Med Genet,* 58, 1-10).
Aims: To evaluate impact of the C677T polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) on the clinical outcomes of patients treated using human leukocyte antigen-matched sibling stem cell transplantation as acute GVHD,VOD,severe oral mucositis (SOM), drug induced hepatic and renal toxicity, transplant related mortality(TRM) and overall survival(OS).
Methods: The study subjects were 46 patients receiving allogeneic HSCT at a bone marrow transplantation unit in Nasser institute for research and treatment from 2010 to 2014,with complete clinical records ; and DNA available for genotyping. MTHFR genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).Acute GVHD was assessed using conventional criteria (Jacobsohn and Vogelsang, 2007 *Orphanet J Rare Dis,* 2, 35), necessity of parenteral nutrition used to identify SOM, and VOD of the liver was defined according to McDonald et al.(1993 *Ann Intern Med,* 118, 255-67).We evaluated renal and hepatic toxicity based on peak serum creatinine and peak total bilirubin, AST, and ALT levels during the first 30 days after allogeneic HSCT.TRM was defined as death by any cause during the course of treatment, other than the relapse of underlying disease.
Results: Median age at the time of HSCT was 22 years (range 3-42 years); 32 patients (69.6%) above ≥18 years, and the median follow-up period of survivors was 21 months. Acute myeloid leukemia (AML) was the most common

underlying diagnosis 23 patients (50%) of them 12 (26%) are in complete remission 1 (CR1).The most commonly used conditioning regimen consisted of Busulfan (BU), and Cyclophosphamide (CY) (n=26, 56.5%). All received peripheral blood stem as a stem cell source with mean CD34+ stem cell dose (6.6±2.3) 10^6/kg. Twenty-seven patients (58.7%) were males. Methotrexate (MTX) in addition to Cyclosporine (CsA) was used as GVHD prophylaxis in 40 patients (87%).The frequencies of the MTHFR C677T genotypes in patients were 43.5% (20 patients) for 677CC, 50% (23 patients) for 677CT, and 6.5% (3 patients) for 677TT ;the allelic frequency of the 677T was 31.5%. Recipient MTHFR677 in CT or TT showed higher incidence of acute GVHD (7/26) 26.9% *versus* (2/20) 10% in CC, but not statistically significant; p=0.26. MTHFR C677T in CT or TT showed higher incidence of hepatic toxicity (11/26) 42.3% *versus* (5/20) 25% in CC; also higher transplant related mortality (5/26) 19.2% *versus* (2/20) 10% in CC, but not statistically significant; p=0.22 & 0.45 respectively.In log rank survival analysis,recipients with variant allele MTHFR 677T were associated with lower non statistically significant overall survival; p=0.15.VOD was diagnosed in 1 patient (2.2%) and had heterogeneous status of the polymorphism, MTHFR 677 CT genotype.

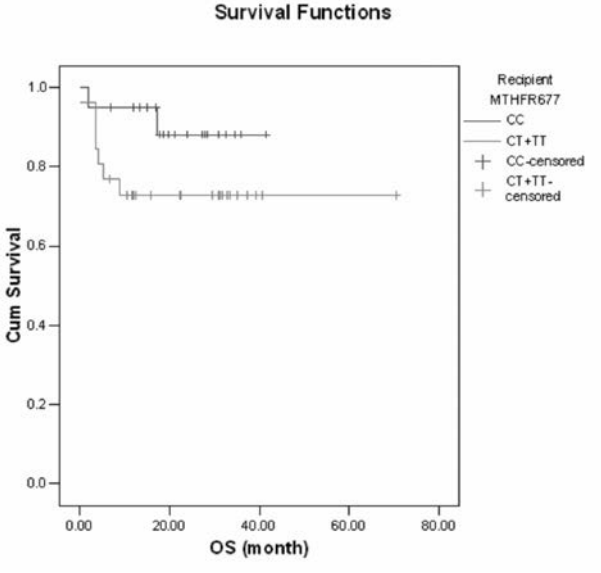

Figure 1.

Summary/Conclusions: Genotyping for MTHFR C677T before HSCT could have clinical significance, not statistically proven in our study, in prediction patients at high risk of developing poor outcomes. Large multicentric, highly standardized prospective studies are needed to identify such potential pharmacogenetic markers with sufficiently strong evidence to be used in clinical practice.

PB2160

TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY IS STRONGLY ASSOCIATED WITH SEVERE GRAFT VS HOST DISEASE POST UNRELATED ALLOGENEIC TRANSPLANT FOR HEMATOLOGIC DISEASES

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Background: Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment in hematologic disorders, but morbidity and mortality remain high. Transplant-associated thrombotic microangiopathy (taTMA) is a multifactorial and often fatal syndrome.
Aims: In a retrospective single-centre study we aimed to investigate the incidence of taTMA in patients (pts) undergone HCT from unrelated donors (URD) and identify prognostic factors and treatment outcome.
Methods: We enrolled consecutive pts who underwent URD HCT from 2003 to 2015. TaTMA diagnosis was based on EBMT criteria: increased (>4%) schistocytes in peripheral blood, thrombocytopenia, increased lactate dehydrogenase and decrease in Hb concentration. Anti-thymocyte globulin (ATG, rabbit) was used as standardised part of the conditioning in almost all pts (total dose of 5-7.5mg/kg). Anti-GVHD prophylaxis consisted of cyclosporine or tacrolimus plus methotrexate in myeloablative and mycophenolate mofetil plus cyclosporine in reduced intensity and toxicity conditioning transplants. Upon identification of taTMA, all possible causative factors were fully investigated. The mainstay of treatment strategy was withdrawal of calcineurin inhibitors, plasma infusion and plasma exchange combined with corticosteroid administration and in refractory cases, humanized anti-CD20 monoclonal antibody (Rituximab).
Results: We studied 179 pts (74 male, 105 female), aged 37±14 years, who underwent HCT from matched HLA A/B/C/DRB1 (8/8, n=88) and allele or antigen mismatched (n=91) URD. Grafts derived from peripheral blood (157), bone marrow (18) and umbilical stem cells (4). Conditioning regimens were myeloablative (139), reduced intensity (28) and toxicity (12). Median follow-up was 11.5 (0.1–147.4) months.TaTMA was diagnosed in 29 (16.2%) pts (12 male, 17 female), aged 34±12 years, 78 (9–721) days post-HCT for acute leukemia (21), Hodgkin (3) and non-Hodgkin (1) lymphoma, MDS (2), MPD (1) and aplastic anemia (1). Conditioning regimens were myeloablative in 24 (12 TBI-based) and reduced intensity in 5 pts. Among pts with taTMA, 10 (34%) presented severe (grade III-IV) acute GVHD, 15 (52%) extensive chronic GVHD, 12 (41%) bacterial, 2 (7%) fungal and 21 (71%) viral infectious episodes. In univariate analysis age, gender, diagnosis, disease phase, previous lines of treatment, conditioning (intensity and TBI-based), ABO and HLA incompatibility, infections, acute and chronic GVHD were studied. The presence of taTMA was associated with severe acute and extensive chronic GVHD (p=0.001 and p=0.035 respectively) and TBI-based conditioning (p=0.043). In multivariate analysis, severe acute GVHD was the only independent factor associated with taTMA (β=3.4, p=0.038). Regarding treatment-outcome, 8 pts responded to cyclosporine cessation and plasma infusions. The rest (21) underwent plasma-exchange sessions; in 14 the syndrome resolved, 1 responded to additional Rituximab treatment, while 6/21 pts were refractory and eventually succumbed. Treatment-related mortality was 33.3% (9/29) in taTMA pts and was directly associated with refractory microangiopathic syndrome in 6/29 (20.6%).
Summary/Conclusions: Despite the progress achieved concerning classic thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome in terms of diagnosis and novel therapies, taTMA seems to be a far more complex syndrome. Since the pathogenetic factors leading to taTMA are not fully elucidated, treatment of this syndrome often fails. According to our findings, GVHD was the most predisposing factor for the development of taTMA, possibly related to associated endothelial damage.

PB2161

THE BENEFITS AND PITFALLS OF PERIPHERAL BLOOD STEM CELLS MOBILIZED BY ETOPOSIDE: THE EFFECT OF CUMULATIVE DOSAGES OF ETOPOSIDE

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Background: Successful collection of adequate hematopoietic stem cells is necessary for autologous stem cell transplantation (AutoSCT). However, the optimal mobilization strategy for transplantation has still not been established. High dose cyclophosphamide with granulocyte-colony stimulating factor (G-CSF) is one of the most widely used mobilization strategies; however, in a proportion of patients, it fails to secure the collection of sufficient cells. Therefore, effective and safe alternative mobilization strategies are required.
Aims: We conducted a retrospective study to determine the impact of etoposide mobilization strategy for AutoSCT in patients with lymphoid malignacies.
Methods: We compared the effectiveness and safety profiles of chemomobilization using three regimens: intermediate-dose etoposide (750 mg/m^2) followed by the late addition of G-CSF, high dose cyclophosphamide and G-CSF, and G-CSF alone. A total of 116 patients (60 multiple myeloma, 56 lymphoma) who underwent autologous stem cell transplantation (autoSCT) were included in this study.

Table 1.

Table 1. Predictive factors for platelet engraftment

	Univariate analysis	Multivariate analysis	
Factor	*P* value	HR (95%CI)	*P* value
Sex	0.472		
Age	0.985		
Underlying disease	**0.001**		
Stage	0.649		
Heavy pretreatment	0.471		
CR at transplantation	0.983		
Mobilization strategies	0.095		0.078
Cyclophosphamide (reference)		1	
Etoposide		0.559 (0.337 – 0.928)	**0.025**
G-CSF		0.91 (0.547 – 1.514)	0.717
Time from diagnosis to mobilization	0.111		
Time from mobilization to transplantation	**0.049**		0.068
≤ 30 days (reference)		1	
> 30 days		0.684 (0.455 – 1.028)	
Infused CD 34⁺ cell count	0.242		**0.009**
≥ 5×10⁶/kg (reference)		1	
< 5×10⁶/kg		0.534 (0.334 – 0.855)	
Cumulative etoposide dose	**< 0.001**		**0.003**
< 2.5g (reference)		1	
≥ 2.5g		0.502 (0.319 – 0.790)	
Type of G-CSF	0.909		

Results: Median CD34+ cell yield was significantly higher in the etoposide group (12.3x10^6/cells/kg) compared to the cyclophosphamide group (4.99x10^6, cells/kg) and the G-CSF group (3.8x10^6/cells/kg) (p<.001). The rate of successful mobilization (≥5x10^6/cells/kg) was also significantly higher in the etoposide group (86.1%) compared to the cyclophosphamide (49.1%) and the G-CSF group (22.2%) (p <.001). There were no significant differences in neutrophil and platelet counts at the nadir between the etoposide and the cyclophosphamide group. Severe febrile neutropenia and mobilization-related mortality were not observed in any group. However, platelet engraftment was slower in the etoposide group compared to the other groups (p=0.029). Furthermore, the proportion of delayed platelet engraftment (≥30 days) was also greater in the etoposide group than in the cyclophosphamide and the G-CSF group (20.6% *vs* 6.7% *vs* 3.8%, p=0.032). In multivariate analysis, the etoposide mobilization strategy (p=0.025) and the high cumulative etoposide dose (≥2500 mg, p=0.003) were significantly associated with delayed platelet recovery.
Summary/Conclusions: Intermediate dose etoposide with the late addition of G-CSF may be an effective mobilization strategy. However, high cumulative doses of etoposide, including systemic chemotherapy, mobilization, and conditioning of autoSCT may delay platelet engraftment.

PB2162

SUCCESSFUL PERIPHERAL BLOOD STEM CELL MOBILIZATION WITH A COST-EFFICIENT FIXED SINGLE-DOSE PLERIXAFOR SCHEDULE IN POOR MOBILIZERS

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Background: Collection of hematopoietic stem cells (HSC) from the peripheral blood (PB) is routinely conducted prior to high-dose chemotherapy and autologous transplantation. Despite safety and efficiency of current apheresis procedures including mobilizing chemotherapy and granulocyte colony-stimulating factor (G-CSF), there is still a significant rate of mobilization failures due to different patient-dependent factors necessitating additional agents like plerixafor. A standardized strategy for its application in poor stem cell mobilizers is still lacking.
Aims: To evaluate the efficacy of a cost-efficient fixed single-dose of plerixafor in poor mobilizers on successful HSC mobilization.
Methods: We analyzed 46 patients who underwent autologous HSC transplantation at our academic center between 2011 and 2015 and received plerixafor because they were expected to be poor mobilizers due to low counts of CD34+ cells in PB samples prior to apheresis or after a first apheresis day with insufficient yield or as a rescue strategy after insufficient harvest with previous mobilizing chemotherapy. We examined CD34+ cell counts in PB and in apheresis products to identify those patients who were able to collect a sufficient CD34+ cell count for transplantation after a single application of plerixafor.
Results: Plerixafor could be safely administered, leading in 83% and 48% to apheresis yields of >2 and >4x10^6 CD34+ cells/kg body weight (bw) and correlating with median CD34+ PB cell counts of 8.0 and 17.8/µl, respectively. Of note, 35/46 (76%) patients showed a substantial benefit of plerixafor *vs* G-CSF alone, with increased PB CD34+ cells (13.3 *vs* 4.7/µl, p=0.0001) prior to apheresis and 4-fold higher CD34+ cell numbers per single apheresis (1.2 *vs* 0.3x10^6 CD34+/kg bw, respectively, p=0.00005). A patient subset of 24% had <5/µl PB CD34+ cells before plerixafor application and profited less from additional plerixafor administration.
Summary/Conclusions: As the number of patients with extensive pretreatment, including new immunomodulatory drugs and with long disease courses will even increase in the future, it is highly important to develop selection criteria for patients with expected benefit of additional plerixafor application, also with regard to cost efficiency. Our data suggest that most patients in preemptive and rescue settings with <5/µl CD34+ cells detectable in PB may fail to substantially benefit and should therefore be carefully selected, whereas those with >5/µl PB CD34+ cells can greatly benefit of a single application with median CD34+ apheresis yield increases of 2.9 and median total apheresis collections of 4.6x10^6/kg bw.

PB2163

AVASCULAR NECROSIS OF BONE IN ADULT PATIENTS SURVIVING MORE THAN 2 YEARS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN YEARS 2001-2012 - EXPERIENCE OF SLOVAK TRANSPLANTATION CENTRE

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Background: Avascular necrosis (AVN) of bone is the most debilitating late skeletal complication of stem cell transplantation (SCT) that often remains underestimated.
Aims: To evaluate potential risk factors of AVN, its clinical manifestation and treatment in patients after SCT.
Methods: One hundred thirty-five patients surviving more than 24 months after allogeneic SCT in years 2001-2012 in Slovak Transplantation Centre were evaluated retrospectively for AVN. All patients were disease-free after SCT for malignant and non-malignant hematological diseases. AVN was confirmed by magnetic resonance imaging or scintigraphy. We investigated correlations between AVN and primary diagnosis (malignant/non-malignant), conditioning regimens, donor and patient gender, type of donor (related/unrelated), HLA match, AB0 compatibility, source of stem cells (peripheral/bone marrow), immunosuppressive therapy, GvHD (acute/chronic), exposure time and cumulative dose of corticosteroids. We analysed time to first symptoms, time to diagnosis of AVN, number of joints, most affected sites, correlation between joint pain and imaging exams, types of treatment and their results.
Results: Seven patients surviving 30-169 months (median, 69 months) after SCT developed AVN (5.2%). We experienced more AVN in male patients (83%), but found no correlation with other factors. Cumulative dose of corticosteroids was 0-5264mg (median, 3960mg) and exposure time was 0-327 days (median, 53days). Patients developed arthralgias 9-56 months (median, 28 months) after SCT. AVN was confirmed 1-17 months (median, 6 months) after onset of the symptoms and 14-62 months (median, 36 months) after SCT. We confirmed AVN of 19 joints (2.71 joints per patient)-hip (12), shoulder (5) and sacroiliacal joints (2). We recognized higher incidence of AVN in patients who suffered from AVN of at least one joint, although the other affected joints weren't always marked as painful (hip: 8 painful joints in correlation with 12 joints with proved AVN in 6 patients; shoulder: 4 painful joints in correlation with 5 joints with proved AVN in 3 patients). We confirmed bilateral occurrence of AVN of hip joints regardless of the number of painful hip joints. Because of lack of MRI of painful knees we weren't able to evaluate the rate of AVN although knees were the second most painful joints. AVN of sacroiliacal joints was asymptomatic. Conservative treatment was performed in shoulder joints, surgical treatment was performed just in hip joints - 4 of 6 patients underwent total hip replacement (THR) of 6 joints. Age median of patients undergoing THR was 26 years. One patient in an early stage of AVN underwent forrage and 1 patient was on conservative treatment while waiting for THR. Out of 6 THRs we experienced no septic or aseptic loosening in an early post-operative period. We followed the patients after THR for 11-143 months (median, 30 months). Out of 6 replaced hip joints we experienced deliberation of acetabular and distal femoral component in 1 patient, 8 years after implantation. Reimplantation was performed without any complications.
Summary/Conclusions: Avascular necrosis occurred more in male patients in our study. We didn't define any other risk factor for AVN. We observed the highest incidence of AVN of hip joints with 100% bilateral occurrence, where imaging exams were more sensitive than corresponding clinical symtomatology. Surgical methods of AVN treatment remain to be gold standard for higher stages of AVN of hip joints with favorable benefit-risk ratio.

PB2164

HIGH FERRITIN LEVELS IN FEVER OF UNKNOWN ORIGIN: POSSIBLE FIRST SIGN OF HEMOPHAGOCYTOSIS IN BMT PATIENTS?

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Background: Fever of unknown origin is a major problem in transplant patients. The management of prolonged fever without any signs and symptoms of a specific microorganism such as bacteria, virus or molds is controversial and debateful.
Aims: While checking other biological parameters of FUO, we also included ferritin to rule out hemophagocytosis.
Methods: Between January 2015-February 2016 out of 37 allogeneic transplantations (21 MFD, 3 haploidentical, 12 MUD, 1 cord blood), 6 patients (4 girls/2 boys, 3 MFD/ 3 MUD, 2 malignant diseases/ 4 nonmalignant disease) developed high fever with hepatosplenomegaly at median day 28 (14-45 days). They were treated with broad spectrum antibiotics, they were all receiving prophylactic viral treatment with acyclovir and prophylactic flucanozol. No microbiological agent causing the fever could be detected. In patients with fever more than 5 days, CT scan of the chest, abdominal ultrasonography were performed, without any diagnostic findings. At the onset of fever none of the patients had GvHD.
Results: The ferritin levels of these patients were found to be very high (median 93.486 ng/ml, 3587-381.300 ng/ml) Other major parameters of hemophagocytosis such as hypertriglyceridemia, pancytopenia etc were not detected. The patients were treated with steroids and fever resolved in all patients within 48 hours. One patient died of graft rejection, one patient received steroid and plasma exchange due to severe multiorgan dysfunction. They received steroids for a median of 29,5 days (24-34 days).
Summary/Conclusions: In transplant patients fever can be sometimes very hard to manage. If a microbiological cause of fever could not be detected we think that checking the ferritin level is very important, even if some of the diagnostic parameters for hemophagocytosis are missing. High ferritin levels could be the first sign of hemophagocytosis in these patients and it can be treated with steroids excluding etoposide to secure the graft.

PB2165

CLINICAL ASSESSMENT OF PATHOGENETIC VIRUSES IN HEMORRHAGIC CYSTITIS AFTER ALLOGENEIC CELL TRANPLANTATION: HOW HIGH IS HIGH FOR URINE BK POLYOMA VIRAL LOAD

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Background: Hemorrhagic cystitis (HC) after allogeneic hematopoetic cell transplantation (allo-HCT) has been recognized to be a relatively common severe complication with increased morbidity requiring hospitalization. Apart from conditioning toxicity, the most common pathogens implicated are cytomegalovirus (CMV) and BK-polyoma virus (BKPV). Data concerning the clinical relevance of molecular monitoring of these viruses in urine are scarce.
Aims: We conducted a retrospective study enrolling patients who developed cystitis after allo-HCT to evaluate the urine viral load of both CMV and BKV that is potentially associated with HC.
Methods: CMV and BKPV viral load in urine and CMV in plasma were measured with RQ-PCR in symptomatic patients with cystitis. Macroscopic hematuria (+/-clots) led to the diagnosis of hemorrhagic cystitis.
Results: Twenty-nine patients (female=14, male=15), aged 16-61 years (median 35) who developed cystitis after allo-HCT and for who viral load data were available for both pathogens were included in the study. Patients suffered from hematological malignancies and underwent allo-HCT following myeloablative (21) or reduced intensity conditioning regimen (8). The majority of patients (17/29) received graft from a matched volunteer, 8/29 from siblings and 4 from a haplo-identical relative donor. Median time for the onset of cystitis was 48 days (7-677). Fourteen out of 29 (48%) patients experienced HC requiring hospitalization. CMV viral load in urine was positive in 7/29 samples (median: 1270 copies/ml, range: 180-1490000). Concordant CMV viremia was documented in 15 patients (median: 2570 copies/ml, range: 26-81000). BKPV viral load in urine was positive in 23/29 patients (median $2{,}5x10^7$ copies/ml, range: $1{,}18x10^2$-$3{,}2x10^{10}$). Bacterial infection by klebsiella pneumonia was documented in one patient. Hemorrhagic cystitis was significantly associated with BKPV viral load: $2{,}5x10^7$ in HC patients *vs* $1{,}9x10^4$ in non-HC, p=0.018. A cut- off of $5{,}1x10^4$ copies/ml could strongly predict the development of hematuria in ROC analysis. CMV viral load in urine and plasma tended to be higher in HC patients without a significant difference: (i) in urine: 106575 *vs* 523 copies/ml and (ii) in plasma: 9004 *vs* 2584 copies/ml for HC *vs* non-HC patients respectively, p=ns. No other clinical or biological factors could predict the development of HC. Cytology was available in 9 patients with HC but only two were found positive (BKPV load $>1x10^8$ copies/ml in both patients). Patients were treated with antiviral agents, mostly val/gancyclovir and foscarnet and cidofovir was added in persisting cases. In 12 patients with HC, >2 consecutive samples were available after the first documentation of BK (median 5, 3-7). In these patients a slow reduction of BK load was observed (median reduction: 0.9 log in 30 days) and all the patients maintained a high BKPV load ($>1x10^5$in 9/12) regardless of the resolution of cystitis.
Summary/Conclusions: In our study BKPV was related with the development of hemorrhagic cystitis after allo-HCT, with viral loads higher than $5{,}1x10^4$ copies/ml strongly predicting hematuria. Despite the fact that CMV was also detected, CMV viruria or viremia were not found to be significant co-factors. Prospective studies are warranted to answer the benefit of viral molecular monitoring in urine among transplanted patients.

PB2166

THE ANALYSIS OF PATIENTS WITH POOR MOBILIZATION AND MOBILIZATION FAILURE: CAN RDW BE A PREDICTIVE FACTOR?

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Background: Autologous hematopoetic stem cell transplantation (AHSCT) has been routinely used in the treatment of hematologic malignancies. Stem cell collection procedure from the patients has been accomplished with apheresis equipments. G-CSF is usually used alone or with chemotherapy for the purpose of the mobilization.
Aims: The aim of the present study is to assess retrospectively the patients who had been mobilized with different chemotherapy protocols between 2012 and 2016 and to analyze the patients with poor mobilization or mobilization failure and to determine the possible causes.
Methods: This study has been planned as a cross sectional study in a center that has been a member of EBMT and had been accreditated by JACIE between the years 2012 and 2016. All the data were obtained from the hospital data bases. Spectra Optia (Terumo BCT, Denver, USA) which utilizes continous flow centrifuge technique was used for the collection of stem cells. Peripheral and product CD 34 counts were done with FACS Canto II (BD Bioscience, USA) device and ISAGE method. Mobilization techniques used in the patients had been planned in accordance to the standart application (SOP number; KIT-KU009) methods. The patients with CD34 counts less than 20 cells/uL had been defined as “poor mobilization” whereas patients with stem cell counts less than 2x106/kg in product had been accepted as “mobilization failure”. In the day of stem cell collection peripheral blood count (Sysmex, XN 1000, Tokyo, Japon) had been performed and RDW, hemoglobin and platelet counts were noted.
Results: In our center there has been a total of 27 poor mobilization cases (8.82%;23 patients) between 2012 and 2016. A total of 279 stem cell apheresis procedures had been performed during the same period and in 35 cases (12.54%) mobilization failure was observed. 5 of the patients in the poor mobilization group had 3 consequtive chemotherapy protocols (1 patient radiotherapy), 12 of them received 2 consequtive and 6 of them received 1 chemotherapy protocolIn this group mobilization was tried to be accomplished with G-CSF alone in 6 patients, with G-CSF and plerixefor in 2 patients and with chemotherapy and G-CSF in 15 patients. For the comparison of RDW, Hb and platelet numbers among the mobilization groups, first day laboratory results of the patients had been used. There were no statistically significant difference with respect to platelet and Hb levels among the groups; however, the RDW was found to be significantly lower in the patients with successfull mobilization when compared with the patients in the poor mobilization and mobilisation failure groups (median [minimum-maximum] values were 15.4(12.08-25.20),16.4 (13.3-21.6) and 16.3(13.5-24.9) in successfully mobilized, poorly mobilized and mobilization failed groups (p=0.029). Mobilization failure was observed during stem cell collection in 6 of the 60 patients in 2012, 5 of 63 patients in 2013, 4 of 44 patients in 2014 and 7 in 49 patients in 2015. The median number of chemotherapy before mobilization procedure was 2 (1-3), and the median platelet levels during peripheral CD34 count was 95500/µL (10000-384000/µL). The median CD34 cell count from the peripheral blood was 9 cell/µL(0-17) and the median percentage of CD34 was 0,03(0,00-0,11).
Summary/Conclusions: Our results support the hypothesis that multiple chemotherapy independent from age results in poor mobilization and mobilization failure. RDW was shown to be lower in patients with successfull mobilization compared with poor mobilization or mobilization failure patients. The role of RDW in prediction of mobilization success has to be clarified in further studies.

PB2167

SUCCESSFUL REDUCE OF 60 PATIENTS FOR REFRACTORY/RECURRENT LEUKEMIA BY ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION AND PROPHYLACTIC IMMUNOTHERAPY

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Background: Refractory /Recurrent leukemia always has a poor prognosis.
Aims: To retrospectively evaluate the results of allogeneic hematopoietic stem cell transplantation and Prophylactic Immunotherapy for Refractory/Recurrent leukemia.
Methods: From June 2012 to January 2015, 60 patients with high-risk leukemia were enrolled, including 18 cases of ALL, 37cases of AML and 5 case of CML-BP. The average leukemia burden was 53(20-95)% in bone marrow. All patients received HLA haplo-identical stem cells transplantation from parent or sibling donors. Myeloablative conditioning regimens consist of 7cases of BuCy, 26 cases of TBI/FLAG, 15 cases of TBI/Cy, and 12 cases of FLAG that followed by reduced-intensified BUCY. All patients received cyclosporine A, MMF and methotrexate for GVHD prophylaxis. All patients received DLI,DC-CIK and NK according to different status after allogeneic HSCT .Analyzed outcomes were hematological engraftment, incidence of acute and chronic GVHD, incidence of relapse, and nonrelapse mortality (NRM), Overall survival and Disease-free survival.
Results: The MNC and CD34+ for transfusion were $9.08(7.02\text{-}24.4)*10^8$/Kg and $3.42(0.8\text{-}12.1)*10^6$/Kg. All 60patients achieved stable engraftment. The median time of ANC≥$0.5*10^9$/L was 16 (8-23) days. And for platelet ≥$20*10^9$/L, the median was 22 (8-150) days.38 patients developed acute GVHD, the accumulative incidence of aGVHD was 66.4%, the accumulative incidence of II-IV grade aGVHD was 35%, and the accumulative incidence of III-IV grade aGVHD was 15%. 26 patients developed cGVHD (12 patients extensive, 14 patients limited), the accumulative incidence of cGVHD was 88.2% and for extensive type, the accumulative incidence was 67.4%. The accumulative incidence of CMV infection was 54.1%, and the accumulative incidence of EBV infection was 16.3%. 10 patients developed virus cystitis. The number of Bacterial and fungal infected patients were51 and 27, respectively. The median follow-up time post transplantation was 11(1-36) months, 14 patients relapsed and the accumulative incidence of relapse was 27%. For AML, ALL and CML-BP patients, the accumulative incidence of relapse were26.6%, 34.8□and 0□, respectively.The median follow-up time post transplantation was 11months, 21 patients died and the main causeswere relapse (11 cases), infection (5 cases), cGVHD(2 cases) and diffuse alveolar hemorrhagediffuse alveolar hemorrhage(3 cases). Among 60 patients, 39 patients survived. The one-year and two-year accumulative incidences of OS were61.8% and 49.5%, respectively. The one-year and two-year accumulative incidences of DFS were 53.8% and 47.8%, respectively.For AML, ALL and CML-BP patients, the two-year accumulative incidence were 52.6%, 34.4% and66.7%, respectively. The non-

relapse mortality was 10. The one-year and two-year accumulative incidences of NRM were 19.4% and 28.4%, respectively.
Summary/Conclusions: Our clinical results have shown that the salvaged HSCT is a promising modality for treatment of high-risk AL with high leukemia burden.

PB2168

STEM CELL MOBILIZATION WITH HIGH DOSE ETOPOSIDE PREDICT AND IMPROVE THE OUTCOME OF AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH REFRACTORY OR RELAPSED LYMPHOMA

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Background: Autologous hematopoietic stem cell transplantation is an effective treatment for refractory and relapsed lymphoma patients.Most studies show that chemotherapy sensitivity is an important prognostic factor for these patients.A significant proportion of patients with relapsed and refractory lymphomas fail to respond to conventional salvage therapy and never proceed to transplantation, most often due to chemotherapy-resistant disease.Etoposide is an epipodophyllotoxin that has a topoisomeraseII-inhibiting function and causes DNA-strand breaks. Several groups have described the effectiveness of etoposide alone or in combination regimens to mobilize PBSCs.
Aims: We report a respective study in treating 88 patients with relapsed or refractory lymphomas who received high-dose etoposide (VP16) in mobilization plus autologous peripheral blood stem cell transplantation as rescue therapy.To investigate the efficacy of stem cell mobilization with high-dose etoposide and granulocyte colony stimulating factor followed by autologous peripheral blood stem cell transplantation in patients with relapsed or refractory lymphoma as salvage therapy. We hypothesized that the response to high-dose etoposide may predict the outcome of ASCT.
Methods: From November 2005to December 2014, 88 patients with refractory or relapsed non-Hodgkin's lymphoma (NHL, n=61) or Hodgkin lymphoma (HD, n=27) received high-dose etoposide (VP16 20~25mg/kg/d ×2) and G-CSF for stem cell mobilization. The median age of 59 male and 29 female patients was 33 years (range 16–60)Patients' remission status prior to mobilization were partial remission (PR) (n=39) and progressive disease (PD) (n=49). All patients underwent autologous peripheral blood stem cell transplantation (auto-PBSCT). Conditioning regimen was BEAM (n=30) or CBV (n=58).
Results: Among 88 patients, 65 (73.9%) patients had response. 36 of 39 (92.3%) PR patients had response, 30 of 49 (61.2%) PD patients had response. Median follow-up was 21.5 (1~85) months after stem cell transplantation. 58 (65.9%) patients achieved CR, 18 (20.45%) patients attained PR, 10 (11.36%) patients suffered disease progression, 1 (1.1%) patients relapsed, and 1 (1.1%) patient died of bone marrow failure. Among PR group, the estimated 2-year DFS for patients who had response and no response to etoposide were 83.6% and 33.0% respectively. In PD group, the estimated 2-year DFS for patients who had response and no response to etoposide were 69.4% and 26.7%, respectively. P <0.01. The estimated 2-year OS for responders and non-responders were 72.0% and 25.9%, respectively. P <0.01 .
Summary/Conclusions: Stem cell mobilization with high dose etoposide and G-CSF followed by auto-PBSCT was beneficial in the treatment of refractory lymphoma. Disease-free survival over 2 years can be achieved in some patients. Patients' response to high dose etoposide was correlated to 2-year DFS and OS, which could also predict the outcome of auto-PBSCT for those patients. Utilizing high dose etoposide, we can select some chemotherapy-sensitive patients from refractory lymphoma. The outcome of autologous transplantation is correlated to the response of high dose etoposide.

PB2169

EFFECT OF TRANSFUSION OF THE THIRD PARTY UMBILICAL CORD BLOOD ON HAPLO-IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Cord blood stem cells have multiple functions in haploidentical stem cell transplantation
Aims: To retrospectively evaluate the effect of the third party umbilical cord blood on haplo-identical hematopoietic stem cell transplantation.
Methods: From June 2012 to May 2015, 125 leukemia patients were enrolled, including 41 cases of ALL, 62cases of AML, 12 cases of MDS, 7 cases of CML-BP, 2 cases of acute mixed leukemia and 1 case of Blastcell dendritic cell tumor. Inclusion criteria: 1) AL patients; 2) halpo-identical HSCT; 3) 3/6 matched cord blood was available. Patients were divided into two groups, ie. groupA (HSCT, n=65) and groupB (HSCT plusumbilical cord blood transfusion group, n=60). Myeloablative conditioning regimens consisted of BuCy, TBI/FLAG, TBI/Cy, and FLAG that followed by reduced-intensified BUCY. The median dose of mononuclear cells in groupA and B were $8.58x10^8$/kg and $9.01x10^8$/kg, respectively. The median dose of CD34+ cells fortransfusion in each group were $3.67x10^6$/kg and $2.94x10^6$/kg, respectively. The dose of grafted UCB MNCsand CD34+ cells for groupB were $3.5x10^7$/kg and $2x10^5$/kg, respectively. All patients received cyclosporineA,MMF and methotrexate for GVHD prophylaxis. The endpoints of this study were hematological engraftment,incidence of acute and chronic GVHD, incidence of relapse, transplant-related mortality(TRM), non-relapsemortality(NRM), Overall survival(OS) and Disease-free survival(DFS) in each group.
Results: The median follow-up time was 17(3-29) months in groupA and 18(3-35) months in groupB.Patients in groupA reached a sustained ANC of more than $0.5*10^9$/L at a median of 11 days, whereas 14 daysin groupB. Platelet more than $20*10^9$/L occurred at a median of 19 days in groupA, whereas 17 days in groupB (P= .4). The rate of aGVHD was not significantly different in the two groups, 56.9% in groupA and 48.3% in groupB (P= .21). The accumulative incidence of II-IV grade aGVHD was 35.4% in groupA and 30% in groupB (P= .42). The incidence of chronic GVHD was 79.2% in groupA and 71% in groupB (P= .47). The incidence of extensive type was lower in groupB, 69.2% *vs* 35%, P=0.09. The incidence of CMV was lower in groupB, 80% *vs* 60% (P= .01). The accumulative incidence of EBV was lower in groupB, 35.4% *vs* 3.3% (P<0.01). At two years, the accumulative incidence of relapse was 24.4% in groupA and 17.2% in groupB, P=0.13. The two-year accumulative incidence of OS was 72.9% in groupA and 84.8% in groupB, P=0.07. The one-year accumulative incidence of DFS in each group were 65.7% and 79.4%, respectively, P=0.03.
Summary/Conclusions: Our clinical results have shown that HSCT with transfusion of the third party umbilical cord bloodis a promising modality for induction of immunity reconstitution. Better survival results may benefit from lower incidence of GVHD and virus infection. $x10^8$/kg and $9.01x10^8$/kg, respectively. The median dose of CD34+ cells fortransfusion in each group were $3.67x10^6$/kg and $2.94x10^6$/kg, respectively. The dose of grafted UCB MNCs and CD34+ cells for groupB were $3.5x10^7$/kg and $2x10^5$/kg, respectively. All patients received cyclosporineA,MMF and methotrexate for GVHD prophylaxis. The endpoints of this study were hematological engraftment,incidence of acute and chronic GVHD, incidence of relapse, transplant-related mortality(TRM), non-relapsemortality(NRM), Overall survival(OS) and Disease-free survival(DFS) in each group.

PB2170

ENGRAFTMENT POST AUTOLOGOUS STEM CELL TRANSPLANT FOR MULTIPLE MYELOMA IS UNAFFECTED BY LENGTH OF STEM-CELL STORAGE- EXPERIENCES FROM THE ROYAL MARSDEN HOSPITAL, UK

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Background: Multiple myeloma(MM) is the is the second most common bone marrow cancer with almost 5000 patients diagnosed in the UK each year, accounting for 10% of all haematological malignancies. Triplet chemotherapy followed by autologous stem cell transplantation (ASCT) is the gold standard induction treatment in younger fit patients and has significantly improved overall survival rates. A second ASCT at relapse can also improve subsequent remission duration. Peripheral blood stem cell (PBSC) harvest and storage is, therefore, routinely performed by transplant centres with the aim to collect enough cells to enable patients to undergo two ASCTs over the course of their treatment. As survival of patients with MM has dramatically improved in the era of novel agents, duration of storage and associated costs incurred have increased. It is therefore important to examine the feasibility and clinical utility of long term cell storage. There is conflicting data on viability of stored stem cells, with some studies reporting reduced $CD34^+$ count and cell viability over time, and others reporting successful transplantation outcomes with stem cells stored for up to 11 years.
Aims: We analysed the effects of stem cell storage duration on time to engraftment in patients undergoing ASCTs for MM in our centre.
Methods: We undertook a retrospective analysis of all patients undergoing ASCT for MM between 2008 and 2015. Patients undergoing tandem ASCTs were excluded. Days to engraftment was defined as number of days from stem cell infusion to neutrophil count >0.5 $X10^9$/L.
Results: 406 patients underwent ASCT between 2008 and 2015, with a median patient age of 65 years (range 25-78 years). 80.3% of patients (n=326) underwent one ASCT and 19.7% (n=80) underwent two. Of those patients receiving a second stem cell transplant, 81.2% percent had stem cells stored for between 0-60 months at the time of second transplant, and 18.8% for between 61-170 months. 80% of patients required only one therapy regimen prior to ASCT and 79% of patients were in a complete remission or very good partial response pre-transplant. Novel agents (bortezomib, lenalidomide and thalidomide) were used in 95% of cases as front-line therapy. PBSCs were stored on average for 32 months before being used. Melphalan 200mg/m^2 was used as conditioning chemotherapy pre-transplant (140mg/m^2 in renal impairment or medical comorbidities) with stem-cell return on Day 0. All patients successfully engrafted.

There was no significant difference in time to engraftment in all patients with PBSCs stored between 0-60 months (11.3 days+/-2.3 days) and 60-170 months (11.0 days+/-2.6 days), p=0.42 (Student's unpaired t test). The longest period of time PBSCs were stored was 165 months with successful engraftment at 12 days. 19.7% of patients had stem cells stored between 60-120 months (n=80) and of these 22.5% of patients went on to have second autograft using stored PBSCs. All patients with PBSCs stored for more than 120 months went on to have a second successful ASCT (n=3). These patients were all treated with novel agents prior to ASCT.
Summary/Conclusions: Our results show that prolonged periods of PBSC storage does not affect stem cell engraftment in MM patients undergoing ASCT. These results support the rationale of ongoing long-term PBSC storage in the era of novel agents as salvage ASCT remains an important treatment option for relapsed or progressive MM.

PB2171

REVIEW OF THE EFFECTIVENESS OF PALONOSETRON IN PREVENTION OF NAUSEAS AND VOMITS INDUCED FOR MELPHALNAN IN AUTOLOGUS TRANSPLANT OF HEMATOPOIETIC PROGENITOR CELLS

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Background: Prevention of nauseas and vomits induced for melphalan in autologus transplant of hematopoietic progenitor cells is an important issue. There is not an standar of care until now. In our work we review the effectiveness of Palonosetron for these patients.
Aims: Assess the effectiveness and security of Palonosetron in the prevention of nauseas and vomits induced for melphalan at high dose (200mg/m2) in autologus transplant of hematopoietic progenitor cells
Methods: Observational study, descriptive and retrospective in which have been included patients older than 18 year old diagnosed of multiple myeloma and with autologus transplant of hematopoietic progenitor cells during the period March 2009-February 2014 which received Melphalan 200mg/m2 in day -4. For prevention of nauseas and vomits a dose of Palonosetron 250mcg was administer intravenous half an hour before the infusion of Mephalan was started. The identification of the subjects was done through the computer program of prescription, validation and preparation of cytostatic mixtures Oncofarm®. To assess the effectiveness a survey was used where patients had reflected nauseas and vomits during the four days after the administration of Mephalan and the notes of nursing and medical staff on the medical records. The variables used were, age, sex, toxic habits (tobacco, alcohol), scale of basal activity ECOG, digestive disease, regular medication, previous chemotherapy and anticipatory nauseas and vomits
Results: 31 patients were included, 20 women (64.5%) and 11 men, mean age 59.4 years old. The 77.4% were not smokers and the 80.6% declared drink less than 30 grams of alcohol per week. At the moment of the transplant one patient had ECOG 1, and all the other patients ECOG 0. Nine patients (29%) were suffering some sort of digestive disease (Dyspepsia, peptic ulcer, hiatus hernia, gastroesophageal reflux disease or obstruction). The 71% (n=22) was been treated with opioids, antacids and/or benzodiazepines. The schemes of chemotherapy previous given were Bortezomid + Dexamethasone (n=24), Bortezomid +Thalidomide + Dexamethasone (n=5), Bortezomid + Dexamethasone + Cyclophosphamide (n=2), lenalidomide (n=3) and Vincristine + Doxorubicin + Dexamethasone (n=1). The 12.9% of the patients had nauseas or vomits in the last cycles. At the moment of the admission for the transplant only two patients had anticipatory nauseas. In the first 24 hours after mephalnan infusion and palonosetron administration 54.8% (n=17) suffered an episode of nauseas (eight patients grade 1, eight grade 2, and one grade 3), and only nine had vomits (seven grade 1, one grade 2 and one grade 3). Between the next 24-96 hours 19 patients (61.3%) had nauseas (14 grade 1, two grade 2 and three grade 3), and 12 patients (38.7%) vomits (nine grade! and three grade 2). As rescue medication levomepromazine was used in the 35.5%. all the others were treated with metoclopramide, dexamethasone, domperidone and/or benzodiazepines. The only adverse effect documented after Palonosetron administration was constipation (n=2, 6.4%).
Summary/Conclusions: We can confirm the effectiveness and security of palonosetron in the prevention of nauseas and vomits induced for high dose of Mephalan. The results are similar to those reported in the available bibliography.

PB2172

STENOTROPHOMONAS MALTOPHILIA BLOODSTREAM INFECTION IN CHILDREN WITH HEMATOLOGIC DISEASES

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Background: Stenotrophomonas maltophilia (S. maltophilia) causes serious infections in immunocompromised hosts which early use of susceptible antibacterial may be lifesaving.
Aims: We investigated the clinical characteristics of S. maltophilia bloodstream infection (BSI) in children with hematologic diseases.
Methods: We retrospectively reviewed the medical and microbiological records of all consecutive episodes of S. maltophilia BSIs in children with hematologic diseases and hematopoietic Stem Cell Transplantation (HCT) recipients at the University Hospital (Samsun, Turkey) between January 2006 and January 2016.
Results: Among 762 patients identified as S. maltophilia BSIs during 10-year period, 26 children were identified as hematologic diseases; 7 ALL, 3 AML, 2 Fanconi anemia, 5 aquired aplastic anemia, 2 Diamonnd Blackfan anemia, 1 Griscelli syndrome, 1 Diskerotozis Congenita, 1 Ataksia telengiektasia, 1 JMML, 1 MPS type I, 1 Hereditery spherocytosis and splenectomy, 1 JRA and macrophage activation syndrome. Fifteen of them were HCT recipients. Other eleven of them have been treated with immunesupresive drugs. Fifteen (58%) patients were female. The median patient age was 8.6 years (range: 0.6–18 years). Five patient have cutaneous infection (ecthyma gangrenosum). *in vitro* susceptibilities to trimethoprim-sulfamethoxazole (TMP-SXT) were 100%.
Summary/Conclusions: S. maltophilia BSIs may cause to sepsis, especially in patient with severe neutropenia, shock, and pneumonia. It should be in mind when fever, sepsis and ecthyma gangrenosum finding continued although the use of standard antibiotics of febril neutropenia. TMZ-SXT should be first treatment choice for S. maltophilia BSIs in hematologic patients based upon drug susceptibility testing.

PB2173

FEASIBILITY AND SAFETY OF BENDAMUSTINE, ETOPOSIDE, CYTARABINE, MELPHALAN (BEEAM) CONDITIONING REGIMEN BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN 33 PATIENTS WITH MALIGNANT LYMPHOMA

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Background: High-Dose Chemotherapy supported by Autologous Stem Cell Transplantation (HDC/ASCT) is considered the treatment of choice for relapsed/refractory or high risk malignant lymphoma. However, several questions on efficacy and feasibility of HDC/ASCT remain, such as the best candidates for this strategy, the optimal conditioning regimen, and acute and late effects. To date, few comparative randomized trials have been performed, and no regimen has demonstrated superiority to another. The commonly used conditioning regimens is BEAM (BICNU...). To overcome the sourcing out of Carmustine in France we have decided to replace this product with Bendamustine, demonstrated significantly superior efficacy compared with standard therapy in B cell lymphoma, and this based on the work published by Visani and al in 2011.
Aims: We report our experience in a retrospective study concerning 33 patients treated with this conditioning regimen
Methods: From July 2012 to February 2016, 33 patients with median age 56 (28 -70) years, 8 had more than 65 years, Sex ratio (M/F) 22/11, underwent this procedure. This regimen BeEAM combined Bendamustine 100 mg/m² d-7 to d-6; Etoposide 200 mg/m² d-5 to d-2; Cytarabine 200 mg/m² twice daily d-5 to d-3; Melphalan 140 mg/m² d-1 and the reinjection of hematopoietic stem cell was done at d0. We did a reduction of 30% of Cytarabine and Etoposide doses because of age (6 patients). The diagnosis was NHL in 30 cases, composite hemopathy in 2 cases, and HD in 1 case. 30 patients had advanced stage disease (III-IV) of Ann Arbor classification, and a Bulky disease in 4 cases. For 10 patients with DLBCL in first line treatment, the IPIaa was ≥2 in all cases. 15 patients received intensification followed by ASCT in first line treatment, 12 in relapse disease and 6 were primary refractory. The median number of previous line therapy was 2 (1-4). The status at the time of ASCT was evaluated by PET/CT in 29 cases. 23 were in complete remission (CR) and 6 in partial remission (PR). 4 patients was evaluated only by CT-scan (3 PR, 1 CR).
Results: Among the 33 patients treated, the number of autologous CD34+ cells infused was systematically >to 2x10^6 CD34+/kg, and we observed that the median time to myeloid engraftment (ANC>0.5x10^9/l) was 8 days (4-16), and the median time to platelet engraftment (≥20x10^9/l) was 15 days (6-39). The digestive toxicity was important with 16 patients who present a diarrhea grade III-IV, and 23 oral mucositis grade III-IV. One patient presented a veno occlusive disease (VOD) treated with Defibrotide®. We observed 3 cases of cardiac toxicity (3 heart failure grade III partially reversible with medical treatment), 5 renal failure grade I-II. One patient died due to a septic choc with multidrug resistant bacteria before hematological recovery after transplant, producing an overall transplant related mortality (TRM) of 0.03%. 2 patients died at distance of intensification by other causes (one patient with multiple infections caused by opportunistic agents, and the other one with secondary myeloid hemopathy) and the others 30 patients still alive, 29 in continuous CR.
Summary/Conclusions: The new effective regimen BeEAM is not safe in this population of patients with High Risk malignant non Hodgkin and Hodgkin lymphoma. The switch of Carmustine to Bendamustine in the intensive conditioning regimen, required a randomized trial to compare BeEAM and BEAM in new conditioning strategies, and advances in the supportive care will probably reduce transplantation related mortality (TRM). Limited data are available to optimize elderly patients selection for transplantation while minimizing the risk of TRM.

PB2174

ACQUIRED HYPER-IGM SYNDROME FOLLOWING CD20 MAB THERAPY FOR EPSTEIN-BARR VIRUS (EBV) REACTIVATION POST-ALLOGENEIC STEM CELL TRANSPLANT (ALLO-SCT)

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Background: EBV reactivation can be a complication of allogeneic stem cell transplant (allo-SCT) that may lead to EBV-related B Cell Post Transplant Lymphoproliferative Disorder (PTLD).Preemptive treatment with the CD20 mAb rituximab eliminates CD20+ lymphocytes, thereby blocking virus replication, is indicated for rising EBV viral load.
Aims: Here we report 2 cases of acquired hyper-IgM syndrome as a late complication in kids who underwent allo-SCT and received rituximab for treatment of EBV reactivation.
Methods: An 11 yo boy and 8 yo girl underwent allo-SCT in 10/2008 and 8/2010 for ALL and Hemophagocytic Lymphohistiocytosis (HLH), respectively. Patient (pt) and allo-SCT characteristics are shown (Table).
Results: EBV reactivation occurred on day +59 and +33 with 34860 and 40325 EBV DNA copies in the pt with ALL and HLH, respectively. Pts received rituximab (Table) with complete elimination of viremia. Both pts developed hypogammaglobulinemia (IgM, IgG and IgA) post-transplant and received IVIG (0.5 g/kg) monthly for the first 3 months after transplant, then every 3 months, thereafter. The pts developed recurrent sino-pulmonary and enteric infections treated with antibiotics beginning 30 and 18 months post-all-SCT for the ALL and HLH pts, respectively (Figure). In the setting of these recurrent infections, and supportive IVIG, both pts developed progressive increase in IgM level. The ALL pt began to lose full donor chimerism, with no evidence of ALL relapse. He also had recurrent autoimmune thrombocytopenia, which responded to IVIG.Neither pt nor respective donor had mutation in CD154. Flow cytometry for the ALL pt showed decreased mature lymphocytes (CD19+ IgG+), memory lymphocytes (CD19+CD27+IgM+), switched memory (CD19+CD27+IgM-), and transitional lymphocytes (CD 19+CD27+IgM+), and no plasma cells in blood. Flow cytometry studies are ongoing for the HLH pt.

Table 1.

	Pt 1	Pt 2
Diagnosis/SEX	ALL/ Male	HLH /Female
Conditioning Regimen	TBI Thyotepa Cyclophospamide ATG	Busulphan Cyclophospamide ATG
GVHD prophylaxis	CSA MTX	CSA
Donor	MRD (10/10)	MRD 9/10
Cell source/ CD 34+ x 10^6/kg	BM / 4.7	BM / 4.5
PLT day / ANC day	+14 /+14	+19 /+19
Infections	None	Staph. Warneri
aGVHD/Grade/organ	II skin	II skin
GVHD treatment	MPDN 0.5mg/kg	MPDN 1mg/kg
EBV reactivation day /copies	+59/34860	+33/40325
EBV treatment	Rituximab 375 mg/m^{2}x 3 weeks	Rituximab 375 mg/m^{2}x 2 weeks

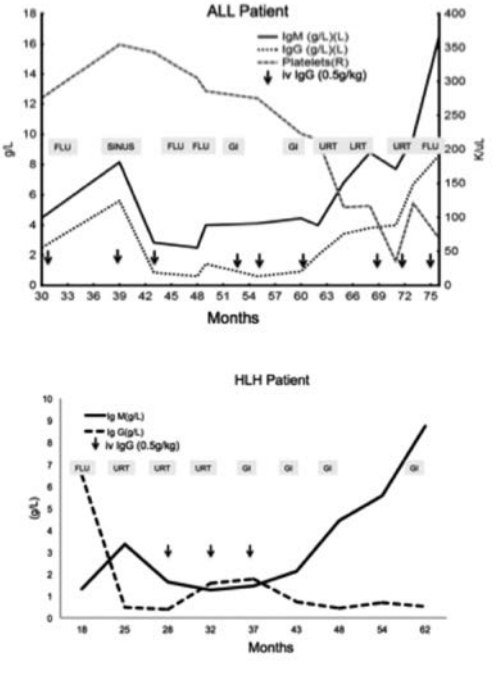

Figure 1.

Summary/Conclusions: We hypothesize that both EBV and rituximab played roles in development of these cases. EBV is responsible of B cell proliferation, maturation arrest, defective DNA repair, leading to hematological neoplasms. EBV infection and reactivation are also associated with autoimmune disorders, transiently elevated IgM during viremia, and monoclonal gammopathy after allo-SCT. Preemptive treatment with rituximab eliminates CD20+ cells where the virus replicates, thereby blocking EBV proliferation, but reduces lymphocyte counts for more than 6 months post BMT, which can alter immunological surveillance. These cases demonstrate development of acquired hyper-IgM syndrome as a late complication of EBV reactivation treated with rituximab post-allo-SCT, which is not previously described. We recommend diligent prolonged monitoring of IgM level post-allo-SCT, particularly in patients who develop autoimmune cytopenia or recurrent infections.

PB2175

BORTEZOMIB AND HIGH-DOSE MELPHALAN AS CONDITIONING REGIMEN BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA: EXPERIENCE IN A UNIVERSITARY HOSPITAL

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Background: The combination of bortezomib and high-dose melphalan (HDM) is an attractive approach to improve the efficacy of the conditioning regimen. Furthermore, this association was expected to be safe because bortezomib and melphalan do not share common toxicities (mainly neurologic toxicity for bortezomib and hematologic toxicity for HDM). We followed a phase 2 study of the Intergroupe Francophone du Myélome (IFM) (Blood 7 Jan 2010, 115 (1); 32-36).
Aims: 1) to evaluate response rates after intensive therapy; 2) to assess the toxicity of this new conditioning regimen.
Methods: We reported 29 Multiple Myeloma (MM) diagnosed patients [18 Males/11 Females, median age 59 years (34-73)]. Patients were treated between February 2010 and December 2015 to receive Bortezomib and melphalan as conditioning regimen (Bor-HDM). Bortezomib was administered subcutaneous at 1 mg/m2 on days -6, -3, 1, and 4. Melphalan was administered intravenously at 200 mg/m2 on day -2. All patients received induction treatment with VCD (Bortezomib 1,3 mg/m2 S.C. D1, D4, D8, D11; Cyclophosphamide 600mg/m2 D1, D8; Dexamethasone 40 mg D1, D4, D8, D11) 6-8 cycles/21 days. Overall, 80% of patients achieved PR, including 8%VGPR and 12% patients with CR before ASCT. Peripheral blood stem cells [median 2.83x106 CD34+ cells/kg (1.97-4.39)] were infused on day 0.
Results: There was no engraftment failure. Neutrophils (ANC ≥0.5x109/L) and platelets (≥20x109/L without transfusion) recovered in median times of 11 days (range, 11-13 days) and 13 days (range, 11-18 days), respectively. Patients were discharged from the transplantation unit in median times of 27.5 days (range, 21-25 days). There were two treatment-related deaths, at +11 day of candidiasis (C. glabrata) and +15 day caused by Enterobacter sepsis. Some serious adverse events were reported: 1) Infections (51% patients): bacteremia was documented: 4 *Staphylococcus epidermidis*, 1 *Enterococcus faecalis*, 1 *Pseudomonas aeruginosa.* 1 Salmonella diarrhea and 1 pneumonia; 2) Mucositis: grade 4 non-hematologic toxicities were mucositis of upper and lower digestive tract sites (10%); the most frequently reported grade 1 or 2 adverse events were digestive (diarrhea, mucositis 55%). It should be noted that peripheral neuropathy (PN) was present at the time of ASCT in one patient and did not get worse after Bor-HDM treatment. At time of reporting, median follow-up time from induction therapy was 30 months (range, 3-72 months). Overall survival was 75%. 2 patients relapsed at 3 and 9 months after ASCT, and they died of progressive disease. Evaluation of response rate at 100 days after ASCT upgraded in 35% patients (reached CR/sCR).
Summary/Conclusions: Our experience shows that Bortezomib can safely be combined with HDM as a preparative regimen followed by ASCT. This regimen was well tolerated with no increased toxicity. Engraftment was not affected by the addition of Bortezomib. PN did not worse after this conditioning regimen.

PB2176

MONITORING MINIMAL RESIDUAL DISEASE IN AUTOLOGOUS CELLULAR THERAPY PRODUCTS COLLECTED BY APHERESIS-EXPERIENCE OF A THERAPY CELLULAR CENTER

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Background: The autologous transplant of peripheral blood stem cells (PBSC), harvested by apheresis on oncologic patients after high-dose chemotherapy and/or radiotherapy, is a therapeutic strategy used in hematologic diseases and solid tumors. Some studies suggest that incomplete disease eradication before collection could lead to the reinfusion of tumor cells and be associated with patient relapse and a lower survival. The search for minimal residual disease (MRD) has been performed in some centers through highly sensitive genetic techniques, contributing to improve the clinical decision and the patient outcome.

Aims: This study aims to evaluate the percentage of MRD-contaminated grafts in patients proposed to hematopoietic transplant with initial genetic alterations in our hospital.
Methods: A retrospective study was performed from May 2006 to February 2016. The inclusion criteria was patients with genetic alterations at diagnosis or relapse. The research was performed using fluorescent *in situ* hybridization (FISH) or/and real time polymerase chain reaction (RT-PCR) techniques. The data was analysed using an excel program.
Results: MRD was researched in samples of PBSC collected from 39 male and 28 female patients (n=67), with a median age of 51 years old (7 months-70 years). Their diagnosis were: non Hodgkin's lymphoma (NHL) n=24, multiple myeloma (MM) n=16, neuroblastoma n=17, acute myeloid leukemia (AML) n=5, acute lymphoblastic leukemia (ALL) n=2, Hodgkin's disease (HD) n=2 and Ewing's sarcoma (Ewing S.) n=1. Only 6 grafts were MRD positive, but with a very low tumour cell contamination (1-2%): 1 AML with *PML-RARA* fusion RNA; 1 ALL with *BCR-ABL* mRNA; 2 MM with deletion of 13q14; 1 Ewing S. with translocation of 22q12; 1 neuroblastoma with deletion of 11q23. Three contaminated grafts were eliminated: 2 patients (1 AML and 1 neuroblastoma) were proposed to different chemotherapy lines, undergone new mobilization and collection program and received a negative cellular therapy product; the ALL patient died before the second mobilization. Two MM patients were infused with the autologous grafts. In the remaining patient (Ewing S.), we performed a positive selection of CD34+ cells after thawing and before graft infusion. At present, 2 patients are alive and in complete remission, 9 and 10 years after treatment (Ewing S. and AML); 3 patients deceased 1, 7 and 4 years after transplant (2 MM and 1 neuroblastoma, respectively).
Summary/Conclusions: As it was a very small and heterogeneous sample, conclusive remarks of the role of MRD significance in stem cells grafts are not possible. In the same way, we only performed one *ex vivo* tumoral purging with identical results to those found on literature. Future prospective trials should address physicians to choose which is the best option to eradicate tumor cells (chemotherapy pretransplantation or immunotherapy postransplantation).

PB2177

VERY LOW INCIDENCE OF NEUROTOXICITY WITHOUT ANTICONVULSANT PROPHYLAXIS DURING THE CONDITIONING REGIMEN WITH BUSULFAN IN PATIENTS UNDERGOING STEM CELL TRANSPLANTATION
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Background: Since neurotoxicities, including seizures, have been reported with the use of busulfan (BU), it is a trend to administer anticonvulsant prophylaxis during a conditioning regimen with this alkylating agent in patients undergoing hematopoietic stem cell transplantation (HSCT). On the other hand, anticonvulsant medications interact with busulfan metabolism, affecting its serum concentration and therapeutic activities, and have their own side effects on the patient which can alter the outcome of the transplant.
Aims: To analyze the frequency of neurotoxicity in patients who underwent stem cell transplantation with conditioning regimen including high doses of busulfan, without anticonvulsant prophylaxis, at INCMNSZ, from November 1998 to January 2016.
Methods: A retrospective analysis was performed in 97 patients receiving high-dose BU as part of their HSCT preparative regimen, at INCMNSZ, determining the frequency of seizures and other neurotoxicities.
Results: Ninety seven patients undergoing stem cell transplantation with conditioning regimen including busulfan, from November 1998 to January 2016, were included. Patients (male, 59%) had a median age of 33 years (range 15-61). The patients had a following range of underlying diseases: myelodysplastic syndrome (n=17, 17.5%), chronic myeloid leukemia (CML, n=13, 13.4%), acute lymphoblastic leukemia (LLA, n=19, 19.6%), acute myeloid leukemia (AML, n=31, 32%), or others (n=17, 17.5%). Patients who underwent an autologous transplant received high doses of busulfan, 16 mg/kg (n=24, 25%), and doses of 12mg/kg (n=73, 75%) were given for allogeneic transplant patients. None of the patients received anticonvulsant prophylaxis. Only one patient (1%), transplanted in 2011, presented seizures after the administration of BU, and was treated with phenytoin and benzodiazepines without further crisis.
Summary/Conclusions: From the beginning of our transplant program no anticonvulsant prophylaxis was given to patients receiving BU-based conditioning regimen for HSCT. Since neurotoxicities were not reported, we continued deferring the anticonvulsant prophylaxis. According to this data, we emphasize that in our experience, seizures are not frequent side effect of conditioning regimens including high dose busulfan, and therefore, anticonvulsant prophylactic regimens are not always necessary.

PB2178

SUCCESFUL STEM CELL COLLECTION IN NEWLY DIAGNOSED MULTIPLE MYELOMA (NDMM) PATIENTS OVER 65 YEARS OLD
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Background: High-dose therapy plus autologous stem cell transplantation (ASCT) is considered the standard of care for front-line treatment of younger patients with newly diagnosed multiple myeloma (NDMM). Eligibility for this procedure are based in almost all health institutions on chronological age more than on biological or functional age.
Aims: Retrospective analysis of characteristics of the mobilisation and collection procedures of NDMM ASCT-candidates patients from 2009 to 2014 comparing 2 groups of age: under *vs* over 65 years old.
Methods: 54 mobilization and apheresis procedures were analysed. 36 patients were under-65y (median 57y, range 48-65y) and 18 in over-65y (median 68y, range 66-71y). Induction Regimen were Bortezomib-based schemes in 50 of 54 patients and 4 received chemotherapy-based scheme before collecting Peripheral Blood Stem Cell (PBSC). PBSC collection was programmed after -at least- 3rd induction cycle. The virtual absence of failures of collection with Bor-Based schemes have permitted us to program mobilisation at any time of induction phase, even after the last cycle, without any complications or failures on the collection. Almost all patients were mobilized with granulocyte colony stimulating factors (G-CSF) alone. Only 1 patient was mobilized with cyclophosphamide and GCSF. Plerixafor was added in predifined by CD34 account poor mobilizers.
Results: G-CSF doses administered was different between the 2 groups of age: group under-65y received a median dose of 10 mcg/Kg/24 hours and the over-65y group a median dose of 10 mcg/Kg/12 hours. Median days of GCSF administration was similar: 5 days. There were no differences on mobilisation failure and/or on the indication of plerixafor administration (7/36 *vs* 2/18, p=ns) The number of aphaeresis needed to reach the CD34 target was similar: median= 1 (range: 1-3). The amount of CD34 cells collected was similar, with no statistical difference: median of 6.35 (2.5-17.67) *vs* 5.8 (2.6-11.6)x10E6 CD34/kg cells between groups. There were no differences in complications after the procedure (bleeding, readmissions ...) between groups. There were no differences in terms of safety and engraftment of ASCT between groups.
Summary/Conclusions: Mobilisation and collection of PBSC in NDMM over-65y patients with current induction schemes is feasible and without differences in terms of efficacy and safety (number apheresis, number of harvested cells, need of plerixafor ...) compared with under-65y group. New induction Bor-Based schemes have a good profile in terms of mobilisation and collection of PBSC, independently of the age Age "per se" is not a restriction to collect stem cells to support an ASCT.

PB2179

DIFFERENCES IN ATTITUDE TOWARDS HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) BETWEEN PHYSICIANS AND NURSES
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Background: Hematopoietic stem cell transplantation (HSCT) is a highly intensive medical procedure and the medical staff at a transplant unit experiences high levels of psychological distress. Since doctors and nurses differ in their clinical training, the level of professional indoctrination and in the duration and nature of exposure to the patients, we hypothesized that they hold different perspective towards the procedure.
Aims: To study how doctors and nurses perceive HCST and whether professional perspectives affect the attitude of physicians and nurses towards the procedure.
Methods: This is a single center study. All the nurses and physicians at our transplant unit were asked to fill out an electronic questionnaire that included 24 items. Five of the items were scenarios, each describing a clinical situation in which transplant is one of the therapeutic options. In order to ensure that the answers do not reflect differences in medical knowledge, there were only minimal or no medical clues to guide participants in these scenarios. Following each scenario, participants had to rank on a 1 to 5 Likert Scale whether they would undergo transplant or recommend it to their close relatives or friends under these circumstances, if this were their doctors' advice. Participants were also asked to rank their level of confidence in the transplant procedure. In addition, we included a work satisfaction questionnaire and basic demographic details were also documented.
Results: We delivered the questionnaire to 51 members of the medical staff, 23 doctors and 28 nurses, who work at our department. The median age was 40 (range: 24 to 78) years and 32 (63%) were females. Nurses were younger, more of them were unmarried and they had less professional experience. Nurses and doctors had similar distributions of male to female ratios, country of origin and both groups expressed similar levels of professional and religious beliefs. When asked directly, physicians and nurses expressed similar level of confidence in the process of HSCT. Intriguingly, only 21 (42%) of responders expressed a high degree of confidence in allogeneic transplantation, while 31 (62%) expressed a high degree of confidence in autologous transplantation. However, almost all doctors (20/21, 96%) but only 50% of nurses (14/28) expressed high levels of confidence in reduced intensity conditioning HSCT (P=.002). When asked if they would undergo transplant or recommend it to their close relatives, in 3 of 5 scenarios presented to responders significantly

more nurses were reluctant to undergo transplant despite their doctors' recommendation.
Summary/Conclusions: At our transplant unit, the attitude of physicians and nurses towards HCST differs significantly. Physicians perceive the procedure in a more positive way and tend to consider undergoing HSCT themselves or recommend it to their relatives if indicated. Nurses more often tend to consider palliative care and express more critical attitude towards the procedure. This study exposes fundamental differences in the professional point-of view and we call for open discussions around these issues in which the various perspectives are expressed and acknowledged. We believe that this approach will ultimately improve the communication and prevent unnecessary confrontations among medical staff.

PB2180

ARTERIAL HYPERTENSION IN LONG-TERM SURVIVORS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Improvement of transplantation techniques and supportive care lead to an increasing number of long-term survivors after allogeneic hematopoietic stem cell transplantation (HSCT). Recipients of allogeneic HSCT have a higher prevalence of cardiovascular risk factors.
Aims: To determine the prevalence of ambulatory hypertension in patients after HSCT.
Methods: Patients ≥1 year after allogeneic HSCT were included during their annual hematological check at University Hospital Basel, Switzerland. Blood pressure (BP) was measured on both arms in the office after 5 minutes rest. Arterial hypertension (HT) was defined as BP ≥140/90 mmHg. 24h ambulatory BP measurement (ABPM) was performed with a device for noninvasive continuous BP monitoring on the same day. 24h-, day- and nighttime HT was defined as ≥130/80, ≥135/85 and ≥120/70mmHg, respectively. Non-dipping was defined as a nocturnal BP decrease of <10%.
Results: 66 patients were enrolled between April 2015 and December 2015. Median age at study entry was 56 (range 22-73) with median 9 (range 1-29) years after transplantation. 47% of the patients were female. Nine (14%) patients had a BMI ≥30kg/m^2, 19 (29%) patients had a sedentary lifestyle and 12 (18%) patients were current smokers. Fifteen (23%) patients had treatment with calcineurin inhibitors and 4 (6%) patients had cortison. Thirty patients (45%) had office HT: Isolated systolic, isolated diastolic and combined HT was documented in 17, 2, and 11 patients, respectively. Systolic BP difference between arms of ≥20mmHg was measured in 3 patients. 24h- HT was documented in 41 patients (62%) with 6 systolic, 8 diastolic and 27 systolic and diastolic HT patterns. 32 (48%) patients had day-time HT and 54 (82%) patients had nighttime HT. 48 (73%) patients were non-dippers. White-coat HT and masked HT was diagnosed in 2 (3%) and 12 patients (18%), respectively. 32 (48%) had treatment with antihypertensive drugs. However, 23 patients were still hypertensive, 17 patients detected with office BP and further 6 patients were hypertensive on ABPM.
Summary/Conclusions: Arterial hypertension is common in patients after allogeneic HSCT and is often missed by office BP measurements only. ABPM detects patients with relevant night-time hypertension. ABPM may lead to better treatment of hypertension.

PB2181

Abstract withdrawn.

PB2182

THE TROUBLE THAT HEMATOLOGISTS DEALING WITH: VARICELLA-ZOSTER AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Reactivation of varicella zoster virus (VZV) is a common event in patients undergoing allogeneic and autologous bone marrow transplantation (BMT).
Aims: The morbidity associated with VZV reactivation and post-herpetic neuralgia results in an impairment of the quality of life, which may also lead to an increase in transplantation related costs and hospitalization. Determining the risk factors for VZV reactivation was aimed.
Methods: We retrospectively analyzed the incidence, clinical outcome, and risk factors for VZV infections in 237 adult patients undergoing BMT between Jan 2011 and Feb 2016 in Inonu University Bone Marrow Transplantation Unit. All of the patients were treated with acyclovir prophylaxis for 3 months after the allogeneic BMT and 30 days for autologous BMT.
Results: Fourteen (5.9%) of the patients developed VZV reactivation. Five (35.7%) of them were autologous BMT and 9 (64.3%) were allogeneic BMT patients. All of the patients were presented with pain and dermatomal zoster. A median day of VZV was 413 days (range 63-820) after transplantation for autologous BMT and 302 days (range 61-1140) for allogeneic BMT. Twenty-one percent (n=3) of VZV reactivation occurred in the first 100 days and 14% (n=2) after the first 24 months. Six (66.6%) of allogeneic BMT patients were under immunsupressive treatment (cyclosporine±mikofenolat mofetil) and 1 (11.1%) had lymphocytopenia when VZV was clinically detected. Sixty-six percent (n=6) of patients with VZV who were treated with allogeneic BMT were acute myeloid leukemia patients. Sixty-four percent (n=9) of the cases was presented in January-march peirod. The most frequent complication was post-herpetic neuralgia. There was no statistically significant difference between the type of hematologic diseases, type of transplantation and lymphocyte count through VZV reactivation. But season of the year (winter) revealed statistically significant difference in VZV reactivation. ($p<0.05$)
Summary/Conclusions: VZV reactivation rates of our BMT unit is significantly lower than the literature especially in autologous BMT. But winter season was defined as an risk factor for VZV reactivation. Longer acyclovir phrophylaxis after autologous BMT and additional phrophylaxis programme for winter months for 2 years after the BMT can be an effective strategy for VZV prevention. Another important key point can be the CD4/CD8 evaluation. Acyclovir phrophylaxis can be maneaged with CD4/CD8 numbers. Phrophylaxis can be continued until the normal CD4/CD8 values.

PB2183

PROPHYLAXIS OF GRAFT VERSUS HOST DISEASE IN PATIENTS WITH APLASTIC ANEMIA AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION WITH BONE MARROW DONOR DERIVED MULTIPOTENT MESENCHYMAL STROMAL CELLS
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Background: Aplastic anemia (AA) is a rare disease characterized by bone marrow failure and pancytopenia. Allogeneic bone marrow transplantation (allo-BMT) is a standard treatment for young patients with a HLA-identical sibling donor. Incidence of acute and chronic graft *versus* host disease (GVHD) is 13% and 14%, respectively. If paroxysmal nocturnal hemoglobinuria clone (PNH) is detected in AA patients the incidence of acute and chronic GVHD increases up to 47% and 70%, respectively. It is known that multipotent mesenchymal stromal cells (MMSC) injection for GVHD prophylaxis after HLA-identical sibling donor transplants significantly decreases incidence of GVHD (ClinicalTrials.gov ID-NCT 01941394).
Aims: Estimate the effectiveness of MMSC injection for GVHD prophylaxis in patients with AA after allo-BMT.
Methods: Nine patients (6 males and 3 females) with AA received allo-BMT from HLA-identical sibling donor in our center between December 2011 and December 2015. Median patients age at the time of transplantation was 24 years (range 17-33). PNH clone was detected in 8 patients (the median granulocytes PNH clone size was 2,5% /range 0,19-97,1%/). PHN clone with intravascular hemolysis (LDH more then 1,5 ULN) was revealed in 2 patients. There were no thrombotic events in all patients. Eight patients received allo-BMT as first line therapy, for one patient allo-BMT was performed for relapse after immunosuppression therapy. The non-myeloablative conditioning regimen was used (ATG (100mg/kg) for 4 days, fludarabine (100mg/m^2) for 4 days, and cyclophosphamide (100mg/kg) for 4 days). For graft *versus* host disease (GVHD) prophylaxis cyclosporine and methotrexate were used. MMSC were infused intravenously at the moment of WBC count recovery to 1*109/l. On average, 1.09 (1.0-1.2)x106 MMSC per kilogram of the patient's body weight were injected.
Results: One patient had graft failure at day + 30 after allo-BMT. Other patients had stable engraftment. Leucocytes recovery occurred at median 21 days (range 17-25). Patients had no complications after MMSC injection. Only in 1 out of 8 patients (12,5%) acute GVHD was diagnosed, despite the presence of PNH clone in 7 out of 8 patients. Acute GVHD with liver involvement (grade III) was developed at 27 days in the patient with chronic hepatitis B virus infection. PNH clone elimination in 6 patients occurred during 1-18 mounts after allo-BMT. All 8 patients had undulating mixed donor chimerism (85-100%) at different time after allo-BMT. One patient with mixed donor chimerism has graft rejection at day + 286. No chronic GVHD in any patients developed. All patients are alive with a median follow-up 16 months (range 1-49).
Summary/Conclusions: We can suggest that in patients with AA bone marrow donor MMSC administration at the moment of leukocytes count recovery after allo-BMT is an effective method for acute GVHD prevention.

PB2184

BK VIRUS INFECTIONS IN PATIENTS WITH UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Hemorrhagic cystitis (HC) is a well-known complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT) that can be categorized as early-onset or late-onset. Early-onset HC is usually caused by adenovirus or cytomegalovirus whereas late-onset HC mainly by polyomavirus BK. BU-containing myeloablative conditioning, unrelated donors and GVHD has been reported as risk factors increasing the chance of infection in bone marrow-transplant patients. Furthermore reactivation of human polyomavirus BK (BKV) may cause polyomavirusassociated nephropathy or polyoma virus-associated hemorrhagic cystitis.
Aims: We aimed to present 17 patients with BK polyoma virus (BKV) ascociated hemorrhagic cystitis and 2 patients with BK polyoma virus associated hemorrhagic cystitis and nephritis.
Methods: Between 2013 and 2015, 90 patients received an allogeneic BMT at Acibadem Adana Hospital Pediatric Bone Marrow Transplantation Unit. 17 patients experienced BKV associated hemorrhagic cystitis and nephritis. BKV was detected in the urine analysis and blood by PCR (polymerase chain reaction) in all patients.
Results: We presented 17 patients with BKV infection, age ranging from 3 to 20 with an average of 11.7 years. They underwent allo-HSCT due to thalassemia major (9 patients), aplastic anemia (3 patients) and leukemia (5 patients). The patients were treated with hydration, continuous bladder irrigation, ciprofloxacin, cidofovir and weekly intravesical hyaluronic acid instillation for four weeks. Ten patients showed complete resolution of hematuria. Three patients with refractory following therapy also received hyperbaric oxygen. Hemodialysis was performed in two patients who developed renal failure due to nephritis.
Summary/Conclusions: Past exposures with the BK virus is widespread but significant consequences of infection are uncommon in the immunocompetent population. Reactivation of infection occurs under conditions of immunosuppression such as during GVHD treatment with patients who underwent HSCT. Early detection and treatment is crucial for successful management of BKV cystitis and nephritis. Neverthless even when treated with all the modalities, in some patients treatment faillure can be observed.

PB2185

IMPACT OF DISEASE STATUS ON OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF REFRACTORY AND RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: Treatment of refractory and relapsed acute lymphoblastic leukemia (ALL) remains challenging. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only potential curative approach for these patients. However, whether patients will benefit by achieving remission before transplant remains controversial.
Aims: To evaluate the impact of disease status on the outcomes of allo-HSCT in the treatment of patients with refractory and relapsed ALL.
Methods: We retrospectively analyzed the outcome of 52 patients with refractory and relapsed ALL, including 19 cases in advanced stage, such as: nonremission (NR) and 33 cases in more than second complete remission (≥CR2), who underwent allo-HSCT after myeloablative conditioning regimen in our department.
Results: 51 patients engrafted successfully, the transplantation-related mortality (TRM) rate of NR and ≥CR2 was 10.5% *vs* 12.1% (P=0.815). The incidence of acute GVHD(aGVHD) was 52.6% *vs* 57.6% (P=0.730), including 42.1% *vs* 33.3% (P=0.527) with mild (grade I-II) aGVHD and 10.5% *vs* 24.3% (P=0.399) with severe (grade III-IV) aGVHD, and chronic GVHD(cGVHD) was 41.6% *vs* 57.9% (P=0.660). With a median follow-up of 12(1.8-44.5) months, the cumulative relapse rate was 47% *vs* 34.3%, P=0.425) of NR and ≥CR2. The estimated 2 year overall survival (OS) and 2 year leukemia-free survival (LFS) rate were 42.6% *vs* 45.7% (P=0.487) and 46.3% *vs* 46.2% (P=0.571) respectively. Multi-parameter analysis results showed that, OS and LFS was significantly better in patients with the appearance of cGVHD□For relapsed patients, OS was significantly better with first CR duration>6 month and time to transplant ≤2 months.
Summary/Conclusions: In this retrospective single center study, we can distinctly note that the survival of patients in NR and ≥CR2 before transplant is similar. Thus, it is suggested that allo-HSCT is an effective salvage therapy for patients with refractory and relapsed ALL and transplantation can be conducted directly for those who could not tolerate salvage chemotherapy. However, given the limited number of patients and observation time, an expanded sample size and prospective cohort study is needed to confirm the results.

PB2186

IMPACT OF THE IL28B RS12979860 CT POLYMORPHISM ON THE CYTOMEGALOVIRUS REACTIVATION IN AUTOLOGOUS STEM CELL TRANSPLANT PATIENTS
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Background: CMV infection represents one of the main cause of morbility and mortality after stem cell transplantation (SCT). Type III interferons (IFNs), including IFNl1 (IL29), IFNl2 (IL28A) and IFNll3 (IL28B), are thought to display potent antiviral and immunomodulatory properties in vivo, which may partially overlap with those exerted by type I IFNs. Type I and Type III IFNsl both generate an antiviral state by triggering the JAK-STAT pathway, ultimately upregulating the expression of interferon-stimulated genes. Rs12979860 single nucleotide polymorphism (SNP) in IL28B gene region is well known to influence the spontaneous and treatment-induced clearance in HCV infection. Although the relevance of such a SNP in other vir. CMV infection represents one of the main cause of morbility and mortality after stem cell transplantation (SCT). Type III interferons (IFNs), including IFNl1 (IL29), IFNl2 (IL28A) and IFNll3 (IL28B), are thought to display potent antiviral and immunomodulatory properties in vivo, which may partially overlap with those exerted by type I IFNs. Type I and Type III IFNsl both generate an antiviral state by triggering the JAK-STAT pathway, ultimately upregulating the expression of interferon-stimulated genes. Rs12979860 single nucleotide polymorphism (SNP) in IL28B gene region is well known to influence the spontaneous and treatment-induced clearance in HCV infection. Although the relevance of such a SNP in other viral infections is still debated, Bravo *et al.* recently documented a protective effect of the T allele against CMV infection in the Allogeneic SCT (Journal of Medical Virology 2014,86:838). Al infections is still debated, Bravo *et al.* recently documented a protective effect of the T allele against CMV infection in the Allogeneic SCT (Journal of Medical Virology 2014,86:838).
Aims: the current study was aimed at investigating whether the IL28B polymorphism Rs12979860 may affect the CMV reactivaction in the setting of Autologous SCT (Auto-SCT).
Methods: From October 2014, 67 patients undergoing an Auto-SCT for hematological malignancies were included in the study. The patients, with a median age of 56 years (range, 16-66 yrs), were distributed according to the underlying disease as follows: 75% had Multiple Myeloma, 16% non Hodgkin Lymphoma, 6% Hodgkin Lymphoma and 3% Acute Myeloid Leukemia. The Rs12979860 IL28B SNP (C/T) genotype was determined by Melthing analysis on DNA derived from peripheral blood samples. CMV DNAemia was determined by quantitative Real-Time PCR with a limit detection of 50 copies/mL (Artus, Qiagen). Patients were monitored for CMV DNAemia weekly for three months after transplantation.
Results: According to the lowest frequency of TT genotype harboring in general population, the detected genotypes were CC in 31 patients (46%) CT in 27 (40%) and TT in only 9 (14%). Although not statistically significant, a CMV reactivation (symptomatic and asymptomatic) was higher in patients carrying TT genotype (n=7 ;77.7%) with respect to patients carrying CC (n21.;67.7%) and CT genotype (n=12; 44.4%). According to the logistic regression analysis, the incidence of CMV reactivation was significantly lower in patients carrying IL-28B CT compared to the cumulative group of patients with CC and TT genotype (44.4% *versus* 70% ; p=0.039, CI 95%, OR 2.91) (codominat genetic model).
Summary/Conclusions: Our data suggest that in the Auto-SCT setting the carriage of IL-28B CT genotype might express a protective effect against CMV infection

PB2187

MAJOR QUALITY PARAMETERS OF LONG-TERM CRYOPRESERVED CORD BLOOD UNITS - A SINGLE CENTER EXPERIENCE
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Background: Cord Blood (CB) is a well-recognized source of stem cells for hematopoietic transplant. Its main advantage is to be stored for long-term with a ready availability for the intended recipient and no risk for the donor.
Aims: The aim of this study was to evaluate the quality of 15 cord blood units cryopreserved in our direct donation bank. for the intended recipient and no risk for the donor.
Methods: All these units were processed without red cells reduction, in a 24 hours period after collection, then were cryopreserved with a controlled-rate freezer and transferred to a liquid nitrogen container. We used cryogenic bags (Kapton/Teflon), highly resistant to very low temperatures. However, due to missing data we do not considered these CB units suitable for transplant. In this study, the CB were thawed and washed based on the New York Blood

Center method in our ISO 7 cleanroom, inside of an air flow cabinet. We performed cellular counts, viability assays (by flow cytometry using 7-aminoactinomycin), sterility testing (bacterial and fungal cultures) and clonogenicity (colony-forming units-granulocyte/macrophage - CFU-GM - growth). The correlation between CD34+ cell and CFU-GM counts was evaluated by linear regression analysis.
Results: The CB units were stored during 11-20 years (mean 16±3). We analyzed 12 CB because 3 bags were broken during storage (20%); this percentage is much higher than that found in our daily practice (4%), which may be caused by the long cryopreservation period. Visual examination of the product showed evidence of hemolysis in 10 CB and fibrin clots in 1. The recovery of total nucleated cells (TNC) was 73±14%; CD34+ cells enumeration 48.3±29.3x10^5 and CFU-GM quantification 28.5±19.3x10^4. The TNC and CD34+ cells viability was 70±8% and 91±5%, respectively. We obtained a strong positive relationship between CD34+ cell and CFU-GM (R^2=0.758). We found 4 contaminated CB, one of them was already positive after processing (*Enterococcus faecalis, Escherichia coli and Streptococcus mitis)*. We hypothesize that the pre-freezing hemocultures were not representative of the product; however, we cannot exclude a cross-contamination during the storage.
Summary/Conclusions: Despite our small serie, the results of cellular viability, purity and potency indicate that long-term cryopreservation does not negatively affect the quality of CB units for further use, even in the presence of contamination, hemolysis signs and aggregates. We think that every cord blood bank should have an expert to help transplant physicians select the best cord blood for his patient based on the control quality results performed before final release.

PB2188

PERIPHERAL BLOOD HEMATOPOETIC STEM CELLS (PBSC) MOBILIZATION WITH GENERIC VERSION OF PLERIXAFOR: AN EXPERIENCE AT A NORTH INDIAN TERTIARY CARE REFERRAL INSTITUTE
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Background: Plerixafor is used to mobilize hematopoietic stem cells in cancer patients into the bloodstream. Developed by AnorMED, plerixafor was approved by the U.S. Food and Drug Administration (FDA) on 15 December 2008. Henceforth, Genzyme (USA based company) took a step forward and launched plerixafor from benchside to bedside. Brilliant success story of this new molecule was hindered by the expensive cost thereby debarring large population of world from the benefit of this drug. Many Indian companies (eg. Hetero and panacea pharmaceutical companies) took the challenge to curb down the cost price and have come up recently with *generic version* of plerixafor. For a successful outcome of Hematopoetic Stem cell transplant a minimum dose of 2x106/Kg CD34+ cells are required. Standard mobilisation procedure involves use of G-CSF or GM-CSF alone in combination with chemotherapy. Those with certain risk factors like old age, previous multiple chemotherapy regimens, radiotherapy, bone marrow involvement with disease and exposure to agents like lenalidomide etc. may not mobilise adequately. We share our experience with *generic* plerixafor which is approximately 4-4.5 times cheaper than the original molecule and yet efficacious.
Aims: To study the efficacy of *generic* plerixafor which is approximately 4-4.5 times cheaper than the original molecule.
Methods: Study was conducted from December 2013 to February 2016 (26 months). In total, 66 transplants were carried out (autologous= 35 and allogeneic= 31) in our institute. Generic plerixafor was used along with G-CSF for in autologous PBSCs mobilisation in 20 out of 36 instances (Multiple myeloma =7, Hodgkins lymphoma=6, Nonhodgkin's lymphoma=6, APML= 1).
Results: Mean age of the patients was 39.5 years (range 17-63 years) with 13 males and 7 females. Average interval from disease diagnosis to transplant interval was 18.4 months (range 6- 96 months). Number of prior chemotherapy regimens used ranged from 2-4 and 6 patients also got radiotherapy in addition. Conditioning regimens used were-(1) Inj. melphalan=7, (2) BEAM regimen=10, (3) LACE regimen =2, (4) CY-TBI=1. Inj. Plerixafor was given in dose of 24 microgram/kg on day 4 and/or day 5 of G-CSF (10 microgram/kg/day) mobilisation depending on peripheral blood CD34+ cells (when available) and the yield of first harvest. Most of the patients (14 out of 20) required a single dose, while the remaining six needed an additional dose. Majority of patients (17 out of 20) underwent two apheresis session while two required single session. One had initial mobilisation failure with GCSF and 2 doses of plerixafor. But he subsequently mobilised successfully after 14 days with repeat GCSF and single plerixafor dose. Mean CD34+ cells obtained was 5.44x10^6/kg (range 2.7-13.13x10^6/kg). Neutrophilic and platelet engraftment occurred on day+13 (range 10 -20 days) and day +16 (range 10-36) respectively. Median units of PRBC and Platelet (SDAP) transfused were 3 (range 0-13) and 7 (2-15) respectively. There was only one death on day +7 due to acute cardiomyopathy.
Summary/Conclusions: Generic version of plerixafor with GCSF is an effective and safe strategy to mobilise stem cells.

PB2189

PREDICTIVE FACTORS FOR RELAPSE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA SUBMITTED TO ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE
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Background: Adults with high risk acute myeloid leukemia features at diagnosis, primary induction failure (PIF) or with recurrent disease have a poor outcome after chemotherapy and are candidates for allogeneic stem cell transplantation (allo SCT). However, the relapse rate in these patients remains a matter of concern.
Aims: Aim of the study is to identify the main factors influencing acute myeloid leukemia (AML) relapse after allo SCT in a single center.
Methods: 118 adult AML patients (median age 42 y [range 16-68], 50 M/ 68 F) received an alloSCT at our Institution from 2000 to 2015. Donors for HCT were HLA-matched related donors in 68/118 (57%) and "alternative donors" (matched unrelated or haploidentical donors) in 50/118 (43%) transplantations. The source of hematopoietic stem cells was peripheral blood in 110/118 (93%) and bone marrow in 8/118(7%) patients; 90% patients underwent a myeloablative conditioning regimen and 10% a reduced intensity regimen. Disease status (1st CR *versus* not[2nd/3nd CR and "active disease"]), molecular-cytogenetic risk(intermediate *versus* poor-risk), response to first chemotherapy(responders *versus* primary induction failure/PIF), time from diagnosis to transplantation (more or less than 6 months), stem cell source (bone marrow or peripheral blood), donor type (matched related or alternative), severe acute GvHD (grade IIII-IV), chronic GvHD, serological risk CMV status were analyzed as predisposing factors for relapse. Multivariate analysis was performed with the variables identified as relevant at univariate analysis.
Results: Transplantation for AML was done in 1st CR in 53/118 (44%) patients and not in 1st CR in 65/118 (56%) patients. Thirty-nine patients (33%) were PIF and 83/118 (70%) transplants were done within 6 months from diagnosis; 28/118(23%)patients were classified as poor molecular-cytogenetic risk. Twenty-six (18%) suffered GvHD grade III-IV and 32%, 15% and 8% of evaluable patients developed limited, moderate and severe cGvHD. The 2-year relapse incidence was 55%: 36% for patients in CR1 and 75% for patients not in CR1(p=0.001). At multivariate analysis the disease status (1st CR)(p=0.02 HR 0.73, 95%CI 1.0-2.8) and cGvHD(p=0.001 HR 0.2, 95%CI 0.1-0.4) were the factors associated with a lower relapse incidence. Donor type, molecular-cytogenetic risk, response to first chemotherapy, stem cell source, acute GvHD did not seem to significantly influence relapse.
Summary/Conclusions: In our experience allogeneic transplants performed in 1stCR and cGvHD were the main factors influencing the risk of relapse in adult patients with AML. Transplanted patients not in 1st CR and without cGvHD may be a "high relapse risk group" that could benefit from pre-emptive therapy post transplantation also with new drugs.

PB2190

COMPARABLE OUTCOME OF ALLOGENEIC VERSUS AUTOLOGOUS HEMATOPOIETIC PERIPHERAL BLOOD STEM CELL TRANSPLANTION IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE AND FLT3-ITD NEGATIVE
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Background: Optimal post-remission treatment for acute myeloid leukemia with normal karyotype (AML-NK) in first complete remission (CR1) is still not well-defined.
Aims: To compare the outcome of allogeneic *versus* autologous peripheral blood stem cell transplantation (PBSCT) in adult AML patients regarding toxicities of transplant procedure, transplant-related mortality (TRM), disease free survival (DFS) and overall survival (OS).
Methods: 43 patients were included; 34 patients (with a median age 28 years) received myeloablative allogeneic PBSCT from a matched sibling donor while 9 patients (with a median age 36 years) received PBSC autograft. All patients had a normal karyotype, FLT3 ITD negative and were in CR1.
Results: After a median follow up of 21.5 months (0.3– 46.5), the cumulative 2-year OS and DFS in the allogeneic group were 73.5% and 70.6% respectively compared to 74.1% and 64.8%, respectively in the autologous group (p=0.690 and 0.768). Increasing number of consolidation cycles (>3) and lower CD34 +ve stem cell dose were associated with lower relapse rates and higher DFS in the autologous group.
Summary/Conclusions: Preliminary data show comparable outcome of autologous compared to allogeneic PBSCT in patients with AML-NK and FLT3 ITD negative in CR1. In absence of matched sibling donor, autologous PBSCT may provide acceptable post-remission therapy for patients with low risk molecular profile.

PB2191

Abstract withdrawn.

PB2192

CAN RED CELL DISTRIBUTION WIDTH BE A NEW PARAMETER FOR PREDICTING HIGHER CD34+ CELL COUNT IN THE HARVEST?

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Background: The red cell distribution width (RDW) is a simple and unexpensive parameter and is traditionally used for differential diagnosis of anemias. However, the number of articles mentioning about the relationship between RDW and human disorders has increased over the past decades. In tranplantation practice, the peripheral blood is the most common source of hematopoietic stem cells. There are many factors affecting the success of stem cell mobilization.
Aims: In this study, we tried to find a relationship between increased RDW and CD34+ cell count in the harvest.
Methods: Fifty patients who underwent stem cell mobilization between March 2014 and October 2015 are included in the study. Mean age was 56.82± 10 years, 28 (%56) patients were male and 22 (%44) patients were female. Diagnosis were multiple myeloma (n=32), non Hodgkin lymphoma (n=9), Hodgkin lymphoma (n=6), primary amyloidosis (n=1), Waldenstrom's macroglobulinemia (n=1) and testicular carcinoma (n=1). Mobilization regimens were cyclophosphamide plus G-CSF in 35 (%70) patients, G-CSF alone in 2 (%4) patients, etoposide plus G-CSF in 2 (%4) patients and salvage chemotherapy plus G-CSF in 11 (%22) patients. Five (%10) patients had a history of radiotherapy. Bone marrow infiltration for patients except myeloma was found in 6 (%35) patients. Patients received mean 1.61± 0.6 lines and 4.94±2.6 courses of chemotherapy.
Results: RDW was not correlated with peripheral blood CD34+ cell count (r=0.064, p=0.75, n=28) and CD34+ cell count in the harvest (r=-0.14, p=0.34, n=50). Other parameters previously reported to affect stem cell mobilization (age, weight, number of chemotherapy courses before mobilization, hemoglobin, white blood cell count, absolute neutrophil count, absolute lymphocyte count, platelet count, albumin, LDH) were also evaluated. Peripheral blood CD34+ cell count (r=0.694, p=0.001, n=50) and the number of chemotherapy courses before mobilization (r=-0.451, p=0.008, n=28) were found as the only parameters that affect CD34+ cell count in the harvest. In order to assess the effect of increased RDW on the CD34+ cell count in the harvest; above 16 was set as a cut-off for increased RDW and patients were divided into 2 groups. Sex, primary diagnosis, stage, bone marrow infiltration, mobilization regimen, chemotherapy courses, radiotherapy, comorbidities, number of leukapheresis days were not different between two groups. LDH was the only parameter different between 2 groups. It was found higher in the increased RDW group (p=0.005). Although peripheral blood CD34+ cell count and CD34+ cell count in the harvest were not different statistically between 2 groups, the numbers were lower in the increased RDW group (10.48±5.94 *vs* 8.07±5.01).RDW was not correlated with peripheral blood CD34+ cell count and CD34+ cell count in the harvest in both groups.
Summary/Conclusions: To our knowledge, this is the first study evaluating the relationship between RDW and stem cell mobilization in cancer patients. Although it has not been definitely established, it seems reasonable to suggest using RDW far beyond the differential diagnosis of anemias. Stem cell mobilization can be a potential candidate in this era.

PB2193

THE INFLUENCE OF HAPLOTYPE HLA-C(C1/X,C2/X) IN EVOLUTION AFTER HSCT

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Background: Haplotypes of patients with acute leukemia like ligands or not for inhibitories and activatories donors KIR allele in HSCT are subjects of studies in a few specialised clinics; it is an evidence that haplotypes can be protective or not against postHSCT complication, easier to try to demonstrate at patients with genoidentical donors.
Aims: Haplotype HLA-C(C1/X,C2/X), homozygot and heterozygot variants (HLA-C1/C1,HLA-C1/C2,HLA-C2/C2) seems to have influence at patients with acute leukemia after HSCT.
Methods: Eighteen pairs patients-donors are evaluated: patients with acute leukemia, lymphoblastic and non-lymphoblastic and their genoidentics donors. One patient have HLA-C1/C1 haplotype, seven HLA-C1/C2, ten HLA-C2/C2. Following the impact of inhibitory KIR2DL1, like ligand for C2,KIR2DL2, KIR2DL3 for C1, activatory KIR2DS1 for C2,KIR2DS4 for C1, on survival and complication development, we proved the protective effect of presence of HLA-C2/X haplotype, HLA-C1/X, respectively. The source of HSCT was PBSC. The method used was PCR-SSP (Innotrain DIAGNOSTIK GMBH, Dynal BIOTECH PEL-FREEZE). The complications like graft *versus* host disease acute and chronic, relapse, TMA and the recovery with leucocytes and thrombocytes are followed.
Results: Presence of HLA-C1 haplotype is protective in presence of KIR2DS4 (activatory allele) with statistical significance for thrombocyte recovery but no influence against aGVHD and relapse; in presence of KIR2DL2, KIR2DL3 (inhibitory allele) against relapse, TMA, a/cGVHD, leucocytes and thrombocytes recovery, without statistical significance. HLA-C2, in presence of KIR2DS1, and also in presence of KIR2DL1 protective against TMA and relapse, cGVHD, also leucocyte and thrombocytes recovery, without statistical significance, except aGVHD (no influence) and observation for absence HLA-C2 with protective effect against relapse (...missing ligand").
Summary/Conclusions: Presence of HLA-C1/X,HLA-C2/X4 improve survival and offer minor protection against most complication at patients with acute leukemia and related donors with 100% allele match, in presence of both types of KIR alleles.

PB2194

HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PEDIATRIC PATIENTS WITH MIXED PHENOTYPE ACUTE LEUKEMIA

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Background: Mixed phenotype acute leukemia (MPAL) is a rare disease accounting for about 2-5% of all acute leukemia, and patients with MPAL have poor prognoses, particularly in patients with T/myeloid immunophenotype. However, there is no standardized treatment for MAPL and no consensus about the incorporation of hematopoietic stem cell transplantation (HCT) as an integral part of therapy. Due to its rarity, outcomes of HCT for pediatric MPAL have not been fully determined.
Aims: We investigated outcomes of HCT for pediatric patients with MPAL with multicenter study.
Methods: We retrospectively analyzed transplant outcomes for 12 pediatric patients with primary MPAL, undergoing HCT between 2001 and 2014 in 10 institutes in Youngnam province, Korea. By WHO 2008 classification, 1 (8%) had MPAL with t(v;11q23); MLL rearranged, 9 (75%) had MPAL, T/myeloid, NOS, and the remaining 2 (17%) had MPAL, B/myeloid, NOS. Before HCT, 10 patients were in first complete remission (CR), 1 patient in second CR, and one patient relapsed and had persistent disease. Donor was matched-related in 6, matched-unrelated in 2, umbilical cord blood (UCB) in 2, and autologous in 2, respectively. Conditioning regimen was busulfan, cyclophosphamide and/or melphalan based myeloablative (MA) in 10 patients and busulfan and fludarabine based nonmyeloablative (NMA) in 1 patients. One case of autologous transplant used cyclophosphamide, etoposide, cytarabine and BCNU as a conditioning regimen. For prophylaxis of GVHD following allogeneic HCT, cyclosporine and short-term methotrexate were used.
Results: Median age at the time of HCT was 9.0 years (range, 1.3-13.3 years), and median interval between diagnosis and transplantation was 6.1 months (range, 2.8-14.2 months). Cumulative incidences of neutrophil and platelet engraftment by day 28 were 100%. After median follow-up of 6.2 years (range, 0.8-17.4), 9 of 12 patients survived. Cumulative incidence of treatment-related mortality (TRM) and relapse were 9% and 35.8%. Event-free survival (EFS) and overall survival (OS) after HCT were 64.2% (50%>78%, 95% CI) and 72.7% (59%>86% 95% CI), respectively. WHO classification did not significantly affected EFS after HCT for pediatric MPAL patients (p=0.371).
Summary/Conclusions: Many studies have reported that patients with MPAL had poor prognosis, and patients with T/myeloid phenotype had a worse outcome than patients with B/myeloid phenotype. We found that WHO classification 2008 for MPAL was not significantly associated with patients' outcome after HCT and that patients with MPAL, T/myeloid, NOS had more favorable outcome compared to historical data. Our data suggested that HCT may improve the outcome of pediatric patients with MPAL, particularly T/myeloid phenotype. This study needs to be confirmed in larger, prospective studies including more pediatric MPAL patients.

PB2195

THE ROLE OF LYMPHOCYTE RECOVERY AFTER HIGH DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA AND MULTIPLE MYELOMA

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Background: In Non Hodgkin lymphoma as well as in Hodgkin lymphoma (HL) and multiple myeloma (MM), lymphocyte recovery after autologous stem cell transplantation (ASCT) has been reported to have association with clinical outcome, but also with contradictory data.
Aims: The aim of study was to evaluate the role of parameters that may be associated with outcome after ASCT.
Methods: The study included 61 patients with relapsed/refractory HL (34 males/ 37 females) aged 30 years (range 18-46) and 85 MM patients (42 males/ 43 females) aged 54 years (range 36-65) who were treated with high dose chemotherapy followed with ASCT. Advanced stage of HL disease (Ann Arbor III-IV) had 41.0% patients. Majority of patients had constitutional B symptoms (88.5%) and bulky disease (54.1%). Low IPS had 15 patients (22.6%). All patients received ABVD therapy and in the course of relapse or refractory disease, DHAP as first salvage therapy, and BEAM (54 patients, 88.5%) or CBV (7 patients, 11.5%) as conditioning regimen due to ASCT. The average value of collected $CD34^+$cells was $11.3x10^6$/kg (range 2-$24x10^6$/kg) in the volume of 250 ml (range 100-900ml). The mean aplasia duration was 11 days (range 6-28 days). After ASCT 29 patients achieved CR (47.5%), 21 patients PR (34.3%), 3 had SD (4.9%) and 8 patients (13.1%) had PD. Median time to recovery of absolute lymphocyte count on $500x10^6$/l (ALC500) was 15 days (range 9-32). Regarding MM population, majority of patients had IgG (59, 69.4%) and IgA (12 pts, 14.1%) type of MM. According to the clinical stage (CS) 6 pts (7.1%) had I, 16 (18.8%) II, and 63 (74.5%) III CS. Renal impairment was present in 15pts (17.6%). MM patients were initially treated with protocol VAD (29 pts, 34.1%) or CTD (56 pts, 65.9%) and high dose Melphalan (200mg/m^2) was conditioning regimen prior to ASCT in the first treatment line. The average value of collected $CD34^+$cells was $6.5x10^6$/kg (range 2-$96x10^6$/kg) in the volume of 300 ml (range 100-660ml). The mean aplasia duration was 8 days (range 4-26 days). After ASCT only 1 patient (1.2%) had PD, while 22 (25.9%) had PR and the rest achieved at least VGPR (52, 73.0%). Median time to ALC500 recovery was 15 days (range 9-23days).
Results: In HL patients survival after ASCT was 39 (2-100) months, and OS was 70 (16-192) months. Patients with low IPS (0-2) had better survival compared to those with high IPS score (≥3) (Log Rank=3.68, p=0.05). Survival after ASCT was markedly influenced by prolonged recovery duration of ALC500≥18 days (Log Rank=4.54, p=0.03). However, the duration of ALC500 recovery was not in correlation with treatment response (p>0.05). Furthermore, survival was significantly inferior in patients with PD or SD after ASCT (Log Rank=6.07, p=0.014). As expected, treatment response after ASCT significantly influenced OS (Log Rank=11.27, p=0.004). Multivariate Cox regression among independent prognostic factors in univariate analysis (IPS, therapeutic outcome and ALC500 recovery) pointed out therapeutic outcome at D+100 and delayed ALC500 recovery as the most important parameters that influenced both OS and EFS (p<0.05). In MM patients survival after ASCT was 29 (2-115) months, and OS was 48 (16- 127) months. However, delayed recovery of ALC500 after 18^{th}. day from ASCT did not influence neither OS nor survival after ASCT.
Summary/Conclusions: According to results of Cox regression analysis in HL, no responders at D+100, and delayed recovery of ALC500 are parameters of inferior prognosis and shorter survival. However, similar cut off values of ALC500 are not predictable in MM patients.

Stem cell transplantation - Experimental

PB2196

COMPARISON BETWEEN SERUM AND FECAL CALPROTECTIN AS MARKER OF GRAFT-VERSUS-HOST DISEASE

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Background: Graft-versus-host disease (GvHD) is one of the complication occurring after hematopoietic stem cell transplantation (SCT) responsible for high morbility and mortality. The main strategy to reduce GvHD-related mortality is the correct diagnosis and prompt steroid administration. In the last few years, different proteins have been evaluated as reliable biomarker of GvHD. In our experience, fecal calprotectin (FC), a heterodimer of two S100 family proteins S100A8/S100A9 secreted by activated macrophages, appeared useful to differentiate gut GvHD from liver/skin GvHD, and to distinguish between gut GvHD and enteritis due to other causes.
Aims: The aim of our study was to compare fecal (FC) and serum (SC) levels of calprotectin in a cohort of patients (pts) submitted to SCT, in order to define if a correspondence there was between the two determinations in the same patient (pt).
Methods: We enrolled 21 pts submitted to SCT in our department since December 2009 al July 2012, 9 females and 12 males, with a median age of 55 years (range 35-63), affected by acute lymphoblastic leukemia in 2 cases, acute myeloid leukemia in 14 cases, non-Hodgkin lymphoma in 1 case, myeloproliferative disease in 1 case and myelodisplastic syndrome in 3 cases. Conditioning regimes was MAC in 2 pts and RIC in 19 pts. GvHD prophylaxis was performed with short course methotrexate plus CSA in 15 cases and with CSA+MMF in 6 pts. Donor was related in 14 cases and unrelated in 7 pts. Serum and stools samples were provided for each pt at the time of GvHD onset and, for pts who did not developed GvHD samples were collected around day +30. Calprotectin detection was made using commercial ELISA kit for Calprotectin assay on stools and serum samples.
Results: Fourteen pts (66,7%) developed acute GvHD after a median time of 25,5 days (range 6-80): grade I in 3 pts (21,4%), grade II in 3 pts (21,4%), grade III in 7 pts (50%) and grade IV in 1 pt (7,2%). Organ involvement was: isolated skin in three pts, skin and liver in one pt, isolated liver in 2 pts, isolated gut in 2 pts, gut and skin in 5 pts, skin and gut and liver in one pt. FC median level was 198,9 mg/Kg (range 58.4-500) in pts with aGvHD and 32.2 mg/Kg (range 15.6-89) in the others (Mann Whitney, p=0.0005, Fig 1A), while SC median level was 2756.5 ng/ml (range 173.3-10690) in pts with aGvHD and 1328 ng/ml (range 410.9-4241) in the others (Mann Whitney, p=0.22, Fig 1B). Among pts with aGvHD, FC median levels was 134.9 mg/Kg (range 58.4-292.3) in pts with grade I-II aGvHD and 396.6 mg/Kg (range 95.2-500) in grade III-IV (Mann Whitney, p=0.029), while SC median level was 231.2 ng/ml (range 173.3-5575) in pts with grade I-II aGvHD and 3601 ng/ml (range 868.5-10390) in grade III-IV (Mann Whitney, p=0.4). Finally, FC median level was 396.6 mg/Kg (range 142.1-500) in pts with gut aGvHD and 115.2 mg/Kg (range 58.4-292.3) for non-gut aGvHD (Mann Whitney, p=0.02, Fig 1C), while SC median level was 3232.25 ng/ml (range 868.5-10390) in pts with gut aGvHD and 2312 ng/ml (range 173.3-5575) in non-gut aGvHD (Mann Whitney, p=0.57, Fig 1D). Figure 1: A) median FC level in pts with (yes) or without (no) aGvHD; B) median SC level in pts with (yes) or without (no) aGvHD; C) median level of FC in pts with gut or liver/skin GvHD; D) median level of SC in pts with gut or liver/skin GvHD.

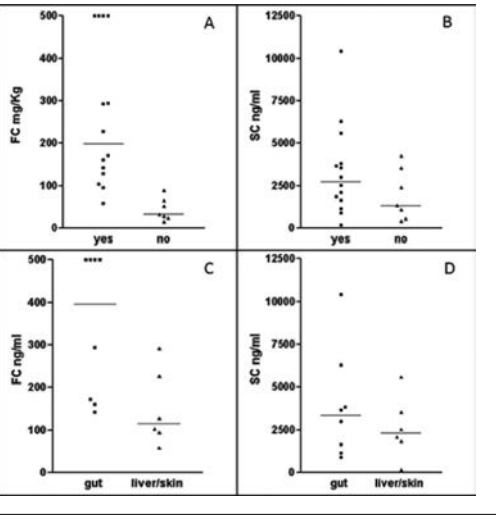

Figure 1.

Summary/Conclusions: There was no correspondence between serum and fecal camprotectin. Our studies suggest that fecal but not serum calprotectin levels may be considered a marker of gut GvHD after SCT.

PB2197

ANALYSIS OF CHIMERISM AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE STUDY OF GENETIC POLYMORPHISMS ASSOCIATED WITH THROMBOPHILIA

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Background: Currently, much attention is paid to the development of simple and relatively inexpensive methods of diagnosing hematopoietic chimerism.
Aims: To study the possibility of using gene allelic variants of thrombophilia as markers chimerism after allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Methods: The results of examination of 10 patients, which in 2015 was performed allogeneic HSCT. Donors of hematopoietic stem cells for 8 patients were siblings, donors for 2 patients were selected from the Bone Marrow Transplant Registry. In all cases the donors and recipients were HLA-identical class I and II. Allo-HSCT utilizing a myeloablative regimen was performed in 7 patients. HSCT in the mode of increased intensity was used in 3 patients. The gene polymorphisms were analyzed by polymerase chain reaction in real time. In order to identify information markers analyzed gene polymorphism F2, F5, F7, F13, FGB, ITGA2, ITGB3, PAI-1, MTHFR: 665, MTHFR: 1286, MTR, MTRR. Chimerism was investigated at 28, 42, 56, 70, 100, 120 days after HSCT.
Results: Genetic differences were detected in 9 of the 10 donor-recipient pairs (from 1 to 7, median -5). The greatest number of distinctions obtained in the study of allelic variants of the gene FGB (7 out of 10 couples). In the analysis of gene MTHFR: 1286 differences were detected in 6 pairs; ITGA2, ITGB3, PAI-1- in 4 pairs, F13, MTHFR: 665, MTRR - in 3 pairs. For each pair were chosen polymorphisms that act as molecular markers allow to differentiate donor and recipient cells after HSCT. In three cases, the donors and recipients were homozygous for different alleles of one gene. In other cases, an identification mark considered polymorphism, in which one allele was common to both donor and recipient, the second - only for specific recipient. Complete donor chimerism was established in 7 patients on day 28 after HSCT.
Summary/Conclusions: Genetic polymorphisms associated with thrombophilia meet the basic requirements of the markers hematopoietic chimerism: analysis is based on the identification of regions of the genome that differ one nucleotide sequence; investigated two allelic variants of the gene; frequency of occurrence in the population of homo- and heterozygous for this is sufficient for the detection of genetic differences at 90% of donor-recipient pairs.

PB2198

IMMUNE RECONSTITUTION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA: THE EFFECT OF CLINICAL FETAURES

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Background: The reconstitution of different immune cell subsets after allogeneic stem cell transplantation (allo-SCT) occurs at different timelines. Anyway, majority of studies following allo-SCT focused on levels of the single cell line while recovery of the entire immune system was generally reviewed. Similarly, few reports addressed on the effect of clinical factors on the restoration of immunity and studies on bone marrow (BM) samples are lacking
Aims: Thus, we prospectively investigated changes in lymphocyte and dendritic subsets after allo-SCT in BM samples with respect to minimal residual disease (MRD), GvHD, infections and HSCT type.
Methods: BM samples from patients (n=25) with acute myeloid leukemia(AML) who underwent to allo-SCT were collected from january 2012 and december 2014 at specific time-points: +1, +3 and +6 months after transplant. Lymphocyte subpopulations (B cells, hematogones,T cells, helper/inducer T cells, cytotoxic/suppressor T cells, regulatory T cells (TREGs), natural killer (NK) cells) and dendritic cells (plasmocytoid and myeloid) were investigated using 6-color flow-cytometry. In order to assess differences in cell subsets between cases and BM healthy controls the Mann-Whitney test was applied. A p-value ≤ 0.05 was considered significant.Variation of cell subsets at + 1,+3, +6 months after transplant was evaluated using the Friedman test.Post-hoc pairwise comparisons with Bonferroni correction for multiple comparison were carried out.
Results:. Significant differences between patients and controls were found with respect to TREGs, T cells, helper/inducer T cells and NK cells. In particular, patients showed a significantly higher level of TREGs and NK and a significantly lower count of T- and helper/inducer T cells at +1 and +3 months from transplant. B cells and hematogones was significantly increased at six months from transplant while no differences between TREGs were found at this time point. Significant effect of timelines from transplant was found for B cells, regulatory T cells, T cells, helper/inducer T cells and cytotoxic/suppressor T cells. A statistically significant raise was found from +1 to +6 months for T cells and cytotoxic/suppressor T cells and from +1 to +3 months for helper/inducer T cells and cytotoxic/suppressor T cells. On the contrary, a statistically significance decrease was observed at any time for TREGs count. Univariate analysis showed a statistically significant association between cytotoxic/suppressor T cell count and the presence of infection (p=0,027) with patients having viral or bacterial infection showing higher counts. No differences were found for any other association.
Summary/Conclusions: In conclusion, immune reconstitution following allo-SCT on BM samples was similar to data reported from peripheral blood.Viral and bacterial infectionsplay an important role in increasing cytotoxic/suppressor T cells.

Thrombosis and vascular biology

PB2199

A SINGLE CENTRE EXPERIENCE MEASURING RIVAROXABAN LEVELS WITH A SPECIFIC ANTI XA ASSAY AND THE EFFECT ON THE STANDARD COAGULATION SCREEN

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Background: Direct oral anticoagulants drugs are now commonly used in clinical practice. Rivaroxaban inhibits free factor Xa and hence prothrombinase activity as well as clot bound Xa, thus effectively blocking thrombin generation. One main advantage over vitamin K antagonists is that blood coagulation monitoring is not necessary. However introducing a static change into a dynamic cascade always raises concern. The concentration may potentially need to be measured in certain clinical situations. These may include urgent surgery, perioperative management, thromboembolic events, bleeding events and suspected overdose. All routine coagulation screens performed in Northern Ireland are undertaken on a Sysmex Coagulaometer using the Innovin reagent. The Regional Specialty Coagulation Laboratory uses a Stago analyser with the STA Neoplastine CI+ reagent. The effect on the coagulation screen by rivaroxaban is known to vary between analysers making interpretation difficult.
Aims: To assess the effect of rivaroxaban on the current coagulation screen to help aid clinicians in making urgent decisions. To assess the variation of rivaroxaban concentrations between patients invivo rather than a controlled dilutional experiment.
Methods: Recording the dose and time from last drug ingestion we were able to measure the effect on the standard coagulation screen with both analysers. On the same samples the rivaroxaban concentration was measured with the specific anti-Xa rivaroxaban assay. Each recruited patient had their age, renal function and other co prescribed medications recorded to assess variation within these parameters. As a control, healthy volunteers on no anticoagulation had their rivaroxaban levels measured. The volunteers' rivaroxaban levels ranged from 15-21ug/l and therefore we assume no drug is present when levels fall into, and below, this range.
Results: The effect on the PT and APTT was variable between analysers. The PT, in line with previous publications, was more sensitive than the APTT at smaller drug concentrations. The Neoplastine CI plus reagent on the Stago analyser was more sensitive than the Innovin reagent to rivaroxaban concentrations. The Neoplastine CI PT was prolonged in all samples taken within 17 hours of rivaroxaban ingestion. Our results compare with University Hospital Hotel Dieu (Paris) which found peak concentrations up to 400ug/l and trough concentrations up to160ug/l. However our results demontrated more variation with some patients have a higher rivaroxaban level than expected 600ug/l. These patients were also taking verapamil. We noted the SPC advises rivaroxaban not to be used in patients who are receiving concomitant combined P-gp and moderate CYP3A4 inhibitors unless the potential benefit justifies the potential risk 2. There is a positive correlation between rivaroxaban concentration and Neoplastine CI PT reagent with prolongation up to a certain concentration and beyond this the effect on Neoplastine CI PT appears to plateau. The patients with a rivaroxaban concentration in excess of 600ug/l had a similar PT than those with a rivaroxaban concentration of 450ug/l.

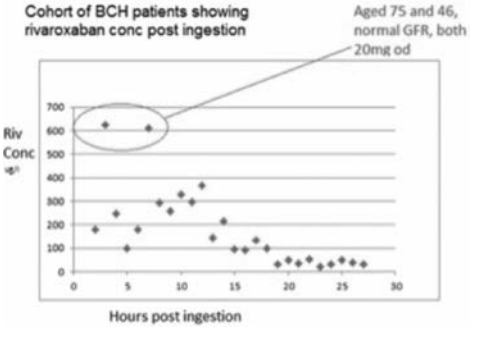

Figure 1.

Summary/Conclusions: The PT, provided it is sensitive to rivaroxaban, is a quick test and may be useful to ascertain rivaroxaban presence or not but given the plateau effect, not necessarily reassurance that rivaroxaban levels are excessive. Although smaller amounts of rivaroxaban (<100ug/l) may still be present when the PT is normal its clinical significance and its anticoagulant needs to be judged on a case by case basis. Rivaroxaban anti Xa assay will quantify the drug concentration however turnaround time is slower. There is significant variation in rivaroxaban when co prescribed with other medications utilising CYP3A4. Although the results demonstrated when used with an inhibitor the elevated rivaroxaban concentration may not necessary have addition anticoagulant effect this raises the concern when the opposite siituation arises. If a patient is found to have a clot while on rivaroxaban it may be appropriate to check the rivaroxaban specific anti Xa to ensure its therapeutic especially when patients have co prescribed CYP3A4 inducers.

PB2200

FREQUENCY AND MANAGEMENT OF VENOUS THROMBOSIS IN ADULT ACUTE LEUKEMIA PATIENTS AT A TERTIARY CARE HOSPITAL OF PAKISTAN

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Background: Solid tumors are well associated with thromboembolic complications, but the incidence of thrombosis is not widely studied in patients with acute leukemia and their management which is a great challenge. However this may be obscured by the significant morbidity and mortality due to other complications such as bleeding and infections.No established guidelines are present to treat these difficult patients. Case-controlled studies of patients with cancer revealed a fourfold increase in thromboembolic occurrence in acute leukemia, with about the same rate in acute myelogenous leukemia (AML) and in acute lymphocytic leukemia (ALL). Among patients with acute leukemia, thrombosis has the highest incidence in acute promyelocytic leukemia (APL) .Of interest, increased thromboembolic events take place even prior to the diagnosis of acute leukemia, similar to the situation seen in solid tumors, indicating that a prothrombotic state is present even at the earliest phase of leukemia. The use of central venous catheter and chemotherapeutic agents such as L-asparaginase and other medicine used in the treatment of hematological malignancies particularly steroids may play an important thrombogenic role.
Aims: To determine the frequency of venous thrombosis and treatment strategy in patients with acute leukemia at a tertiary care Hospital of Pakistan.
Methods: Retrospective, observational study of case charts of hospitalized patients with diagnosed case of acute leukemia at department of oncology Aga Khan University Hospital Karachi during the 18 months period (January 2014 to June 2015). Data was retrieved by using ICD 9 coding for acute leukemia patients. Investigations were obtained from electronic medical record system. Finally data was analyzed for frequencies and percentages by using SPSS version 19.
Results: Total of 107 patients presented during the study period. Among them 76 were males and 31 were females with median age ranges from 18 to 60 years. These patients were stratified into 2 major groups according to type of leukemia.63.5% patients were with Acute myeloid leukemia in which 4.7% patient developed venous thrombosis among them highest in APML 22.2% while 36.4% patients were with acute lymphocytic leukemia(ALL) in which 2.5% of patients developed venous thrombosis. Three patients were treated successfully with LMWH during their consolidation phase of chemotherapy.
Summary/Conclusions: Venous thrombosis in acute leukemia is not uncommon which can lead to fatal results if left untreated. Anticoagulation with intermittent use of LMWH for 3-6 months with close monitoring of platelet counts would be the appropriate option for treatment.

PB2201

CYTOMETRY OF HBA1C

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Background: In the field of routine diabetes testing, flow cytometry does not play an important role.
Aims: In an innovative effort we try to show that the application of flow cytometry of a widely accepted parameter as HbA1c can improve the understanding of the course of the disease and can be applied to other diseases as well.
Methods: In a new experimental assay, blood was diluted in an environment that removed the red cell membrane and precipitated hemoglobin. The particles of precipitated and condensate hemoglobin look similar to normal red cells in flow cytometry. The precipitation is irreversible and the particles in physiological conditions can be stained with a fluorescent conjugated monoclonal antibody against HbA1c. In an inhibition assay with hemoglobin in solution it was shown that the accessibility of the HbA1c antigen did not differ between soluble and condensate hemoglobin. Another property of the condensate particle in flow cytometry is that the side scatter of the particles reflects the hemoglobin content of the original red cell. The cellular hemoglobin content of samples that were analyzed on a Sysmex hematologic analyzer and the mean values of the side scatter of the same samples that were treated as above for flow cytometry showed good correlation.
Results: A good correlation was also found with conventional HbA1c measurements. From 120 diabetic patients, the HbA1c percentage values were obtained by taking the mean of three different methods as measured by a ref-

erence laboratory. The blood samples of these patients were also analyzed by cytometry and the quotients of the side scatter and the HbA1c staining reflecting the HbA1c percentage were compared with the HbA1c reference values. Frozen hemoglobin particles with a known HbA1c percentage were used as a reference. The novelty of this assay is that using flow cytometry, the HbA1c percentage can be measured cell by cell. This permits to see beyond the mean value of HbA1c percentage of a sample. Presenting a lower deformability, cells with the highest HbA1C concentration may play a crucial role toward microvasculature obstruction. To further assess the validity of the approach of red cell age measurement, we collected from 26 diabetic patients the booklets in which they had administrated several glucose values every day over a time-span of several months. At the moment of collection of the booklets, blood samples were taken and cytometry of HbA1c was performed. The glucose values of the booklets, showed considerable varieties during a day. Therefore a mean daily glucose was used. The comparison of the two sets of data showed a striking correlation.

Summary/Conclusions: Showing proof of principle of HbA1c cytometry and the possibility to read the memory of the blood, other parameters can be read. Within the assay cellular hemoglobin content can be read against time. Also the representation of cells of a certain age can be established. Both these parameters could be of interest for diseases like anemia.

PB2202

INHERITED AND AQUIRED THROMBOPHILLIAS IN WOMEN WITH RECCURENT PREGNANCY LOSS- 5 YEAR EXPERIENCE

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Background: Inherited thrombophilias are the leading cause of maternal thromboembolism and are associated with an increased risk of certain adversy pregnancy outcomes including second and third trimester fetall loss, abruptions, severe intrauterine growth restriction and early onset severe preeclampsia. The antiphospholipid antibody syndrome (APS) is an acquired autoimmune thrombophyllia in which vascular thrombosis and/or reccurent pregnancy losses occur in patients having laboratory evidence for antibodies againts phospholipids or phospholipid-binding protein cofactors in their blood. Pregnant women with these highly thrombogenic conditions are at very high risk for both thromboembolism and adverse pregnancy outcomes.

Aims: Aim was to determine the presense of inherited or acquired thrombophillias in women with reccurent pregnancy losses (RPL).

Methods: Women with RPL were tested for heterozygosity for the factor V Leiden and prothrombin G 20210A mutations, homo and heterozygosity in the type 1 plasminogen activator inhibitor gene (PAI-1) and the thermolabile variant of the methylentetrahydrofolate reductase gene (MTHFR). They were also tested for deficiencies of protein C, protein S and antithrombin, as well as for antiphospholipid antibodies-anti beta 2 glycoprotein I antibodies, anticardiolipin antibodies and lupus anticoagulant assays.

Results: From January 2011 till January 2016, 445 women were tested for the presense of inherited or acquired thrombophillias because of reccurent pregnancy loss or treatment of infertility. Median age of patients was 33.2 (19-46). 18 women (4%) was negative for both inherited or APS and others 427 (96%) were positive. Criteria for APS fullfilled 68 patinets (15.2%), 10 patients (2.2%) had only APS and 58 patients (13%) had APS with some of inherited thrombophillias. 246 patients (94.6%) were positive for one or more inherited thrombophillias. The most common inherited thrombophillia was PAI1 mutation, found in 313 women (70%).then MTHFR mutation found in 303 women (68%).Heterozygous mutation for Factor V Leiden was found in 45 women (10%) and heterozygous mutation for protrombin G 201210A in 16 women (3.6%). 173 women were positive for 1 inherited thrombophillia (38.9%), 222 for 2 (49.9%) and 21 women (4.7%) for 3 inherited thrombophillias. 105 patients (40.3%) with low molecular weight heparin plus aspirin (LMWH/ASA) or ASA alone had successfully pregnancy outcome-live birth.

Summary/Conclusions: Some form of thrombophillia-inherited or acquired was found in most of tested women with reccurent pregnancy loss (96%) and 273 patients (61.3%) had more then one thrombophillia. With adequate anticoagulant therapy patients with these conditions had chance for successfully pregnancy outcome.

PB2203

THROMBOLYTIC THERAPY USING UROKINASE FOR MANAGEMENT OF CVC IN HEMODIALYSIS: OUR EXPERIENCE

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Background: The need to use the CVC (central venous catheter) for hemodialysis is an occurrence in growth, related to the aging of the population on dialysis and the high prevalence of the disease and thrombotic angiosclerotica-uremic coaugulativa of the subject. The CVC is almost always a second best solution precisely in those subjects with situations of vascular compromise and/or coagulation disorder that does not allow the creation of the FAV (arteriovenous fistula). For this reason, the CVC meets very often malfunction situations for intraluminal thrombotic events. The malfunction of the CVC is directly related to the duration of use, for a higher probability of thrombotic affixing on the inner wall with the passage of time. However, some patients show that they are prepared to meet very early in the CVC occlusive events, probably due to altered Hemorheological situations, not always clearly defined. In the case of thrombotic occlusion of the CVC is sweeping the use of thrombolytic agents to try to ricanalizzarli. Among the various substances being used for the urokinase thrombolytic there is, even if the bleeding risk potentially related to its use has hampered the use on a large scale.

Aims: We conducted a retrospective case series of patients on hemodialysis with CVCs pertaining to a single dialysis center over the past 15 years, from 2001 to 2016, analyzing the effectiveness of the use of urokinase for "declotting" of CVC for hemodialysis and the manner of its use, in the search for better therapeutic efficacy with the lowest risk of adverse events.

Methods: We have analyzed 48 cases of patients with CVC, inserted in 35 patients, for whom it was deemed appropriate to proceed with declotting urokinase due to malfunction for thrombotic occlusion. Even in our series, despite the routine filling of the lumen of the catheter with heparin sodium or sodium citrate between a dialysis and the next, the thrombotic complication of the CVC was very common, affecting in time 27% of the CVC. Our usual therapeutic protocol for the declotting of the CVC with urokinase included the following steps 1) slow-fill with positive pressure of each lumen of the CVC with a solution of 25,000 IU/ml of urokinase, by reason of the priming volume indicated by the manufacturer of the CVC and reported in writing on the same; 2) wait/bus station 30 minutes, carefully avoiding the marketing of the drug circle; 3) the total intake of the luminal contents and a few ounces of blood; 4) at a high pressure irrigation with saline solution.

Results: In our experience, 75% (36/48) of cases of CVC occluded by thrombosis were already passed on successfully with urokinase. We detected in some cases the need for repeated short-term treatment of thrombolytic therapy with urokinase, in subjects in which the position of the tip and or the side holes of the catheter had assumed a decubitus position on the vessel wall in which the CVC was allocated. In these people, the success rate of the individual thrombolytic treatment resulted however very high.

Summary/Conclusions: In our series, the unblocking of the CVC for hemodialysis with thrombolytic therapy using urokinase has proved very effective, without being burdened by any adverse reactions and/or side effects related to treatment.

PB2204

THROMBOTIC COMPLICATIONS IN HEMATOLOGIC MALIGNANCIES

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Background: In patients with cancer there is an increased risk of thromboembolism, especially in certain types of solid tumors and hematologic malignancies. Occurrence of venous thromboembolism in patients with cancer is linked to a hypercoagulable state due to synthesis of pro-coagulants factors or local hypoxia. The risk of thrombotic complications increases during chemotherapy, hospitalization or surgery. Thrombosis is a frequent complication in acute leukemia as well, but its pathogenesis is not entirely known, being probably determined by a combination of factors related to the patient, disease and treatment regimens used.

Aims: To analyze the causes of thrombotic complications in patients with hematologic malignancies, excluding those with myeloproliferative neoplasms, multiple myeloma treated with immunomodulatory agents and acute promyelocytic leukemia, known to be associated with increased risk of thrombosis. Also, we excluded cases of catheter-related thrombosis.

Methods: We analyzed data of 41 patients with hematologic malignancies admitted in our institution during a period of 9 years which presented with at least one episode of thrombosis.

Results: There were 23 females and 18 males. The diagnosis distribution was: 4 cases of acute myeloid leukemia, 1 case of hairy cell leukemia, 1 case of acute lymphoblastic leukemia, 1 case of myelodysplastic syndrome, 2 cases of Hodgkin Lymphoma, 27 cases of Non-Hodgkin Lymphoma, 5 cases of multiple myeloma. The median age was 65 years (ranges from 37-81 years). After location of thrombosis, there were 37 cases of venous thrombosis, 4 cases of arterial thrombosis, 2 cases of DIC. Additional risk factors besides cancer were identified in 8 cases, including recent history of splenectomy, secondary thrombocytosis, phospholipidic syndrome, presence of clone of paroxysmal nocturnal hemoglobinuria, status post-autologous stem cell transplantation and chronic venous insufficiency. No additional risk factors were identified in the other patients. Inherited thrombophilia was not tested, but personal and family history was not suggestive for thrombophilia in the reported cases.

Summary/Conclusions: Thrombosis is the second cause of death and a major cause of morbidity in cancer patients, and improving antithrombotic prophylaxis

and treatment in this subset of patients may have important prognostic implications. An important issue is to identify patients at risk in order to establish proper prophylaxis. Score models were elaborated based on site of cancer, hemoglobin value, platelet and leukocyte count, and body mass index. However, in a subset of patients additional risk factors besides cancer could not be identified. Furthermore, in some patients thrombosis occur in the setting of severe thrombocytopenia. In this context, it could be important to identify some biological markers for thrombosis, such as the well known and used D-Dimer, and other studied markers, but not yet in clinical use, such as tissue factor, P-selectin or VEGF. We present an important lot of patients with hematologic malignancies and thrombosis, of note that most of these patients had no identifiable risk factors of thrombosis. Moreover some of these patients developed thrombosis in the setting of severe thrombocytopenia in the absence of DIC. We find important to identify biological markers useful in patients with cancer to predict thrombosis. We intend to screen patients with hematologic malignancies and thrombosis for D-Dimer, tissue factor and VEGF.

PB2205

TREATMENT AND OUTCOME OF PEDIATRIC CEREBRAL SINOVENOUS THROMBOSIS: A SINGLE CENTER EXPERIENCE

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Background: Pediatric cerebral sinevenous thrombosis(CVST) is a rare but potentially life threatining condition with a 3-12% reported mortality rate. Clinical findings mostly depend on the initial age at presentation, anatomic localization and extent of thrombosis, presence of infarct and /or symptomps of increased intracranial pressure, intracranial hemorrhage. Moreover, it was reported that most of those children with CVST have diffuse neurologic findings on admission.
Aims: We aim to analyse treatment and prognosis of childhood CVST.
Methods: In this study we retrospectively evaluate clinical presentation, underlying disorders, risk factors (genetic and acquired), anticoagulant treatments and prognosis of our 34 patients with CVST diagnosed between January 2009 to June 2015 at Hacettepe University Faculty of Medicine, Ihsan Doğramacı Children's Hospital.
Results: In this study we retrospectively evaluate clinical presentation, underlying disorders, risk factors (genetic and acquired), anticoagulant treatments and prognosis of our 34 patients with CVST diagnosed between January 2009 to June 2015 at Hacettepe University Faculty of Medicine, Ihsan Doğramacı Children's Hospital. There were 14 female and 20 male children with a median age of 7.3 years(1 month to 17,5 years). When we grouped initial ages 9 (26%) patients were under one year of age and 14(41%) patients were older than 10 years of age on admission. Initial complaints include headage(n=15;44%), seizures(n=5;15%), loss of consciousness (n=4;12%), focal neurological impairment (n=6;%17), diffuse neurological impairment (n=1; 3%) and pseudotumor cerebri (n=3; 9%). Risk factors included underlying disorders (Nephrotic syndrome, Acute leukemia, homocysteinemia, Behçet disorders, etc.) were found to be positive in 59% of the children and 15(44%) of them had hereditary prothrombotic risk factors (Heterozygous Factor V G1691A mutation (n=4), Homozygous Factor V G1691A mutation(n= 2), Heterozygous Prothrombin G20210A mutation(n=2), homozygous MTHFR C 677T mutation (n=4), PAI 4G/5G mutation(n=2)). Nearly all patients were given anticoagulant treatment and initial treatment were mostly included LMWH(73%) with an median 6 months (1 month to 9 months)of treatment period and 66% of them obtained partial or complete resolution of thrombosis with treatment. Mortality rate was 6%.
Summary/Conclusions: This study shows that CVST was more common under 10 years of age (60%)in our group however there was no increased rate of CVST for the neonatal period which was found to be 11% inconsistently lower than the reported rate(57%). Neonatal CVST occured more than half of the patient without a known prothrombotic or underlying risk factors.

PB2206

MANAGEMENT OF PEDIATRIC THROMBOSIS IN A SINGLE TERTIARY CARE MEDICAL CENTER

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Background: Venous and arterial thrombosis is increasingly encountered in the pediatric population.
Aims: The aim of this retrospective study was to summarize our experience regarding clinical characteristics, diagnosis and management of pediatric thrombosis in a single tertiary care medical center.
Methods: We reviewed clinical files of patients (pts) under 18 years old diagnosed of arterial thrombosis (AT) and/or venous thromboembolic (VTE) events in our institution between March 2006 and June 2015. Clinical data, thrombotic risk factors (TRF), thrombophilia test results, thrombosis location and therapy were analized.
Results: 49 AT and/or VTE events were diagnosed in 48 pts, median age at diagnosis was 1 year (0-17); 24 pts were infants (25% neonates) and 24 (50%) >1year; out of 48 pts, 29 were ♂ and 19 ♀. Clinical presentation: arterial isquemic stroke 19 (38.7%), lower extremities deep venous thrombosis (DVT) 13 (26.6%), cerebral venous sinus thrombosis 8 (16.3%), portal and inferior vena cava thrombosis 4 (8.2%), lower extremity arterial ischemia 2 (4.08%), purpura fulminans 1 (2.04%), subclavian vein thrombosis 1 (2.04%) and suprahepatic vein thrombosis 1 (2.04%). Age classification of pts was: 18 (37.5%) newborns: <1 month; 6 (12.5%) infants: <1 year, 12 (25%) children: 1-13 years and 12 (25%) adolescents: 14-17 years. According to the age classification the most common clinical presentations were arterial isquemic stroke in newborns 12 (66.6%); DVT 2 (33%) and portal/ inferior vena cava thrombosis 2 (33%) in infants; cerebral sinus thrombosis 4 (30%) in children and DVT 6 (50%) in adolescents. Adquired risk factors included systemic sepsis and oncologic diseases in 15 and 6 pts, 4 central venous catheter carriers, 3 congenital heart disease (CHD), 2 systemic sepsis + CHD, 4 artery malformations, 1 immobilization after ankle injury, 1 hormonal contraceptives therapy + sepsis, and 1 *perinatal hypoxic*-ischemic *encephalopathy*. The most important risk factor for thrombosis was systemic sepsis. In 12 cases no risk factors for thrombosis were found. Thrombophilia testing was performed in 36 cases (73.4%). Thrombophilic risk factors were found in 9 pts 25%: 2 prothrombin G20210A mutation, 1 prothrombin G20210A + MTHFR mutations, 1 R506Q mutation, 1 Protein S decreased, 1 lupus anticoagulant, 1 Protein S decreased + lupus anticoagulant and 1 antiphospholipid-like syndrome. Anticoagulation therapy was initiated in 3 pts with unfractioned heparin to achieve therapeutic aPTT and 30 pts received enoxaparin at a mean dose of 1 mg/kg/12 h to achieve anti-Xa therapeutic level. Median duration of anticoagulation was 3 months. Median number of anti-Xa level was 6.96 (1-22), and range controls 4.61 (0-19). Intraarterial fibrinolytic therapy followed by enoxaparin was administered in 1 pt with stroke, 4 pts with stroke were antiaggregated with AAS. No anticoagulation/ antiaggregation was given to 15 pts. Overall recurrence rate of thrombosis was 6.2% (3/48); mortality rate 4.1% (2/48). Mortality was related to underlying diseases: 1 pt perinatal hypoxic encephalopathy and 1 pt tumor progression. Gastrointestinal bleeding linked to anticoagulation was observed in 1 pt.
Summary/Conclusions: The distribution of thrombosis was similar to already published. Acquired thrombotic risk factors were associated with both AT and VTE. We must be cautious about interpretation of thrombophilia test because the haemostatic system is still developing. Adjustment in enoxaparin dosage to target anti-Xa level is a valuable tool for anticoagulant therapy in pediatric thrombosis.

PB2207

CEREBRAL STROKE IN A CHILD WITH CONGENITAL DYSERYTHROPOIETIC ANEMIA TYPE II

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Background: Congenital Dyserythropoietic Anemia type II (CDA II) belongs to a subtype of bone marrow failure syndromes characterized by monolineage involvement and typical morphological abnormalities in erythroid precursor cells resulting with different degree of hyporegenerative anemia. Moreover reticulocytosis, which is not corresponding to the degree of anemia (ineffective erythropoiesis) with jaundice and splenomegaly are major diagnostic criteria. Causative gene is located at *SEC23B*. Although stroke among children is rare, it can cause significant morbidity and mortality.
Aims: Herein we present three years old boy who had diagnosed with CDA II and eventually experienced stoke.
Methods: A newborn male baby referred to us with complaints of icterus and anemia. From his medical history it was learned that his parents were consanguineous. Initial physical examination showed pallor, icterus, hepatosplenomegaly and cryptorchidism. Laboratory finding showed anemia, reticulocytosis, hyperbilurinemia. Bone marrow aspiration showed morphological abnormalities of the erythroblasts. The genetic studies showed double heterozygous mutations in SEC23B.Regular transfusions were started. At age of four he admitted to emergency department with complaints of aphasia and physical examination showed facial paralysis. The MRI revealed acute infarcts at left frontal lobe and digital subtraction angiograph showed occlusion of left internal carotid artery suggestive of fibromusculer dysplasia. Enoxiparine was started. And he is under outpatient control without any neurological sequel.
Results: Pediatric stroke is an important cause of long-term disability, In our recent manuscript we observed seizures in 53%, long-term significant neurological deficits in 67%, and death in 14%. Risk factors for stroke in childhood are different from those traditionally observed in adults. Over 100 risk factors for stroke in children have been reported, but in up to one third of patients, no cause is identified, and these cases are classified as idiopathic. In literature search we did not encounter any individual with CDA II who had stroke.
Summary/Conclusions: To best of our knowledge this case presentation reports an interesting combination of CDA and stroke. This combination can

be coincidental but clinicians who manage patients with CDA must be vigilant about the neurological complications including stroke.

PB2208

CROATIAN EXPERIENCE OF THROMBOPHILIA TESTING-A HUGE VARIETY OF REFERRING PRIMARY PHYSICIANS, DIFFERENT INDICATIONS, AND PATIENTS' CHARACTERISTICS

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Background: Thrombophilia is a predisposition to thrombosis and is not disease per se, but exposes carriers to increased risks for thrombosis compared with non-carriers. Some earlier studies showed also association between thrombophilia and adverse pregnancy outcomes. However, it is still a matter of intense debate who should undergo comprehensive and expensive laboratory testing for thrombophilia and its usefulness in clinical practice.
Aims: The aim of this work was to analyze indications, patients' characteristics and specializations of referring primary physicians who requested thrombophilia testing with hematology consultation.
Methods: We analyzed patients referred for thrombophilia testing with hematology consultation to the Coagulation Outpatient Unit of the Division of Hematology, Department of Internal Medicine, University Hospital Center Zagreb, Zagreb, Croatia, from 2013-2014. Study was approved by the Medical ethic committee.
Results: There were 351 new patients sent from different specialists for thrombophilia work-up, median age 38 years (range 18-82 years), and among them majority were female patients (68.1%). The most frequent specialists who requested testing of the patients were gynecologists (30.2%), followed by internal medicine specialists with different subspecialisations (20.5%), cardiologists (11.96%), hematologists from other institutions (9.4%), family physicians (9.1%), neurologists (7.7%), vascular surgeons (5.1%), ophthalmologists (1.7%), and others. The most common indication for thrombophilia testing was personal history of venous thromboembolism (VTE) (deep vein thrombosis, pulmonary embolism or both) in 50.9% of analyzed patients. Among patients who had VTE, 64.4% had idiopathic VTE and 18.9% had recurrent VTE. The most common causes of secondary VTE were trauma, surgery and immobilization (34.3% of secondary VTE). The second most common indication for thrombophilia testing were gynecology/obstetric reasons (recurrent or even the first time miscarriages, pregnancy complications (intrauterine fetal growth restriction, preeclampsia, abruption placentae), and sterility) among 28.5% of all patients (41.8% of female patients). Other indications for request for thrombophilia testing with hematology consultation were stroke (5.7%), varicose veins (5.4%), recurrent thrombophlebitis (5.1%), myocardial infarction (1.9%), retinal vein thrombosis (1.7%), and other. More than 1 reason for testing had 14.5% of patients, and 25.15% of patients had positive family history for VTE. In addition to request for laboratory hypercoagulable work-up, 27.9% of the patients confirmed that were active smokers.
Summary/Conclusions: Although in general majority of patients sent for thrombophilia testing with hematology consultation were younger people with previously unprovoked VTE, there was huge variety of indications and different kind of specializations of primary physicians who requested testing. Gynecologists as referring physicians and gynecology/obstetric reasons were especially frequent, what might change in the following years with new data about clinical indications and usefulness of thrombophilia testing.

PB2209

ANTIPHOSPHOLIPID ANTIBODIES, PREGNANCY AND SPONTANEOUS MISCARRIAGES. EXPERIENCE FROM A RECENTLY OPENED CENTER

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Background: Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the appearance of arterial or venous thrombotic events and/or obstetric complications in the presence of antiphospholipid antibodies (aPL). These include: lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and anti-beta2 glycoprotein I antibodies (aβ2GPI). APLs are found in up to 40% of women who suffer recurrent miscarriages and/or fetal deaths. LA has been associated with preeclampsia, intrauterine growth retardation (IUGR) and fetal loss. ACA are linked to preeclampsia and late fetal loss. The aβ2GPI to preeclampsia, IUGR and late fetal loss. The presence of any of them in pregnancy is a predictor of adverse outcome and should be considered a high risk factor for venous thromboembolism (VTE). The management of these patients could be controversial, especially in cases with moderate-high titles of LA and/or aCL with neither thrombotic nor obstetric signs, although this approach has not been tested in prospective clinical trials. The combined therapy of acetylsalicylic acid (ASA) and low molecular weight heparin (LMWH) has shown efficacy and birth rates close to 80%, with less toxicity than other schemes including corticosteroids and/or immunoglobulins
Aims: determine the prevalence and clinical significance of the aPL in women with recurrent miscarriages and obstetric complications
Methods: Retrospective analysis of patients with a previous history of recurrent miscarriages or fetal deaths, between 2009 and 2015, referred to the hematology consultation in which the presence of aPL was confirmed in two separate determinations at least 12 weeks apart
Results: The presence of aPL was confirmed in 20 patients and 13 were diagnosed according to the Sydney classification criteria for APS. The accompanying clinical manifestations in those patients with positive aPL were: recurrent miscarriages in 18/20 (90%), median of 3 abortions <12 weeks; VTE disease 2/20 (10%); 1 patient with a history of fetal loss at 24 weeks; 1case of eclampsia in previous pregnancy and 1 case of IUGR (See table). The antibody most frequently involved was aCL, present in 13/20 (65%). The LA was observed in 9/20 (45%), including this group, the only 2 cases with VTE in the series and the only case with IUGR. 25% (5/20) of the patients had more than one positive aPL, one of these patients had having a previous history of pulmonary embolism (PE) before the diagnosis of APS. After the diagnosis of APS 18/20 cases (90%) had new pregnancies including 15 full-term newborns and 3 ongoing pregnancies. All these patients were treated with ASA and LMWH at prophylactic doses, with monitoring levels of anti-Xa. Once treatment was established the only complication was one pregnancy loss at weeks eight of gestation in an obese patient who did not reach levels of anti-Xa at prophylactic range, but later had a successful pregnancy by increasing the dose of LMWH. There were no fetal deaths or new cases of VTE or hemorrhagic complications.

Table 1.

Table. Patient's Characteristics

Patients	Age	aPL type	Previous miscarriage s	Thrombosis	Associated thrombophilia	Others
1	36	LA+, aCL IgG+	4			
2	33	aCL IgG, IgM+	3			Autoimmune hepatitis
3	41	aCL IgM+	3			
4	46	aCL IgG+	3			
5	35	aCL IgM+	1			
6	41	LA+, aβ2GPI IgG+	2			IUGR*
7	38	aCL IgG+	1			
8	32	LA+,aCL IgM+	2			
9	38	LA+, aCL IgG+	1	PE	Prothrombin 20210A heterozygous gene	
10	28	LA+	3			
11	31	aCL IgG+IgM+	3			
12	40	aβ2GPI IgG+	1		Suspected Protein S deficit	ANAS+, anti SS-, anti Fib
13	46	aCL IgG+IgM+	6			
14	34	LA+	4			Gestational diabetes. Obesity
15	38	aCL IgG+, IgM+	0			Eclampsia in previous pregnancy (32 weeks preterm newborn)
16	26	LA+	0	Postpartum DVT		
17	35	LA+	3			
18	36	aβ2GPI IgM+	2			
19	45	LA+,aCL IgM+	2			
20	40	aCL IgG+	2			

*presented when the patient was evaluated by hematology; treatment was initiated and it was resolved before the end of the pregnancy.

Summary/Conclusions: Combination therapy with ASA and LMWH is safe and effective in the secondary prevention of obstetric complications in patients with APS. As in other series, our study confirms that more than one positive aPL determination and LA positivity are associated with an increase of the number and severity of complications in patients with APS. Further studies to improve the current evidence for the management of these patients are needed.

PB2210

INFLUENCE ADRENOCORTICOTROPIC HORMONE ANALOG SEMAX ON FUNCTIONAL ACTIVITY OF BLOOD CELLS DURING IMMOBILIZATION STRESS

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Background: Bokgraund It is known that many disease states are associated with impaired red blood cell membrane. It is the activity of these blood cells associated normal microcirculation. At present, special attention is given to researchers semax neuropeptide derived from pituitary adrenocorticotropic hormone. The peptide penetrates the blood-brain barrier and has an impact on a number of functions of the central nervous system. Experiments have shown that peptide can affect blood cells, particularly red blood cells. The red blood cells increase the biochemical reactivity and functional only activated platelets. Change erythrocytes platelet biochemistry is very important for understanding the interaction of blood cells in hemostasis. It was found that the immobilization of the animal's body are also changes of erythrocyte membrane.
Aims: The aim of this work was to determine the effect of Semax on the functional activity of red blood cells and their interaction with platelets and the influence of these processes immobilization stress.
Methods: To reproduce the experimental stress reaction used immobilization

stress by fixing the rats on their backs for 60 minutes. In *in vitro* experiments semax in the concentration range 10-1 - 10-10 M is added to a pool of platelet-rich plasma (PRP), or PPP and the mixture washed erythrocytes (e) and change in aggregation was determined by the action of ADP. In experiments *in vivo* semaks administered intravenously prior to or after the immobilization was determined and osmotic fragility of erythrocytes and the platelet aggregation in normal and by animals immobilized.
Results: *in vitro* experiments have shown that, when added to semax pool PRP concentration of 10-10M only caused a significant enhancement of platelet aggregation. Adding PRP mixture of washed red blood cells significantly increased platelet aggregation. When added to the mixture PRP + erythrocyte as a peptide, and directivity of physiological saline retained reactions. *in vivo* experiments demonstrated that intravenous administration of semax at a dose of 1 mg/ml in a physiological standard had no significant effect on platelet aggregation. In the study of changes in the wholesale electricity market it has shown that semaks increases resistance of the membrane of red blood cells, reducing the percentage of haemolysed cells. The prophylactic administration of semax as well as the administration of the peptide on the background of immobilization stress significantly increased the resistance of erythrocytes to hemolysis.
Summary/Conclusions:. Our studies have shown that neuropeptide semaks, on the one hand, no significant effect on hemostasis in a physiological condition, and with another - increased stability of the erythrocyte membrane to the stressor exposure.

PB2211

A CASE REPORT OF FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS WITH CNS DEMYELINATION COMPLICATED WITH THROMBOSIS
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Background: Hemophagocytic lymphohistiocytosis (HLH) is a fatal disease affecting infants and very young children, including high fever, hepatosplenomegaly and pancytopenia. Hemophagocytic syndrome may be spontaneous or secondary to infection, malignancy or autoimmune disease, and mechanisms involved are poorly understood. The main histopathologic feature is increased proliferation and activation of macrophages with hemophagocytic lymphohistiocytosis throughout the reticuloendothelial system.
Aims: We present a case with cranial involvement of HLH showing diffuse infiltration of white matter complicated with intracranial thrombosis.
Methods: A 5 year-old girl with fever, and pancytopenia was referred to our hematology unit. Also she had a history of recurrent infections. Her parents were consanguine. Lymphadenopathy, and hepatosplenomegaly were detected in physical examination. Ultrasound examination displayed hepatosplenomegaly and intraabdominal free fluid. Hemophagocytic lymphohistiocytosis was revealed on bone marrow aspiration biopsy. Anomaly in NK and T lymphocyte cytotoxicity and degranulation tests was determined. İn genetic analysis, syntaxin gene mutation was depicted. Immunosuppressive therapy was performed to the patient, diagnosed with familial HLH. Brain MR imaging was performed because of the suspicion of cranial involvement. On MRI diffuse hyperintense signal changes of cerebral white matter on T2-W and T2 FLAIR images, showing demiyelination were detected. There wasn't any mass effect, contrast enhancement and restricted diffusion on MRI. A repeated brain MR performed a month after the first cranial imaging, showed an acute infarct involving left temporooccipital region. Follow up images showed that the infarct was disappeared but white matter lesions was stable on the brain MR imagines. The cerebral white matter lesions were stable but hyperintense signal changes were appeared in cerebellar white matter, accepted as progression. She was died in despite of immunosuppressive therapy.
Results: Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of pathologic immune activation, in association with a variety of triggers and is prominently associated with cytopenias and combination of clinical signs and symptoms of extreme inflammation. The histopathologic findings of HLH in pediatric patients with cranial involvement is various. The common CNS involvement of HLH is leptomeningeal infiltration of lymphocytes and histiocytes/macrophages with a sterile CSF lymphocytosis. If parenchymal involvement occurs perivascular infiltrations are seen. In more severe cases, demiyelination and tissue necrosis of the cerebral white matter may be seen. The previously reported MR findings of HLH with cranial involvement, include diffuse leptomeningeal and perivascular enhancement showing infiltrations of histiocytes and lymphocytes, T2W hyperintense white matter lesions on cerebrum, cerebellum and spine, parenchymal necrotic lesions with ring enhancement and diffuse atrophy of the cerebrum and cerebellum. In our patient we displayed diffuse white matter lesions in cerebrum and cerebellum showing demiyelination on T2W MR sequences. There wasn't any contrast enhancement or restricted diffusion. The spine was normal.
Summary/Conclusions: The HLH treatment is an induction therapy including steroid amd chemotheuropetics. Without therapy, survival of patients with HLH is very short, especially in familial form and cranial involvement. CNS involvement may occur at the beginning or during the treatment. Patients with CNS involvement should be treated with intrathecal agents. Depiction of the cranial involvement, is important for patient's survival and treatment. All patients with HLH should had brain MRI, even if asymptomatic.

PB2212

VENOUS THROMBOEMBOLISM IN WOMEN USING HORMONAL CONTRACEPTION. FINDINGS FROM A SINGLE CENTER
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Background: Venous thrombosis including deep-vein thrombosis (DVT) and pulmonary embolism (PE) is considered a multifactorial disease associated with genetic and acquired risk factors. In women of reproductive age, the main cause of venous thromboembolic disease (VTD) is the hormonal contraception. However, other risk factors interact to produce VTD.
Aims: Identifying the risk factors for DVT and PE, may intervene in risk situations and limit the occurrence of VTD with its potential morbidity and mortality.
Methods: We reviewed the characteristics of our series of 103 women with objectively confirmed VTD associated with hormonal contraception. We analyzed its clinical data and thrombophilia studies which were performed in more than 80% of them and included anticoagulant proteins, genetic tests, antiphospholipid antibodies and factor VIII level. Statistical analysis was performed with SPSS 18.0 software. bidi-font-size:12.0pt;font-family:Calibri;mso-ansi-language:EN'>.
Results: The mean age of our patients was 29±7.4 years old. A high percent of them (46%) had a body mass index >25 Kg/ m². The most important clinical risk factors for venous thromboembolism were inmmobility (31%), family history (27%) and polycystic syndrome (9.8%). The combined oral contraceptive including antiandrogen and third generation progestogen were the most frecuently used for our patients. Thrombophilia test revealed factor V Leiden in 13%, prothrombin mutation in 20%, antiphospholipid syndrome in 3%, protein C deficiency in 0%, protein S deficiency in 2.4%, antithrombin deficiency in 0% and increased factor VIII (>195%) in 18.7%.

Table 1.

Patients, N	103
Clinical characteristics	
Mean age (years ± SD)	29 ± 7.4
Body mass index (Kg/m2 ± SD)	25.8 ± 5.9
Body mass index >25 Kg/m²	25 (46.3%)
Risk factors for venous thromboembolism	
Immobility	29 (30.9%)
Family history	23 (27.4%)
Polycystic ovary syndrome	9 (9.8%)
Type of combined oral contraceptive	
Including antiandrogen	27 (52.9%)
Including second generation progestogen	3 (5.9%)
Including third generation progestogen	21 (41.2%)
Venous thromboembolism characteristics	
Pulmonary embolism	45 (44.6%)
Proximal deep vein thrombosis	53 (52.5%)
Upper-extremity deep vein thrombosis	3 (3%)
Thrombophilia tests	
Factor V Leiden	11 (12.9%)
Prothrombin mutation	18 (20.5%)
Antiphospholipid syndrome	3 (2.9%)
Protein C deficiency	0 (0%)
Protein S deficiency	2 (2.4%)
Antithrombin deficiency	0 (0%)
Increased factor VIII (>195%)	14 (18.7%)

Summary/Conclusions: Contraceptive use remains the most important risk factor for VTD in women of reproductive age. We observe additional risk factors such as obesity, immobilization, polycystic ovary syndrome or family history of VTD and certain thrombophilic defects. G20210A prothrombin and G1691A factor V Leiden mutations as well as elevated factor VIII (which has been associated to blood group non O) are frequent in women at a fertile age and should be part of thrombophilia study. Defining high risk patients may improve interventions in risk situations (e.g. administering antithrombotic prophylaxis or discontinuing contraceptive use after lower limb trauma) in order to prevent VTD. Finally, the increased thrombotic risk of antiandrogen and third generation contraceptives should be also taken into account.

PB2213

THE RICK OF THROMBOEMBOLISM IN SURGICAL AND ONCOLOGICAL PATIENTS WITH ANTITHROMBIN III, PROTEIN S AND PROTEIN C DEFICIENCY
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Background: Thrombophilia is a condition where the blood has an increased tendency to form clots. Blood clots can cause problems such as deep vein thrombosis (DVT) or pulmonary embolism. Natural coagulation inhibitors in hemostasis are Antithrombin III (ATIII), Protein S (PS) and Protein C (PC). Congenital AT III deficiency is an autosomal dominant disorder in which an individual inherits one copy which encodes AT III. This condition leads to increased risk of venous and arterial thrombosis. This form is most commonly diagnosed during childhood after a child has had a thrombotic event. Severe congenital AT III deficiency, in which the individual inherits 2 defective genes, is a rare autosomal recessive condition associated with increased thrombogenesis, typically noted in the neonatal period or early infancy. This condition is rarely compatible with life. Acquired AT III deficiency is a deficiency of antithrombin primarily due to consumption, such as disseminated intravascular coagulation (DIC). Protein S deficiency may be hereditary or acquired; the latter is usually due to hepatic diseases or a vitamin K deficiency. Protein S deficiency usually manifests clinically as venous thromboembolism (VTE). Protein C deficiency is a congenital or acquired condition that leads to increased risk for thrombosis. Congenital PC deficiency is one of several inherited thrombophilias, which are a heterogeneous group of genetic disorders associated with an elevated risk of venous thromboembolism.
Aims: The aim of this study is to measure AT III, P S and P C in patients from oncology department, urology department and the department of obstetrics and gynecology.
Methods: The study took place of observation at the general hospital of Patras (Greece) and included a total of 280 patients (male and female). The blood of patients was examined and the ATIII, P S and P C values were recorded. AT III measurement is a chromogenic determination based on an amydolitic method. Moreover the control of P C include the study of the functionality and antigenicity with chromogenic method. Also, the control of P S is based on the study of the functionality and the antigenicity of free P S (batch method).
Results: We studied 280 patients. From these patients, 200 were surgery patients from gynecological and urological department and 80 were patients from oncology department (table 1). Results for surgical patients were: AT III deficiency 4 patients (2%), P C deficiency 2 patients (1%) and P S deficiency 1 patient (0,5%). Furthermore, results for oncological patients were: AT III deficiency 3 patients (3.75%), P C deficiency 2 patients (2,5%) and P S deficiency 1 patient (1,25%).

Table 1.

Total patients		AT III deficiency	P C deficiency	P S deficiency
	110 female	3(2,7%)	2(2%)	0(0%)
200 surgical				
	90 male	1(1%)	0(0%)	1(1%)
	47 female	2(4%)	1(2,1%)	1(2,1%)
80 oncological				
	33 male	1(3%)	1(3%)	0(0%)

Summary/Conclusions: It is a fact that, either oncological or surgical patients are associated with a number of complications, mainly thrombosis. In addition deficiency in a classic coagulation factor is a high rick of venous thrombosis. The obvious conclusion to be drawn is that, measurement of AT III, P S and P C should be performed. Thus we should evaluate the impact of VTD or pulmonary embolism on the survival of these patients.

PB2214

ANTICOAGULANT TREATMENT WITH APIXABAN IN INHERITED ANTITHROMBIN DEFICIENCY

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Background: Apixaban, a direct inhibitor of activated factor X, has at least the same efficacy and safety as vitamin K antagonists (VKA) for secundary prevention of recurrent venous thrombosis in adults, but no data in patients with inherited Antithrombin Deficiency are available.
Aims: Evaluate apixaban as a valid anticoagulation alternative in adults patients with inherited Antithrombin Deficiency and difficulty for treatment with VKA.
Methods: We report a case of a 49-year-old man with Crohn disease, on long-term anticoagulation with low molecular weight heparin (LMWH) by recurrent episodes of venous thrombosis due to inherited Antithrombin Deficiency. Due with the difficulty for treatment with VKA because his severe Crohn disease, it was decided to maintain anticoagulation with intermedie doses of LMWH (therapeutic doses were associated with rectal bleeding). To obviate LMWH-associated osteoporosis, the use of calcium and vitamin D supplementation, and weight-bearing exercise were recommended. Proximal femur bone and lumbar spine density measurements were taken at baseline and each 6 months. Two years later, significant osteopenia was observed at the proximal femur bone, so heparin was replaced by 2.5mg/12h apixaban. Nine months later, he has not suffered thrombotic recurrence neither bleeding complications, but there were not any changes in bone densitometry.
Results: This is the first description of the use of apixaban in secundary prevention of recurrent venous thrombosis induced by Inherited Antithrombin Deficiency. Our patient on long-term anticoagulation with apixaban has not experienced bleeding or thrombotic complications months after transitioning to apixaban, but osteopenia has stayed without changes.
Summary/Conclusions: We believe that apixaban may be considered as a valid anticoagulant alternative in adults with inherited Antithrombin Deficiency and difficulty for treatment with VKA, although until results of prospective randomised trials are available, we recommend caution in using apixaban in patients with inherited thrombophilia. However, LMWH-associated osteopenia does not seem to improve after transitioning to apixaban.

PB2215

RELATIONSHIP BETWEEN TYPE AND INTENSITY OF ANTICOAGULANT THERAPY AND RECURRENCE OF ARTERIAL THROMBOSIS ON ANTIPOSPHOLIPID SYNDROME

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Background: The optimal type and intensity of anticoagulant therapy for the treatment of Antipospholipid Syndrome (APS) remain controversial issues, particularly for arterial thrombosis.
Aims: We investigated if there are difference between the development of recurrence of arterial thrombosis on APS taking anticoagulant therapy with vitamin K antagonist (VKA), antiplatelet therapy with ASA, or both VKA and ASA.
Methods: Retrospective we investigated the development of recurrence arterial thrombosis in 23 patients consecutive diagnosed in our hospital of APS with previous arterial thrombosis, from 1 February 2000 to 31 December of 2015. The patients ages were between 25 to 88, gender 39.1% male and 60.9%. Patients were treated well with ASA 100 or 300 mg either VKA for objective 2.5 INR, adding or ASA 100 mg depending on the thrombotic risk (depending on the location of thrombosis and the presence of thrombophilia and established cardiovascular risk factors). 10 patients present Stroke, including Cryptogenic Ischemic Stroke, 6 present Transient Ischemic Attack, 4 Myocardial Infarction, and 3 other periferical arterial thrombosis (ocular, laberintical and popliteal). Of this 23 patients, suffered recurrence arterial thrombosis 4 of 12 were taking AAS, 4 of 8 patients taking VKA, and 1 of 3 taking VKA and ASA. The statistical analysis was performed with Pearson test realized by JMP 9.
Results: We not found statistically significant difference between the APS patients taking VKA, ASA or VKA and AAS and the development of recurrence arterial thrombosis (OR 0.12, IC 95% -0.21 -0.46, p=0.73).
Summary/Conclusions: Although we found more patients with APS development recurrence arterial thrombosis taking AAS than VKA, we cannot establish an association between recurrence of arterial thrombosis and treatment without VKA. The location of thrombosis and the presence of classical cardiovascular risk factors do not seem to be good predictors of thrombotic recurrence. Future studies that delineate thrombotic risk in APS and evaluate current and novel anticoagulants as well as non anticoagulant therapies are required.

Transfusion medicine

PB2216

HOME BLOOD TRANSFUSION, A SAFETY OPTION IN SPAIN

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Background: The terms "Home medical care" generically designate a wide range of support services for palliative patients and people with disabilities. The designation includes everything, to improve quality of life for both the patient and the family. Quite often chronic and terminally ill patients who are being treated with Domiciliary Hospitalization usually require a blood transfusion. Commonly the form of action is to transfer the patient to the hospital. In many cases, this transfer can be uncomfortable and painful for the patient and implies costs of medical transport or transfer costs assumed by the patient.
Aims: San Rafael Hospital in Granada, Spain provides a Home medical service for chronic patients and palliative patients. A recent initiative of the Haematology Department of the Hospital is to offer home transfusions which aims to reduce the demand for emergency services and the need for hospital admission. To carry out this activity we must emphasize that all blood transfusion must ensure the patient safety, always carried out by qualified personnel of Home Hospitalization units (HHU).
Methods: San Rafael Hospital has a written a standard operating procedure, reviewed by the hematologist and the personal of the Medical Blood Bank. Before performing the blood transfusion, the patient (or the person authorized to sign Relation to Patient) has signed Informed Consent Form.
Results:, 100% of home transfusions in our Center have signed informed consent. A copy is recorded in the patient's history. Before the blood transfusion, the patient is visited by the HHU which is composed by a Physician and a qualified nurse, which has verified that the place where it is going to be performed the activity, has the minimum requirements. Patients who have requested this service had oncological diseases and are palliative patients. 88% transfusions of the total had been red blood cells and 11% platelet units. No reaction has been registered. After transfusion, the doctor UHD had visit the patient to assess the improvement and to make the following diagnostic tests.
Summary/Conclusions: The Home blood transfusion is an accessible and safety alternative for chronic patients and palliative patients in Granada Spain that decrease associated costs, reduce the hospital admissions and demand on acute services.

PB2217

EVALUATING THE EVALUATORS. EXPERIENCE OF A CENTER AND STATE OF ACCREDITATION IN TRANSFUSION MEDICINE IN ANDALUSIA

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Background: In transfusion medicine implementing a quality management system certified by an external entity,allows the development of a safe and efficient activity, while contributing to the involvement of all staff to improving a continuous quality system. In Spain there are two entities accredited byENAC for certification in transfusion: Transfusion Accreditation Committee (CAT) and the Agency for Health Quality of Andalusia (ACSA), although the latter with a specific program limited to transfusion centers
Aims: To describe the situation related to quality management system in transfusion medicine in Andalusia, a region in the south of Spain.
Methods: On the basis of data published by the CAT and ACSA we exposed the number of certified centers in Andalusia compared to national data. The experience of the local hospital was also evaluated Baza as externally certified center.
Results: 1. Although only half transfusion centers are certified by CAT, 100% of them are validated by the ACSA specific program. 2. 34% of centers accredited in Spain are accredited in Andalusia. 3. The relationship with the referral hospital and transfusion center seems to be the most important predisposing factor for a certification program. With respect to the certification program in the form of management is notfully adapted to "little" hospital, because if it's true that there is a hospital responsible for haemovigilance, hemotherapy isn't an exclusive dedication for him. In the aspect of non-conformities, the effort in a program of continuous improvement quality is very significant. Comparing our data center initial certification (2010) in which the 3rd edition of the standards was used, with reaccreditation (2014) which use the 4th. Table 2 shows comparative nonconformity according to the specific field deviations observed: it is noteworthy that the percentage remains the same, the most frequent on the requirements of quality control system.
Summary/Conclusions: 1. The implementation of a management system certified qualityby an external entity is a clear need in all transfusional centers involved, although the percentage of certified centers in Andalusia is less than 50%, representing a 34% of the certified centers nationwide. 2. Certification of CAT hospital is the only feasible option, but its adaptation to community hospitals is so complicated which is evident in the low number of certified centers. 3. Continuous quality improvement represents an additional workload sometimes unacceptable, it would be required a limited version adapted to district centers.

Table 1. Transfusion centers and hospital of public national health service in Andalusia, classified in provinces. Between parentheses certified centers.

	Huelva	Sevilla	Córdoba	Cádiz	Málaga	Jaén	Granada	Almería	TOTAL
Transfusion Centers	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	1(1)	8(8)
Regional Hospitals	1	2(2)	1(1)		1(1)		1(1)		6(5)
Speciality hospitals		1(1)		4(2)	2(1)	1		1	9(4)
Community hospitals	1	2(1)	3	1(1)	3(1)	3	2(2)	2	17(5)

Table 2. Evaluation of non-compliance related to, in two consecutives external audits.

	2010 AUDIT	2014 AUDIT	TOTAL
Quality control system	5	8	13
Equipment control	4	5	9
Reactive control	1	1	2
Transfusion procedures	4	6	10
Registers	4	3	7
Hemosurveillance	-	1	1
Comments of improvement	2	2	4
TOTAL	20	26	46

PB2218

ERYTHROCYTE'S GENOMIC IN RUSSIAN MULTITRANSFUSED HEMATOLOGICAL PATIENTS

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Background: The patients suffering with ineffective hematopoiesis and peripheral cytopenias need frequent RBC transfusions. There are minimum two populations of red blood cells (RBC) in transfused within the four previous month patients-own and donor's. This situation is called posttransfusion chimerism and serological blood typing becomes unreliable. It's known about the immunogenicity of such blood group antigens as A, B, D, c, C, E. e in antigen-negative patients. DNA analysis is available for clinical use in various situations, especially in identification of blood group antigens in multitransfused hematological patients.
Aims: true blood group identification in multitransfused hematological patients
Methods: The blood samples drawn into citrate, EDTA anticoagulant were serotyped for RhD, Rh C/c and RhE/e antigens by anti-D, anti-C, anti-C^w, anti-c, anti-E and anti-e monoclonal antibodies (Moabs) (Hematolog, Russia). Hemagglutination typing in gel columns ID-Cards "DiaClon ABO/D+Reverse Grouping" and "DiaClon Rh-subgroups+K" (Bio-Rad, Switzerland) were used for confirmation of mixed chimerism in transfused patients: agglutinated cells forming a red line on the surface of the gel and no agglutinated cells formed compact button on the bottom of the microtube. DNA extraction was followed by commercial PCR-SSP kits with primers for typing ABO, *RHD, and RHCE (BAG,* Germany).
Results: We serotyped blood samples from 2055 Russians hematological patients (HP) in 2015. The problems in blood group (BG) identification for ABO system were caused by posttransfused chimerism (PTCh) in 32 primary patients (1,6%) and for Rhesus system-in 64 primary patients (3,1%). 24 patients provided informed consent for genotyping. All patients suffered with idiopathic or symptomatic cytopenia or separate anemia (aplastic anemia -5, different forms of acute leukemia -3, myelodisplastic syndrome -4, multiple myeloma-2, β-thalassemia -1, PNH-1, unidentified anemia-6, partial red cell aplasia-1, with renal transplant -1) and were transfused before hospitalization to National Research Centre for Hematology. Two patients had a problem with identification in ABO BG: 50% chimerism was detected with anti-A and anti-B Moabs in one and 50% no agglutinated RBC with anti-A-in another one. Genotyping revealed B1B1 and A1B1 BG in these patients. We couldn't identified Rhesus phenotype in 23 HP: RhC-in 17 (percent of agglutinated RBC ranged from 40 to 90), Rhc-in 13 (agglutinated RBC 50%>90%), RhD-in 4 (agglutinated RBC 20%>90%), RhE-in 18 (agglutinated RBC 30%>90%), Rhe-in 7 (agglutinated RBC 50%>95%), RhC^w-in 3 (agglutinated RBC 5%>50%). So PTCh for 1 Rh antigen was detected in 5 HP, for 2 Rh antigens-in 7, for 3-in 6, for 4-in 5 and for 5 Rh antigens-in one. The presence of RhC^w was confirmed in two HP with 50% RhC^w chimerism by genotyping. Molecular method predicted the presence of RhC in 5 HP with chimerism from 20% to 95%, Rhc-in 9 HP (chimerism ranged from 40% to 90%), RhD-in 3 (chimerism ranged from 20% to 90%), RhE-in 9 (chimerism ranged from 30% to 90%) and Rhe-in 3 HP with chimerism 95%. Monitorig of BG in all HP after four month.
Summary/Conclusions: The determination of ABO and Rhesus phenotypes is very important for previously transfused patients and for patients with known irregular antibodies. The erythrocyte's genomics has enabled the application

of molecular methods to transfusion medicine and allows preventing the alloimmunization to the most clinically significant blood group antigens.

PB2219

RHD VARIANTS INCLUDING REGULATORY TYPE RHNULL IN RUSSIANS

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Background: In clinical transfusions the D antigen of the Rhesus blood group system (BGS) is the third most relevant behind the A and B antigens of the ABO BGS Approximately 1%>2% of Caucasians show weak expression of D antigen on the RBC surface, which is caused by various types of mutations in the *RHD* gene. There are three D antigen variants in RH system: weak D, partial D and Del. It's essential to know D variant distribution in each population in order to define whether a patient has a high or low probability to produce anti-D alloimmune antibodies during erythrocyte's transfusion therapy.
Aims: to determine the distribution of D antigen variants in Russians.
Methods: Blood samples from 5100 Russians. 65 people have been investigated in our lab due to diminished D expression, discordant results with different anti-D reagents, or D negative samples with atypical Rh phenotype. D antigen was typed with 5 serological methods saline and indirect antiglobulin tests. The blood samples were serotyped for Rh C/c and Rh E/e antigens by corresponding Moabs. DNA extraction was followed by commercial SSP-PCR kits with primers for typing *RHD, RHCE,* 11 weak D types, partial D (BAG, Germany). Mutations in *RHD* (for confirmations of weak D type 3 - two cases and weak D type 15 - one case) and *RHAG* genes was detected by direct Taq cycle-sequencing, computer based sequence analysis and comparison with reference sequences from Genbank™.
Results: In 2014-2015 years we revealed low expression of RhD antigen in 109 (2%) persons by screening. Genotyping 65 of them revealed six weak D types in 62 persons: weak D type 3 (47,7%; n=31; phenotypes-C+c+E-e+ in 28 cases, C+c-E-e+ in two and atypical one C-c+E-e+) and weak D type 1 (27,7%; n=18; phenotypes-C+c+E-e+) were the most frequent. Weak D type 2 was detected in 12,3% (n=8; phenotypes-C-c+E+e+), weak D type 15-in 4,6% (n=3; phenotypes-C+c-E-e+, C+c+E-e+ and atypical one C-c+E+e+), weak D types 4.2 (DAR; phenotype C-c+E-e+) and weak D type 6 (phenotype C+c+E-e+)-each in one person (1,5% accordingly). All except 2 weak D types were identified by serological methods, and the most sensitive method was antiglobulin test in gel columns. Two cases with RhCe and RhcE phenotype were D-negative in all serological methods, and only molecular method allowed identifying weak D type 15. More one sample with diminished D expression was partial D variant DNB with phenotype C+c-E-e+. Two erythrocyte's samples had true D-negative phenotype: one - *RHD*ψ with phenotype C-c+E-e+, and first in Russia (since 1926) we found regulatory type Rh_{null} with predicted phenotype D+C+c+E-e+ in woman with meningioma. *RHAG* sequencing shows polymorphism in exon 4 of the *RHAG* gene at position 571 (C >T) which leads to an earlier Stop-codon (amino acid position 191 [R191]) and the additional mutation at position 724 of exon 5 (G >A).
Summary/Conclusions: The distribution of D variants in Russians was identified. These data may help to improve the transfusion strategy of the D variant.

PB2220

EVALUATION OF PROTEINURIA IN B-THALASSEMIA MAJOR PATIENTS WITH AND WITHOUT DIABETES MELLITUS TAKING DEFERASIROX

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Background: Beta-Thalassemia is the most common heredity disease in Iran. Regular blood transfusion is critical to sustain life and normal growth. Deferasirox is an oral chelator. One of the side effects of the Deferasirox is proteinuria.
Aims: This study aimed to investigate the safety of deferasirox on kidney function in diabetic and non-diabetic Beta-Thalassemia Major patients.
Methods: In this cross-sectional study, 70 patients who take deferasirox (Exjade-Novartis) 20-40mg/kg/day were studied. Exclusion criteria included patient with renal failure, proteinuria, hepatitis B, hepatitis C and the patients who refused to continue the study to the end. Subjects were divided into diabetics and non-diabetics groups. Spot urine protein/creatinine ratio, Urinary analysis, Alanine transaminase, Aspartate Transaminase, Creatinine, Fasting Blood Sugar, Blood Urea Nitrogen and serum ferritin were checked every 3 months. Patients were followed for a period of one year.
Results: In the 9th month after therapy there was a significant relationship in mean change of Spot urine protein/creatinine ratio between diabetic and non-diabetic (p=0.011). Spot urine protein/creatinine ratio in diabetic and non-diabetic group was 0.19±0.18 and 0.1±0.05, respectively which showed no significant relationship between the two groups at the end of study (P=0.162). E, Fasting Blood Sugar, Blood Urea Nitrogen and serum ferritin were checked every 3 months. Patients were followed for a period of one year.
Summary/Conclusions: The results of our study showed that consumption of deferasirox is safe since there was no significant relationship between Spot urine protein/creatinine ratio in diabetic and non-diabetic group. Deferasirox consumption is not associated with increased proteinuria in diabetic patients compared to non-diabetic group having only a transient proteinuria.

PB2221

COMPARISON AMONG PERIODS FOR HEMOGLOBIN EQUILIBRATION AFTER BLOOD TRANSFUSION

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Background: Target hemoglobin (Hb) varies among different clinical situations but mainly at 7 g/dL. Many institutes include ours follow Hb and hematocrit (Hct) after transfusion at 4 hours for achieving target level. But previous study showed that equilibration after red cell transfusion was at 24 hours. We sought to find out the appropriate time point after red cell transfusion. The result may help physicians to provide earlier and proper treatment.
Aims: This research was aimed to determine the equilibration time point of Hb and Hct after red cell transfusion.
Methods: This is a prospective randomized trial conducted from December 2014 to August 2015 at Chiang Mai University Hospital. Inclusion criteria was adult patient who received 1 unit of red cell without active bleeding and hemolysis. Number of patient was calculated by match pair method from previous study. Hb and Hct were measured at baseline before transfusion. Patients were randomized into 3 groups according to baseline characteristics to follow Hb and Hct at 1, 4 or 24 hours after transfusion. Mean difference of Hb and Hct were compared between each group.
Results: Sixty patients were eligible in this study. Median age was 51 years old. There was male predominance (83.33%). Most common indication for transfusion was post-operative anemia (88.33%). There were 20 patients per each group. All baseline characteristics were not significantly different in each group which included age, sex, body weight, body surface area, transfusion indication, creatinine clearance, transfusion volume, congestive heart failure history, diuretics use, fever, intravenous fluid, blood group, baseline Hb and Hct. The median volume of red cell transfusion was 260.00, 262.50 and 257.50 ml for each group (P=0.638), Baseline median Hb before transfusion was 8.60, 8.50 and 8.60 g/dL for each group (P=0.553). Baseline median Hct before transfusion was 26.65, 26.30 and 27.05% for each group (P=0.570). Mean difference of Hb level among different time point at 1, 4 and 24 hours was 1.21, 1.19 and 0.95 g/dL for each group (P=0.109), Mean difference of Hct level among different time point at 1, 4 and 24 hours was 3.62, 3.63 and 3.09% for each group (P=0.398). The equilibration of Hb and Hct after red cell transfusion was not statistically different between these three groups.
Summary/Conclusions: The equilibration of Hb and Hct after transfusion was not different at 1, 4 and 24 hours. Target Hb and Hct can be determined at 1 hour after transfusion which can provide early further treatment.

PB2222

WHY ARE WE LOSING OUR PRECIOUS BLOOD DONORS?. A SYSTEMATIC REVIEW FROM PAKISTAN

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Background: Worldwide, a great majority of blood donors are deferred and many of these deferrals are for temporary reasons. This may cause loss of precious blood donors. This is also a common practice in Pakistan. Given the limited number of blood donors in our setting it is undesirable to defer a significant number of blood donors. This systematic review was conducted to document the various reasons for temporary deferral in Pakistan.
Aims: To carry out a systematic review of the surveys addressing the reasons of temporary blood donor deferral in Pakistan.
Methods: PAKMEDINET, GOOGLE SCHOLAR, PUBMED, CINAHIL and EMBASE search engines were used for literature search by using following terms '' pre donation deferrals Pakistan'', ''temporary reasons for blood donation deferrals'', ''questionnaire related deferrals in Pakistan'' and'' temporary blood donor deferrals Pakistan'' from January 2010 to December 2015. Studies which addressed transfusion transmitted infections and permanent causes of donor deferrals were excluded. Case reports were also excluded. Finally 4 relevant

studies were selected and reviewed and results were analyzed by using SPSS version 21. Forest plot was made to analyze these studies and p value <0.05 was taken significance.
Results: The search generated 8459 records for the year 2010 to 2015. 4 relevant studies were selected and reviewed. These studies were carried out in various blood banks located in different provinces of Pakistan. Based on the collective findings of these studies; anemia 31%, low blood pressure 21%, medications 8%, donation in last 3 months 8% and low weight for age 5% were identified to be the commonly observed factors for temporary blood donor deferral.
Summary/Conclusions: Anemia was found to be the most frequent cause of temporary blood donation deferral in our population. It is important to provide donors with a clear message regarding their deferral status. They should be encouraged to come again after their deferral period. Public awareness and education regarding blood donation may decrease deferral rates and can prevent an unfavorable impact on the donor as well as on blood donations.

PB2223

RESULTS OF AN ANEMIA PREOPERATIVE'S CIRCUIT CORRECTION AS TECHNIQUE TO SAVE BLOOD IN MAJOR GYNECOLOGICAL SURGERY SINCE 2010 TO 2015 IN A THIRD NEVEL HOSPITAL
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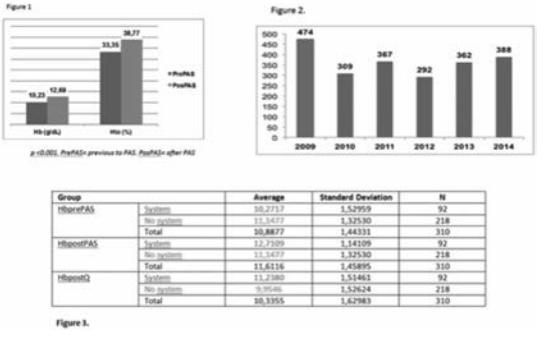
Background: Anemia is not only the biggest risk factor for transfusion, but also an independent predictor of perioperative mortality and morbidity, including more infectious complications and increased hospital stay. Its prevalence in surgical patients varies in the range from 10 to 70%, depending on their definition and the type of intervention. Iron-deficiency anemia is the most common cause in the patient's proposal for mayor gynecological surgery (MGS).
Aims: This surgery presents a transfusion rate of 14% and secondary to perioperative anemia without bleeding complications of 13%. Since late 2010, with the creation of a multidisciplinary group of saving blood (PAS). We implemented a circuit for the correction of preoperative anemia in patients scheduled for this surgery with the aim to reduce the rate transfusional. We propose a retrospective study to analyze the effectiveness of this strategy saving blood.
Methods: The proposed patients for MGS with Hb<12 g/dl, we programed, in susceptible cases, the administration of iv iron (sucrose and carboxymaltose) for 2-4 weeks before surgery. The day before the intervention, we monitored a preoperative blood count to assess treatment response and we measured the proportion of transfused patients. 103 patients were included in PAS until February 2015 (Figure 1). 5 patients were transfused: 2 due to hemorrhagic postoperative complications and 3 due to preoperative anemia.



Group		Average	Standard Deviation	N
HbprePAS	System	10,2717	1,52959	92
	No system	11,1477	1,32530	218
	Total	10,8877	1,44331	310
HbpostPAS	System	12,7109	1,14109	92
	No system	11,1477	1,32530	218
	Total	11,6116	1,45895	310
HbpostIQ	System	11,2380	1,53461	92
	No system	9,9546	1,52624	218
	Total	10,3355	1,62983	310

Figure 3.

Figures.

Results: Hemoglobin of the patients, which were included in our protocol, an average of 2.46 g/dl and 5.42% hematocrit increased, being the response to treatment statistically significant. We study the evolution of the number of red blood cells concentrates transfused in the gynecology before and after the implementation of our strategy. We observed a decrease of 34.8% in 2010, 22.6% in 2011, 38.4% in 2012, 23.6% in 2013 and 18% in 2014 compared to 2009. (Figure 2). Regarding the incidence of perioperative transfusion, the percentage of patients transfused included in the PAS is 4.9%, while in the historical cohort is 26.5% and this difference being also statistically significant (p <0.001). We compare our patients with a historical cohort obtained from the database at our center, selecting all patients undergoing major surgery gynecological (MSG) available from 2008 to the implementation circuit that meet the same criteria for our group. Preoperative hemoglobin (Hbpre) before (HbprePAS) was analyzed and after our intervention (HbpostPAS), postoperative hemoglobin (HbpostIQ) and the incidence of perioperative transfusion in both groups. Hb levels were compared with a general linear model and the percentage of transfusion with the chi-square test. P value <0.05 was considered significant. The historical cohort was constituted with 223 patients. Before treatment with iv iron patients exhibit inferior circuit Hbpre the cohort historical statistically significant (p <0.001). After PAS, the change in Hb is statistically significant (p <0.001), so that the Hbpre of treated patients is 12.7 g/dl *versus* 11.14 g/dl group untreated. The average value of the HbpostQ is also superior in our group significantly (p <0.001). Figure 3.
Summary/Conclusions: This circuit is effective to correct the preoperative anemia of the patients proposed for MSG. This protocol has improved Hb levels and preoperative hematocrit of these patients. The results were statistically significant. Treatment with intravenous iron for the correction of preoperative anemia in GMS as blood conservation strategy is effective to increase Hbpre statistically significant. Patients included in the circuit have a lower incidence of transfusion and have greater HbpostQI that patients not treated with iv iron. This protocol has improved Hb levels and preoperative hematocrit of these patients. The results were statistically significant. This measure seems effective in reducing the transfusion risk.

PB2224

EFFECT OF THIOCYANATE ON THE ACTIVITY OF BLOOD CLOTTING FACTOR VIII
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Background: The transmissions of viral agents have been associated with blood transfusion, particularly with the factor coagulation concentrates (for example, factor VIII, vWF, factor IX and prothrombin complex). The blood clotting concentrates should have not only a high specific activity, but to be viral safety. The safety of the final plasma protein concentrates has a paramount importance. The specific steps for the inactivation of the virus have been included in each process to enhance the safety of the products by reducing the virus load during manufacturing. Currently, there are several technologies to help ensure the safety of plasma products: solvent-detergent method, heat treatment, sodium caprylate and low pH, chromatography, nanofiltration and chemical reagents (for example, sodium hydroxide, guanidine chloride, sodium or ammonium thiocyanate) for purification of proteins and sanitizing chromatography systems.
Aims: studies of the effect of ammonium thiocyanate (NH_4SCN) on the activity of blood clotting factor VIII (FVIII).
Methods: The commercial concentrate of FVIII («Immunate») was initial raw material for the work (working activity 1.6 IU/ml). Determination of activity of FVIII conducted the one-stage clotting method which is based upon the activated partial thromboplastin time (APTT) and using of congenital severe hemophilia A plasma or artificially prepared of FVIII deficiency plasma as a substrate. To investigate the effect of NH_4SCN on the activity of FVIII, to enzyme working solution were added a buffered solutions of different concentrations of reagent. Removing of NH_4SCN was performed in stages by ultrafiltration (used the filters Amicon Ultra-0.5 10k; Centrifuge Eppendorf 5702-R).
Results: Virus inactivation by NH_4SCN is shown earlier in studies of various scientists. NH_4SCN was used as an antiviral agent in the manufacture of factors coagulation from plasma of blood. We conducted the study of this effect on the activity of clotting FVIII, to determine the possibility of its use in the manufacture of investigational factor. Experimental studies were performed in stages: in the reaction mixture was added buffered solutions of various concentrations of NH_4SCN and was measured activity of FVIII. As a result of the research we showed that thiocyanate reversibly inhibits the activity of the investigated FVIII (Table.1). When it had removed from the reaction mixture, factor activity recovered.

Table 1. Comparative characteristics of FVIII activity depending on the concentration of thiocyanate.

№	Concentration, NH_4SCN, M	Time, s	Activity of factor VIII, IU/ml
1	0.1230	> 180	not determined
2	0.0610	>180	not determined
3	0.0240	90	0.16
4	0.0122	69	0.74
5	0.0024	63	1.24
6 (control)	0	60	1.6

Summary/Conclusions: Thiocyanate may be used for antiviral treatment and as the eluting agent of coagulation FVIII in chromatographic process purification.

PB2225

TRANSFUSION PROTOCOLS OF BLOOD CENTRE OF ASL CASERTA (MANAGEMENT AND MONITORING)
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Background: The red blood cell is an immunologically competent cell for the presence of various exposed antigens, inherited genetically. Transfusion is a medical procedure extremely important, and certainly not without risks. Our Trasfusion Centre implemented transfusion protocols to ensure that all patients

have best treatment possible; these protocols provide for the identification of two groups of patients:chronic and acute. The first transfusing compatibility with the Rh, kell Duffy, Kidd, MNS systems with apheresis or filtered red blood cell, the seconds one with Rh and Kell systems compatibility, by decomposition of red blood cells. This is possible thanks to the availability of blood guaranteed by the excellent cooperation with voluntary associations, particularly for the production of red blood cells by apheresis.
Aims: The aim of our study was to evaluate the effectiveness of our transfusion protocols and their impact on health patients (immunological complications)
Methods: A retrospective study was done on all patients transfused in 2015 year, the patients were divided in chronic and acute and we studied the percentage of immunization and the most frequent antibody (computer system Eliot Enginnering Health)
Results: In the year 2015 were carried out 14,277 transfusions to 7000 patients (2500 chronic and 4500 acute). There were no immunizations in chronic patients while in the acute patient the percentage was 0.6%. The identified antibodies were: anti-D: 36.4%, anti-K: 27%, anti-E: 15%, anti-C: 1%, anti-c :6%, anti-e: 3%, anti FyA :2%, anti-Fyb: 1%, anti M: 3.4%, anti LeA :2.2%.
Summary/Conclusions: The results show that the cases of immunizations are all at the expense of acute patients, this confirming as the transfusion protocols guarantee the best possible therapy trying to preserve all chronic patients from ineffective transfusions. The frequencies of the detected antibodies reflect the immunogenicity scale. The choice to transfuse the chronich patients respecting such scale have preserved to immunizations. These protocols may seem expensive for the company budget assigned to the blood transfusion service, the savings are long-term evident in reality as it reduces the number of immunized patients and then the cost of their management.

PB2226

INDICATIONS FOR TRANSFUSING NAT UNTESTED BLOOD PRODUCTS ARE THESE RATIONAL?

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Background: Blood transfusion is a life-saving therapeutic option in many conditions. However, blood transfusion is never safe and is associated with risks of transfusion transmitted infections (TTI). To minimize the risk of TTI, all blood donations are screened for infectious diseases as per W.H.O standard. In emergency situation where blood products are required urgently and screened products are unavailable, it may be necessary to transfuse unscreened or partially screened blood products. Some of the indications where use of such products can be justified include: Critical Laboratory Results (defined as Hb <7gm/dl and platelets <20x10^9/L), active bleeding, emergency surgical/ radiological intervention and sepsis. Irrational transfusion of unscreened blood puts a patient at risk of acquiring an infectious disease which can cause severe long term complications. So it is very important they must only be transfused in dire need when screened products are not available and transfusion is the only life-saving option. In our hospital all products are serologically tested and then all seronegative samples undergo molecular testing known as Nucleic acid testing (NAT). The rate and indications of dispensing NAT untested blood products in our hospital is unknown
Aims: The aim of this study is to recognize the indications for transfusing NAT untested blood products and assess whether these are rational or not.
Methods: This was a clinical audit conducted by Blood Bank, Section of Haematology, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital. The Study duration was from May-July 2015. All the request forms received to Blood bank requesting release of NAT untested blood products were reviewed. Additional information regarding the need and indication was extracted from the online integrated laboratory data and medical record chart of patients. Data was recorded on a predesigned proforma. Statistical package for social sciences version 19 was used for data analysis.
Results: During the study period, 254 requisition slips were received to blood bank for 234 patients. Six hundred and seventy two NAT untested blood products were released during study period. The products released included: 154 packed red cell, 493 random donor platelets and 25 whole blood units. Most of the cases where blood bank was not able to provide screened blood were from Section of Clinical Haematology (n=61, 24**%**) followed by General Surgery (n=46, 18%) and Internal Medicine (n=40, 16%). The location of patients was Emergency room (n=58, 23%), Operating room (n=46, 18%), Wards (n=45, 18%) and special care units (n=28, 11%). In 224 out of 254 (88.2%) requests, the indications for transfusion were rational. The most common indication for the release of NAT untested products was Critical Laboratory results (n=106, 42%) followed by Surgical/Radiological Intervention (n=65, 26%) active bleeding (n=53, 21%) and sepsis (n=12, 5%). Seven percent (18/254) of the requests were not fulfilling any of the indication and transfusion in these cases was not justified. Majority of these requests (n=14, 77.8%) were from oncology day care transfusion area.
Summary/Conclusions: Majority of the indications for transfusion of NAT untested products were rational (93%). Few cases of irrational transfusion of NAT untested products were identified. The audit identified the areas in the hospital which need re-inforcement on the rational use of NAT untested blood products.

PB2227

A NEW PROTOCOL FOR OBTAINING PLATELET RICH IN GROWTH FACTORS (PRP). A DESCRIPTIVE STUDY IN 15 PATIENTS AND COMPARISON WITH RESULTS PUBLISHED IN LITERATURE

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Background: The diversity of procedures for obtaining platelet and plasmatic growth factors, the absence of control in most of them and the growing field of clinical application, makes them necessary methods adequately structured, documented, controlled and tested, playable by any author. The present series of clinical cases aims to introduce and test a specific technique for obtaining PRP, with precise characteristics both production and final composition of compound got, in 15 hematological healthy patients, comparing our results with those obtained by other procedures scientifically tested.
Aims: introduce and test a specific technique for obtaining PRP, with precise characteristics both production and final composition of compound got, in 15 hematological healthy patients, comparing our results with those obtained by other procedures scientifically tested.
Methods: 15 caucasian patients were selected, 8 male and 7 female with age range between 35 and 65, healthy haematologically. The procedure for obtaining the PRP, consisted of a single centrifugation of the blood sample for 30 minutes at 3500 rpm in a angular shaft of 16 tubes centrifuge serie (CEMCON 2) and micropipetting the protein fraction rich in platelet and plasmatic growth factors and cell through open technique under aseptic conditions in horizontal laminar flow hood Grade A at a temperature of 22 ° C, with the use of leucoplatelet or Buffy-coat layer (PRP rich in leukocytes).
Results: No correlation between the amount of concentrated platelets and the amount of growth factors finally obtained was observed. The protocol set forth concentrated levels of platelets and leukocytes approximately 3 to 5 times higher than baseline levels with a predominance of mononuclear. Levels of growth factors from 7-10 times greater than the patient's baseline levels, with little variation in them. The growth factor levels were stable in the blood of each patient within 24 h of treatment between 7 and 9 times higher compared to the previous baseline. Compared with other procedures discussed in the literature; This method achieves concentration between 1.5 and 3 times more platelets in the final product, with a purification of growth factors overall type VEGF and TGF-B clearly superior.
Summary/Conclusions: the technique disclosed is more effective since concentrate achieves greater amount of platelets and growth factors and efficient since it maintains a serum protein in these stable sera of patients after 24 hours of administration thereof.

PB2228

PEDIATRIC PATIENT WITH BOTH LEUKOCYTE ADHESION DEFICIENCY II AND BOMBAY BLOOD GROUP

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Background: Leukocyte adhesion deficiency type II (LAD II) is a rare autosomal recessive inherited immunodeficiency disease that induces frequent, recurrent infections, persistent leukocytosis, severe mental and growth retardation and impaired wound healing. Therewithal, Bombay blood group is rare phenotype that is characterized by deficiency of H, A and B antigens on red cell surface.
Aims: LAD II and Bombay blood group have rarely been present together because both of them associated with a global defect in common pathway of fucose metabolism.
Methods: 11-year-old boy was evaluated due to diarrhea. He was born to healthy first degree consanguineous parents at term with a birth weight of 1.250 gr (<3th). He had a history of hospitalization for intrauterine growth retardation during first three weeks of life. As an infant, he suffered from delayed separation of the umbilical cord. He developed multiple recurrence infections such as pneumonia, diarrhea, fever and recurence skin abscess. On the examination, his height and weight were both below the third percentile for age. He had 2 cm of hepatomegaly and 4 cm of splenomegaly. Physical examination revealed the dysmorphic features such as coarse facial appearance, flattened nose, long prominent philtrum. He had numerous scars of abscess in the bilateral inguinale region. On neurological examination, he had severe growth and psychomotor retardation (Figure 1). İnitial laboratory evaluation revealed elevated white blood cell count (72.1x10^3/µL) with neutrophilia (80% neutrophils, 20% lymphocytes). His hemoglobin level was 11.6 g/dL and his platelet was

456,000/mm3. The other blood parameters of the patient were normale. His disease was identified Leukocyte Adhesion Deficiency (LAD) which was characterized by recurrent infections, persistent leukocytosis, delayed separation of the umbilical cord and severe mental and growth retardation.
Results: The presence of Lea, Leb, and H antigens on the erythrocytes of patient was investigated by hemagglutination tests performed with commercial monoclonal antiserums (ALBAclone, Alba Bioscience, United Kingdom) derived from mouse. At same hospital, his blood grouping was again interpreted as O Rh D positive by the ABO and Rh typing on gel card. Forward reverse grouping on tube showed the blood group to be O Rh D positive. Indirect Coomb's Test and antibody screening was 4+ reactivity, direct Coomb's Test (DCT) of patient was negative. It was found to be incompatible with strength of 4+ agglutination in all cross match by reverse analysis conducted with erythrocytes of A1, A2, B and O blood group. Reaction with anti H lectin was negative. The blood group was finally interpreted as *Bombay blood group with naturally occuring anti-H antibodies* in plasma. The moleculer basis of LAD II was demonstrated according flow cytometric results; the deficient expression of the CD15 adhesion molecules on the surface of leukocytes while expression LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18), p150/95 (CD11c/CD18) are normal.
Summary/Conclusions: When a patient with Bombay Phenomenon is detected, the investigation for leukocyte adhesion deficiency type 2 must been done.

PB2229

TRANSFUSION IN THE PRESENCE OF MULTIPLE ALLOANTIBODIES PLUS COLD-REACTIVE AGGLUTININS/DAT+
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Background: Transfusion in the presence of allloantibodies is a common procedure in blood-banks. The challenge appears when alloantibodies are multiple and associated with "benign" antibodies or another inespecific laboratory finding that cover up the clinically significant antibodies. The grade of difficulty grow up if the presentation of alloantibodies is detected at the same time rather than in succession.
Aims: We exposed two cases of multiple clinically significant alloantibodies, classified as group I (antibodies that react at 37ºC and that cause a significant majority of transfusion reactions, related to systems: AB0, Rh, Kell, Duffy, Kidd and Ss).
Methods: We describe the last two complex positive inmunohematological studies perfomed in our community hospital
Results: Case1: Pluripathological woman 86 years old, without pregnancies and without previous studies in our hospital. She has received just one transfusion of 2 red cells concentrates, two years before in another hospital. IAT Positive in emergency room with subsequent study in table 1. Probability to find compatible 0 positive blood: 2%. Case 2: Man 68 years old with sever hepatic disease, multiple blood transfusion in the past 7 years due to gastrointestinal bleeding. He needs urgent transfusion for the same reason. Previously he has developed several alloantibodies successively as reported in table 1. Probability to find compatible 0 positive blood: 6%. The challenge of transfusion in these cases are associated with four facts: 1. Number and specificity of alloantibodies; 2. Timing of apparition of alloantibodies; 3. Absolute necessity of blood transfusion in life-threatening situations; 4. Presence of others events that difficult the identification of alloantibodies: cold-reactive antibodies, positive direct antiglobulin test.

Table 1. Antibodies detected in blood transfusion recipients.

	Blood Group	Fenotyphe	Antibodies	Other facts
Patient1 Woman 86 y	O POS DcEe	K(-) k(+) Fya(-)Fyb(+) JKa(-)JKb(+) M(-)N(+) S(-)s(+)	Anti-C Anti-K Anti-Fya Anti-JKa	DAT + Autocontrol +
Patient 2 Man 68 y	0 POS DCe	K(-) k(+) Fya(+)Fyb(+) JKa(+)JKb(-) M(+)N(+) S(-)s(+)	Anti-E Anti-c Anti-JKb Anti-S	Cold-reactive antibodies + (Anti-I)

Summary/Conclusions: The presentation of multiple anti-erythrocytes alloantibodies associated with non-specific/bening serological findings in life-threatening situations required and appropriated and immediate evaluation of the patient to decide if transfusion can be delayed or not, simultaneously efforts can be made in order to identify the facts clinically significatives to avoid serological related conditions that mask the real problem focussed in antibodies group I related. We think that transfusion of incompatible blood, in a hemodynamic unstable patient due to others diseases, should be avoided. If anaemia is the exclusively cause of instability we should consider the risk of transfusing incompatible blood.

PB2230

ANALYSIS OF PLATELETPHERESIS DONOR DEFERRAL RATE, CHARACTERISTICS AND ITS PREVENTABILITY
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Background: Plateletpheresis is a procedure included in donor apheresis in which the donor blood is passed through an extraction machine, the platelets are extracted, and the remainder is returned into the circulation. Donor selection is important because donors are the only source for meeting blood product needs.
Aims: In our study we aimed to evaluate deferral reasons in applications for plateletpheresis donation and reveal determine the preventable ones among them.
Methods: The persons who were admitted to our Blood Center to be plateletpheresis donors during the period between August 2013-February 2014, after a demand/request for platelet suspension was issued by the clinics. Their eligibility for being plateletapeheresis donors was decided
Results: A total of 1098 persons have applied to be plateletpheresis donors; 1045 were male and 53 female. A total of 787 persons were found to be eligible for being plateletpheresis donors; 772 were male and 15 female. 891 units of apheresis platelets were obtained from apheresis donors, including double units collected from 104 of them. Applications of 311 persons were deferred due to various reasons. The most common reason for donor deferral was inappropriate vascular access in 80 persons (25.7%, 69 males, 11 females). This is followed by abnormal laboratory values in 57 persons (18.3%) and drug use (most commonly analgesics) in 46 persons (14.7%).
Summary/Conclusions: During the assessment of blood product donors, proper selection of the candidates should be considered very carefully in order to not to harm the donor but to supply the demand of the recipient properly. When the deferral reasons were evaluated, improvement of in-hospital technical reasons and education sessions for people in order to increase the social awareness may substantially decrease the deferral rates.

PB2231

RED CELL PHENOTYPING AMONG MULTITRANSFUSED EGYPTIAN THALASSEMIC PATIENTS
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Background: Thalassemia is an inherited hemolytic disorder caused by partial or complete deficiency of α or β-globin chain synthesis. Regular transfusions are essential for patients with thalassemia to maintain growth and development during childhood and to sustain good quality of life during adulthood. The development of hemolytic alloantibodies and red blood cell (RBC) autoantibodies complicates transfusion therapy in patients with thalassemia.
Aims: of this research is to determine the prevalence of red blood cells antigens among multitransfused thalassemic patients attending Mansoura University Children Hospital, Mansoura, Egypt and a sample of blood donors, as an approach for prevention of alloimmunization.
Methods: The present study comprised 70 mulitransfused thalassemic patients. They were 38 males and 32 females and in addition 65 adult male donors. All were subjected to Laboratory tests for blood group antigens (ABO), and (Rh system, Kell system), using ID-cards, Also a CBC was done.
Results: 1) Blood group O is the most prevalent blood group in our patients but B group is the most prevalent in our donors. 2) Kell negative group is prevalent in the patients compared to the donors. 3) There is significant difference between the patients and the donors in Rosenfield: (1,2,3,4,5) and (1-2-3,4,5), More prevalent in the donors, But Rh (1,2-3-4,5) is prevalent in the patients *versus* the donors. 4) There is significant increase in (c), (E) and marginal increase of (k) antigens in donors *versus* the patients. 5) Patients below five years old showed significant increase of negative kell group and Rosenfield Rh (1,2-3-4,5). 6) No relation between sex and blood group phenotypes of the patients. 7) Significant increase of kell negative group and Rosenfield Rh (1, 2-3-4,5) in patients who were younger in age of first transfusion and who received frequent transfusions. 8) High incidence of pallor in patients with blood group O. Also patients with Rosenfield Rh (1,2-3-4,5). 9) Splenectomizd patients had significantly higher incidence of negative kell group and Rosenfield Rh (1,2-3-4,5).
Summary/Conclusions: From the present study, in order to minimize the hazards of blood transfusion, especially the immunological reactions the following could be done: 1) Pre transfusion matching of blood for ABO and Rh D antigens is important as a routine work, which helps in determining the true blood groups and it assists in the identification of suspected alloantibodies and selection of antigen-negative RBCs for transfusion. 2) Extended phenotype matching of blood for Rh (other than D) and Kell have been proposed as effective method which could reduce morbidity in thalassemic patients.

PB2232

SHOULD BLOOD DONORS WITH HARRIS PLATELET SYNDROME BE EXCLUDED FROM PLATELET DONATION?
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Background: Harris platelet syndrome (HPS) is an autosomal dominant macrothrombocytopenia with mild to moderately severe thrombocytopenia and normal platelet function. HPS has been reported from Indian subcontinent blood donors, especially from the northeastern part of India. Approximately a third of blood donors from state of West Bengal have been found to have HPS.The Indian NACO 2007 guideline recommends one bag of PRC from 350 ml of whole blood should have a platelet count above 3.5 $X10^{9/l}$.

Aims: We designed a study to assess whether PRC (platelet rich concentrate) obtained from those with HPS contained adequate platelets as recommended by the Indian guidelines.

Methods: HPS is defined as healthy blood donors having a mean platelet volume (MPV) greater than 12 fL (normal <10 fL) with or without concomitant thrombocytopenia in the absence of any significant past history of bleeding disorder in either themselves or their first degree relatives. In our study, we collected 350 ml of blood from each donor and via Platelet Rich Plasma Separation Method (PRPSM) we separated out PRC and PRBC in separate bags.A CBC was performed at the time of donation and from PRC bag blood collected in EDTA tubes, and analyzed using SYSMEX XP-100.

Results: A total of 28 donors were included. The median age of the donors was 33 years(21-62) and 21 (75%) were male.Pre donation CBC showed a median platelet count of 1.93±0.54 $X10^{9}$/ L (0.99-2.98 $X10^{9}$) and platelet count from PRC showed a median platelet count 1.16±1.77x10^{9}/ L(0.03-8.25$X10^{9}$) which is much below the national guidelines. It is possible that platelet yield is lower in these donors as during separation, the platelet concentration gradient was influenced by the larger platelet size [1].

Table 1.

Platelet Indices:	Pre Donation Platelet Indices (n=28)	Platelet indices from PRP (n=28)
MPV(Mean)	13.66±1.99 fl	10.00±2.02 fl
Platelet Count (Median)	1.93±0.54 x10^{9}/L (0.99-2.98x10^{9}/L)	1.16±1.77 $X10^{9}$/L (0.03-8.25x10^{9}/L)
PDW(Mean)	19.80±2.95	13.87±4.64
Platelet Biomass(Mean)	26.91±1.16	15.4±9.41

Summary/Conclusions: This study shows that PRC from HPS donors are not able to keep the international standard and should not be used in the future. Our study is the first one to date to assess the candidacy of those with inherited giant platelet disorders being blood donors.

Reference

1. Dhurat R, Sukesh M. Principles and Methods of Preparation of Platelet-Rich Plasma: A Review and Author's Perspective. J Cutan Aesthetic Surg. 2014;7(4):189–97.

Index of authors

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Late breaking authors

G

H

I

J

K

L

M

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Median time to MMR in CP-CML responder: 5.5 months[1,2]

Deep response

38 % of CP-CML patients reached MMR or even better[1]

Durable response

87 % of CP-CML responders maintained MCyR at 28 months[1]

* **Iclusig** is a tyrosine kinase inhibitor (TKI) indicated in adult patients with[1]

- chronic phase, accelerated phase, or blast phase chronic myeloid leukaemia (CML)
  - who are resistant to dasatinib or nilotinib;
  - who are intolerant to dasatinib or nilotinib and for whom subsequent treatment with imatinib is not clinically appropriate;
  - or who have the T315I mutation
- Philadelphia chromosome positive acute lymphoblastic leukaemia (Ph+ ALL)
  - who are resistant to dasatinib;
  - who are intolerant to dasatinib and for whom subsequent treatment with imatinib is not clinically appropriate;
  - or who have the T315I mutation

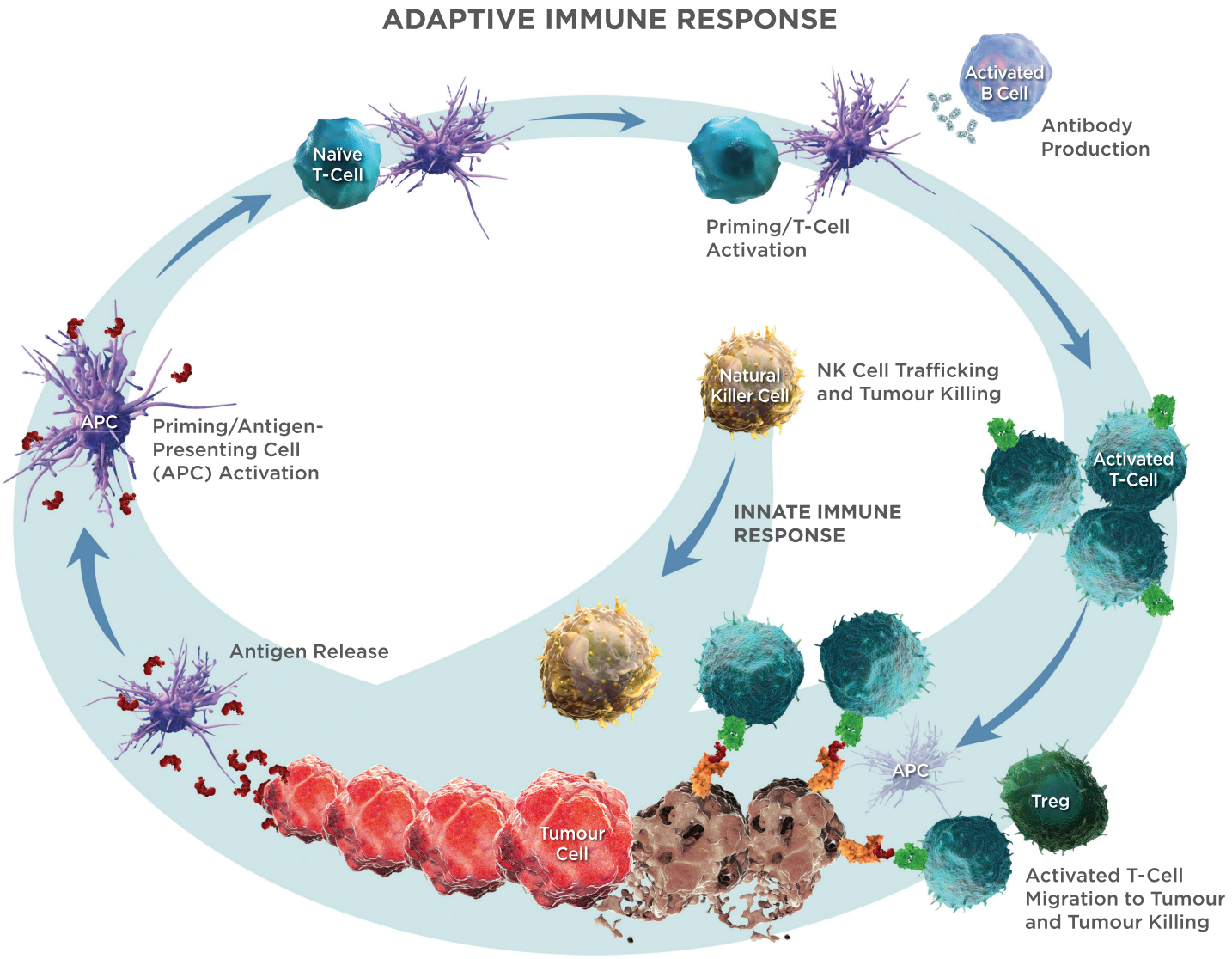
References: 1. Iclusig (ponatinib). Latest Summary of Product Characteristics (March 2016). 2. Cortes JE, Kim DW, Pinilla-Ibarz J, le Coutre P, Paquette R, Chuah C et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. N Engl J Med2013;369:1783-96.

PRESCRIBING INFORMATION – Iclusig® ▼ (ponatinib) ▼ This medicinal product is subject to additional monitoring. Antineoplastic agent, protein kinase inhibitor, ATC code: L01XE24.
Presentation: Film-coated tablets containing 15 mg or 45 mg of ponatinib (as hydrochloride). **Indications:** Iclusig is indicated in adult patients with chronic phase (CP), accelerated phase (AP), or blast phase (BP) chronic myeloid leukaemia (CML) who are resistant to dasatinib or nilotinib; who are intolerant to dasatinib or nilotinib and for whom subsequent treatment with imatinib is not clinically appropriate; or who have the T315I mutation, and in adult patients with Philadelphia chromosome positive acute lymphoblastic leukaemia (Ph+ ALL) who are resistant to dasatinib; who are intolerant to dasatinib and for whom subsequent treatment with imatinib is not clinically appropriate; or who have the T315I mutation. **Dosage and administration:** Therapy should be initiated by a physician experienced in the diagnosis and treatment of patients with leukaemia. Haematologic support such as platelet transfusion and haematopoietic growth factors can be used during treatment if clinically indicated. Before starting treatment with ponatinib, the cardiovascular status of the patient should be assessed, including history and physical examination and cardiovascular risk factors should be actively managed. Cardiovascular status should continue to be monitored and medical and supportive therapy for risk factors optimised during treatment with ponatinib. The recommended starting dose is 45 mg once daily. Response should be monitored according to standard clinical guidelines and treatment discontinuation considered if a complete haematological response has not occurred by 3 months. It should also be discontinued when there is evidence of disease progression or severe adverse reactions. Tablets should be swallowed whole and not crushed or dissolved, and may be taken with or without food. Dose adjustments – The risk of vascular occlusive events is likely to be dose related. In a patient suspected of developing an arterial or venous occlusive event, Iclusig should be immediately interrupted. After the event is resolved, the decision to re-start treatment should be directed by a benefit-risk assessment. Dose modifications, or interruptions, should be considered for haematological and non-haematological toxicities. Treatment should be withheld in the case of severe adverse reactions. Dose modifications are recommended for neutropenia or thrombocytopenia unrelated to leukaemia, pancreatitis and elevation of lipase/amylase and prescribers should consult the SmPC for full details. When adverse reactions have resolved, or become less severe, dose escalation back to the prior daily dose may be considered, if clinically appropriate. Dose reductions may be considered for patients who have achieved a Major Cytogenetic Response and the SmPC should be consulted for risk-benefit and monitoring details. **Contraindications:** Hypersensitivity to ponatinib or to any of the excipients. **Warnings and precautions:** *Myelosuppression* – Iclusig is associated with severe thrombocytopenia, neutropenia, and anaemia. The frequency of these events is greater in patients with AP-CML or BP-CML/Ph+ ALL than in CP-CML. A complete blood count should be performed every 2 weeks for the first 3 months and then monthly or as clinically indicated. *Vascular occlusion* – Arterial and venous thrombosis and occlusions, including fatal myocardial infarction (MI), stroke, retinal vascular occlusions associated in some cases with permanent visual impairment or vision loss, stenosis of large arterial vessels of the brain, severe peripheral vascular disease, kidney artery stenosis, the need for urgent revascularization procedures have occurred with Iclusig; these events were more frequent with increasing age, or in patients with prior history of ischaemia, hypertension, diabetes, or hyperlipidaemia. Iclusig should not be used in patients who have suffered an MI, prior revascularisation or stroke, unless there is a positive benefit-risk assessment; other treatment options should also be considered. Monitoring for thromboembolism and vascular occlusion should occur throughout treatment with Iclusig, which should be stopped immediately if an event occurs. If decreased vision or blurred vision occurs, an ophthalmic examination (including fundoscopy) should be performed. Hypertension (including hypertensive-crisis) has occurred with Iclusig and can contribute to the risk of arterial thrombotic events, so blood pressure should be monitored at each visit and any associated symptoms treated urgently. Iclusig should be interrupted in the event of uncontrolled or treatment resistant hypertension. *Congestive heart failure* – Fatal and serious events have occurred with Iclusig including events associated with prior vascular occlusion. Patients should be monitored and treated as clinically indicated, including interruption of Iclusig. If serious heart failure develops, Iclusig should be discontinued. *Pancreatitis and serum lipase* – Iclusig is associated with pancreatitis. The frequency of pancreatitis is greater in the first 2 months of use. Check serum lipase every 2 weeks for the first 2 months and then periodically thereafter. Dose interruption or reduction may be required. If lipase elevations are accompanied by abdominal symptoms, Iclusig should be withheld and patients evaluated for evidence of pancreatitis. Caution is recommended in patients with a history of pancreatitis or alcohol abuse. Patients with severe or very severe hypertriglyceridaemia should be appropriately managed to reduce the risk of pancreatitis. *Lactose* – Iclusig contains lactose monohydrate. Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicinal product. *Effects on ability to drive and use machines* – Adverse reactions such as lethargy, dizziness and vision blurred have been associated with Iclusig. Therefore, caution should be recommended when driving or operating machines. *QT prolongation* – a thorough QT study has not been performed; therefore a clinically significant effect on QT cannot be excluded. *Hepatotoxicity* – Iclusig may result in elevation in ALT, AST, bilirubin, and alkaline phosphatase. Hepatic failure (including fatal outcome) has been observed. Liver function tests should be performed prior to treatment and periodically, as clinically indicated. *Haemorrhage* – Serious and fatal haemorrhagic events have occurred with Iclusig, most commonly cerebral and gastric bleeding. Most events were associated with grade 3 & 4 thrombocytopaenia. Iclusig should be interrupted for serious and severe events. *Hepatic impairment* – Caution is recommended when administering Iclusig to patients with severe hepatic impairment. *Renal impairment* – Caution is recommended when administering Iclusig to patients with estimated creatinine clearance of <50 mL/min or end-stage renal disease. *Elderly patients* – Are more likely to experience adverse reactions. *Paediatric population* – The safety and efficacy of Iclusig in patients less than 18 years of age have not been established. **Interactions: Co-administration of Iclusig with strong CYP3A4 inducers should be avoided;** caution should be exercised with concurrent use of Iclusig and strong CYP3A inhibitors and a starting dose of 30 mg considered; caution should also be exercised with concurrent administration of substrates of P-glycoprotein (P-gp) or breast cancer resistant protein (BCRP). Caution should be taken with concomitant use of anti-clotting agents in patients who may be at risk of bleeding. Pregnancy and breastfeeding: Women of childbearing age should be advised not to become pregnant, and men should be advised not to father a child during treatment. An effective method of contraception should be used during treatment - **it is unknown if Iclusig affects systemic hormonal contraceptives so an alternative or additional method should be used.** There are no adequate data from the use of Iclusig in pregnant women. Studies in animals have shown reproductive toxicity. If Iclusig is used during pregnancy, the patient must be informed of the potential risk to the foetus. Breastfeeding should be stopped during treatment. **Undesirable effects:** *Very common ADRs (≥1/10)* – Upper respiratory tract infection, insomnia, anaemia, decreased platelet count, decreased neutrophil count, decreased appetite, headache, dizziness, hypertension, dyspnoea, cough, abdominal pain, diarrhoea, vomiting, constipation, nausea, increased lipase, increased alanine aminotransferase, increased aspartate aminotransferase, rash, dry skin, bone pain, arthralgia, myalgia, pain in extremity, back pain, muscle spasms, fatigue, asthenia, peripheral oedema, pyrexia, pain. *Common ADRs (≥1/100 to <1/10)* – Pneumonia, sepsis, folliculitis, pancytopenia, febrile neutropenia, decreased white blood cell count, hypothyroidism, dehydration, fluid retention, hypocalcaemia, hyperglycaemia, hyperuricaemia, hypophosphataemia, hypertriglyceridaemia, hypokalaemia, decreased weight, cerebrovascular accident, cerebral infarction, peripheral neuropathy, lethargy, migraine, hyperaesthesia, hypoaesthesia, paraesthesia, transient ischaemic attack, blurred vision, dry eye, periorbital oedema, eyelid oedema, cardiac failure, myocardial infarction, congestive cardiac failure, coronary artery disease, angina pectoris, pericardial effusion, atrial fibrillation, decreased ejection fraction, peripheral arterial occlusive disease, peripheral ischaemia, peripheral artery stenosis, intermittent claudication, deep vein thrombosis, hot flush, flushing, pulmonary embolism, pleural effusion, epistaxis, dysphonia, pulmonary hypertension, pancreatitis, increased blood amylase, gastrooesophageal reflux disease, stomatitis, dyspepsia, abdominal distension, abdominal discomfort, dry mouth, increased blood bilirubin, increased blood alkaline phosphatase, increased gamma-glutamyltransferase, pruritic rash, exfoliative rash, erythema, alopecia, pruritis, skin exfoliation, night sweats, hyperhidrosis, petechia, ecchymosis, skin pain, exfoliative dermatitis, musculoskeletal pain, neck pain, musculoskeletal chest pain, erectile dysfunction, chills, influenza-like illness, non-cardiac chest pain, mass, face oedema. *Uncommon ADRs (≥1/1000 to <1/100)* – Tumour lysis syndrome, cerebral artery stenosis, retinal vein thrombosis, retinal vein occlusion, retinal artery occlusion, visual impairment, myocardial ischaemia, acute coronary syndrome, cardiac discomfort, ischemic cardiomyopathy, arteriospasm coronary, left ventricular dysfunction, atrial flutter, poor peripheral circulation, splenic infarction, venous embolism, venous thrombosis, hypertensive crisis, kidney artery stenosis, gastric haemorrhage, hepatotoxicity, hepatic failure, jaundice. **Denmark packages & prices:** 15 mg: 60 tabl. 45 mg: 30 tabl. See prices at www.medicinpriser.dk Danish prescription group: BEGR, hospitals only. **Marketing Authorisation Holder:** ARIAD Pharma Ltd., Riverbridge House, Guildford Road, Leatherhead, KT22 9AD, United Kingdom. **This text has been rewritten and/or abbreviated compared to the authorised summary of product characteristics (2016-03-22). The summary of product characteristics can be ordered free of charge from ARIAD. Product information may differ internationally. Date of preparation:** April 2016; UK/ICLG/15/0060.

The Science Behind **Immuno-Oncology**

The science behind Immuno-Oncology is based upon the understanding of the mechanisms tumours use to escape the immune system and how these can be modulated to promote tumour destruction.[1]

At Bristol-Myers Squibb, we are committed to furthering the science and understanding of Immuno-Oncology through our research and development, as well as by supporting educational activities.



For more information, please visit the I-O section of Booth 202

Reference:
1. Drake CG et al. *Adv Immunol*. 2006;90:51-81.

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